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ORIGINAL ARTICLE

Antioxidant Effectiveness Test of Olive Oil on Malondialdehyde in Hyperglycemic Rats

Uji Efektifitas Antioksidan Minyak Zaitun terhadap Malondialdehyde (MDA) pada Tikus Hiperglikemia

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ABSTRACT

Background

Hyperglycemia is a medical condition that increases blood glucose levels beyond normal limits because the body lacks enough insulin or insulin does not work properly and is known to trigger oxidative stress. In cells sensitive to Hyperglycemia, such as endothelial cells, excessive glucose loads trigger the formation of Reactive Oxygen Species (ROS) in mitochondria, impairing mitochondrial function. ROS are essential mediators for activating pro-inflammatory signaling pathways; obesity and hyperglycemia-induced ROS production may support the induction of pro-inflammatory macrophages such as M1 during the onset and development of diabetes. Consumption of olive oil can increase plasma antioxidant capacity and reduce oxidative stress markers.

Methods

This study aimed to determine the effectiveness of olive oil antioxidants against Malondialdehyde (MDA) in hyperglycemic rats. This is an experimental design with a post-test-only group design. Adult male Wistar rats were divided into three groups (n = 8): negative control (K-), positive control (K+), and treatment (P). The normality test was conducted using the Shapiro-Wilk test, followed by Kruskal Wallis.

Results

The olive oil was proven to reduce levels of Malondialdehyde in the P group, with an average of 2.29 nmol/mL lower than the K+ group. Although the olive oil reduced the average MDA level in hyperglycemic rats, the post-hoc test showed no difference in MDA between the K+ and P groups (p-value = 0.226).

Conclusions

Antioxidants in olive oil may effectively lower blood glucose levels, reducing levels of Malondialdehyde in hyperglycemic rats.

Keywords: Hyperglycemia; Malondialdehyde; Reactive Oxygen Species; Olive Oil; Oxidative Stress

Markers

ABSTRAK

Latar Belakang

Hiperglikemia merupakan suatu kondisi peningkatan kadar glukosa darah melebihi batas normal dikarenakan tubuh tidak memproduksi insulin atau insulin tidak bekerja dengan baik yang dapat memicu stres oksidatif. Pada sel yang sensitif terhadap hiperglikemia, seperti sel endotel, beban glukosa yang berlebihan memicu pembentukan *Reactive Oxygen Species* (ROS) di mitokondria yang dapat merusak fungsi mitokondria. ROS adalah mediator penting untuk aktivasi jalur pensinyalan pro-inflamasi, obesitas dan produksi ROS yang diinduksi hiperglikemia dapat mendukung induksi makrofag pro-inflamasi seperti M1 selama onset dan perkembangan diabetes. Konsumsi minyak dari buah zaitun dapat meningkatkan kapasitas antioksidan plasma dan menurunkan penanda stres oksidatif. Penelitian ini bertujuan untuk mengetahui efektivitas antioksidan minyak zaitun terhadap malondialdehid (MDA) pada tikus hiperglikemik.

Metode

Ini adalah desain eksperimental dengan desain grup post test only. Tikus wistar jantan dewasa dibagi menjadi 3 kelompok (n=8), kontrol negatif (K-), kontrol positif (K+), dan perlakuan (P). Uji normalitas dilakukan dengan menggunakan uji Shapiro-Wilk dilanjutkan dengan Kruskal Wallis.

Hasil

Minyak zaitun terbukti menurunkan kadar malondialdehid pada kelompok P dengan rata-rata 2,29 nmol/mL lebih rendah dibandingkan kelompok K+. Meskipun minyak zaitun terbukti menurunkan kadar MDA rata-rata pada tikus hiperglikemik, uji Post-Hoc menunjukkan tidak ada perbedaan MDA antara kelompok K+ dan P (nilai p = 0,226).

Kesimpulan

Antioksidan dalam minyak zaitun dapat menurunkan kadar glukosa darah, menurunkan kadar malondialdehid pada tikus hiperglikemik.

Kata Kunci: Hiperglikemia; Malondialdehyde; Reactive Oxygen Species; Minyak Zaitun; Marker Stress Oksidatif

INTRODUCTION

Hyperglycemia is a characteristic of diabetes mellitus, a medical condition characterized by increased blood glucose levels beyond normal limits because the body cannot produce insulin or insulin does not work properly.[1,2] Hyperglycemia in diabetes mellitus is associated with long-term damage, dysfunction, and failure in various organs.[2,3]

Diabetes mellitus (DM) is a complex disease because it is a metabolic disorder associated with oxidation and inflammation. It is the most common endocrine disease globally, with the highest prevalence and mortality rates in the world.[4–6]. The number of people with diabetes mellitus continues to increase every year. In 2019, 463 million adults (8.8% of the global population) were diagnosed with diabetes mellitus; it is estimated that this number will reach 700 million in 2045.[7] In 2021, in Indonesia, as many as 19.5 million adults suffer from diabetes mellitus, and Indonesia is in fifth place out of 10 countries with the highest prevalence of DM worldwide. It is estimated that this number will increase to 28.6 million in 2045.[8]

Hyperglycemia in diabetes mellitus is known to trigger oxidative stress.[9] The cells sensitive to Hyperglycemia, such as endothelial cells, excessive glucose loads trigger the formation of reactive oxygen species (ROS) in mitochondria, impairing mitochondrial function.[10,11] ROS formation is exacerbated by diabetes and macrophages due to shifts in glycolytic metabolism. Macrophages play an essential role in developing diabetes and promote inflammation by releasing pro-inflammatory cytokines and proteases. ROS are important mediators for activating pro-inflammatory signaling pathways; obesity and hyperglycemia-induced ROS production may support the induction of pro-inflammatory macrophages such as M1 during the onset and development of diabetes.[12] ROS react with various cellular constituents, including DNA, lipids, and proteins, to cause cell damage. Excessive ROS activate pro-inflammatory transcription factors such as NFkB and activator protein-1 (AP-1), which increase the expression of pro-inflammatory chemokines/cytokines and adhesion molecules. Activated endothelial cells attract monocytes, increasing inflammation and triggering macrovascular and microvascular injury.[11] Inflammation can be marked as one of them with levels of Malondialdehyde. Malondialdehyde is the end product of lipid peroxidation, a good marker of free radical-mediated damage and oxidative stress. MDA measurement has been used as an indicator of lipid peroxidation.

Malondialdehyde is the most frequently used compound as an indication of lipid peroxidation. MDA is also a compound that can describe the activity of free radicals in cells, so it is used to indicate oxidative stress due to free radicals. The high level of MDA is affected by the l peroxidation, which indirectly shows an increase of free radicals and an indication process in the cell membrane. High antioxidant status usually causes a decrease in MDA levels.[13,14] The components of polyphenolic compounds from olive oil can capture free radicals and reduce oxidative stress so that they can affect MDA levels.[14,15] MDA is a measure of lipid peroxidation of membrane lipids, which is directly proportional to the oxidative stress on the cell membrane. The correlation between MDA and adenosine deaminase (ADA) levels in relation to diabetes mellitus control based on HbA1C levels showed that there is auto-oxidation of glucose, which results in persistent production of MDA and ROS, which will release further glycation end products and further lipoxidation end products.[16] Normally, the body has a systematic strategy to combat the formation of free radicals or to accelerate the degradation of these compounds. One of them is a preventive defense system such as superoxide dismutase and catalase enzymes. However, due to this condition of hypercholesterolemia and Hyperglycemia, it can increase the occurrence of a number of reactive oxygen species. This excessive amount of ROS causes lipid peroxidation, which produces MDA and can reduce the capacity of intracellular antioxidant enzymes, superoxide dismutase, and catalase.[17]

Consumption of foods that contain high levels of antioxidants and polyphenols can increase plasma antioxidant capacity and reduce oxidative stress markers in people with diabetes, obesity, hypertension, and hypertriglyceridemia.[9] One of the foods with high levels of antioxidants and polyphenols is olive oil, which contains components and acts as a natural antioxidant in the body. Olive oil is extracted from olives, which has many health benefits.[18] The olive oil contains the primary antioxidants, namely polyphenols. The oil from olives can modulate genes related to insulin sensitivity and increase antioxidant defenses in the body. In polyphenols, several compounds are associated with increased antioxidant activity, such as flavonols, phenolic acids, phenolic alcohols, and secoiridoids.[19] Several components of olive oil that are considered to have

antioxidant activity are oleuropein and hydroxytyrosol. It was found that hydroxytyrosol and oleuropein can counteract free radicals and reduce intracellular ROS levels, as well as prevent oxidative DNA damage in breast cancer cell lines.[20] Extra antioxidant activity of olive oil increases significantly in high phenol concentrations.[21] The polyphenols from plants have various potential health benefits and act as hypoglycemic agents to reduce blood glucose levels in Hyperglycemia.[22]

The components of polyphenolic compounds from olive oil can capture free radicals and reduce oxidative stress so that they can affect MDA levels.[14,23] MDA is a measure of lipid peroxidation of membrane lipids directly proportional to the oxidative stress on the cell membrane. The correlation between MDA and adenosine deaminase (ADA) levels in relation to diabetes mellitus control based on HbA1C levels showed that there is auto-oxidation of glucose, which results in persistent production of MDA and ROS, which will release further glycation end products and further lipoxidation end products.[16] Normally, the body has a systematic strategy to combat the formation of free radicals or to accelerate the degradation of these compounds. One of them is a preventive defense system such as superoxide dismutase and catalase enzymes. However, due to this condition of hypercholesterolemia and Hyperglycemia, it can increase the occurrence of a number of reactive oxygen species. This excessive amount of ROS causes lipid peroxidation, which produces MDA and can reduce the capacity of intracellular antioxidant enzymes, superoxide dismutase, and catalase.[17]

This research aimed to determine the effectiveness of antioxidants from olive oil to Malondialdehyde in Hyperglycemic Rats. The information obtained from this study can be used as basic data for other researchers to research further the effect of giving olive oil antioxidants. It can increase public awareness about the benefits of olive oil antioxidants for health.

METHODS

This study uses a post-test-only group design following a Completely Randomized Design. This study consisted of 3 treatment groups with the number of experimental animals 8 K- (negative control), which were not induced by alloxan and not given the olive oil, 8 K+ (positive control,) which were given intraperitoneal alloxan induction with one dose of 100 mg/kg BW and were not given the olive oil, and 8 P groups (treatments) received alloxan intraperitoneally at 100 mg/kg BW and the olive oil orally at 25 mL/day for 14 days. The experimental animals were 24 adult male Wistar rats. In the animal house of Andalas University, objects were treated, maintained, and weighed. MDA level examinations were conducted at the Biomedical and Biochemical Laboratory, Faculty of Medicine, Andalas University, Padang. Inclusion criteria included adult male Wistars aged 2-3 months with a weight of 200-250 kg and healthy rats of the same type. The exclusion criteria included mice that had received treatment interventions in previous studies, sick mice (characterized by hair loss/baldness, dullness, inactivity, exudate discharge from eyes, mouth, genitals, and anus), and physically disabled mice anatomy. Rats that died during the study period were considered dropouts activity indicator, MDA levels obtained from mice blood serum. Examination of rat MDA levels was carried out on the 15th day after treatment in all groups. The MDA inspection method has been modified as follows:[24,25]

a. Reagen: blood ± 3 mL, TBA Reagen, TCA 5% Reagen, and standard MDA
b. Procedure: Centrifuge the blood, then separate the serum and prepare the tube according to the following procedure:

Table 1. MDA Sampling Procedure

REAGEN	BLANKO	STANDARD	SAMPLES (1,2, etc)
Aquadest	500 µl	-	-
Standard	-	500 µl	-
Sample Serum/ Homogenate	-	-	500 µl
Add to each tube as much as 2.5 ml of 5% TCA			
Mix using a vortex mixer			
Centrifuge at 10,000 RPM for 15 minutes			
Pipette the filtrate (each tube) as much as 1 ml			
Add 1 ml of TBA reagent			
Incubate in a Water Bath for 30 minutes at 100 °C			
Was cooled			
Read Absorbance with Spectrophotometer at 530 nm			
Separate the filtrate			

$$\text{Sample MDA Levels} = \frac{\text{Sample Absorbance}}{\text{Standard absorbent}} \text{ X C. Standard}$$

Data analysis was performed using a computerized system, and all the data were presented as mean ± standard deviation. Due to the small number of samples, a normality test was also conducted using the Shapiro-Wilk test, followed by a homogeneity test using the Levene test. The data is not usually heterogeneous, so non-parametric statistical analysis is continued, namely Kruskal Wallis, followed by a post hoc analysis using the Mann-Whitney test with a 95% confidence level to determine the differences between the control groups and each treatment. In this analysis, if the average difference is $\alpha \leq 0.05$, the research results are considered significant.

This research was presented to the Faculty of Medicine Ethics Committee, Andalas University, Padang, and received a certificate of passing the ethical review with number 1051/UN.16.2/KEP-FK/2022.

RESULTS

This study aims to determine the relationship of antioxidant olive oil to Malondialdehyde in hyperglycemic rats. The experimental animals were 24 rats aged 2-3 months, which were divided into three groups, namely the negative control group (K-), the positive control group (K+), and the treatment group (P).

Table 2. Examination Results of Glucose Levels (mg/dl)

Category	Initial Glucose Levels	Levels of Glucose I	Levels of Glucose II	Levels of Glucose III
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
K-	62.00 ± 10.16	72.75 ± 5.55	71.88 ± 5.06	72.50 ± 3.42
K+	65.38 ± 6.55	254.38 ± 34.38	284.38 ± 28.67	299.88 ± 20.54
P	70.00 ± 7.65	215.25 ± 18.13	155.63 ± 15.45	144.75 ± 14.06

*Examination of glucose levels consists of:

1. Initial glucose levels: Before being given alloxan and olive oil treatment,
2. Level of glucose I : After being given alloxan,
3. Levels of glucose II : On the 10th day of treatment,
4. Glucose level III : after completion of treatment.

The average glucose level of rats in the early stages was higher in rats induced by alloxan and given the olive oil at a dose of 0.45 mL/day (P) compared to K+ and K. However, after doing measurements I, II, and III, it was found that the levels The highest blood glucose in rats induced by alloxan (K+), namely 254.38 mg/dl (measurement I), 284.38 mg/dl (measurement II), and 299.88 mg/dl (measurement III). Rats induced by alloxan and given the olive oil at a dose of 0.45 mL/day had lower glucose levels, namely 215.25 mg/dl (measurement I), 155.63 mg/dl (measurement II), and 144.75 mg /dl (measurement III) compared to the positive control group.

Table 3. Examination Results of Glucose Levels (mg/dl)

Measurement	N	Negative control (K-)	Positive control group (K+)	Treatment (P)	P *value*
Initial glucose levels	24	62.00 ± 10.12	65.38 ± 6.55	70.00 ± 7.65	0.176[a]
Levels of glucose I	24	72.75 ± 5.55	254.36± 34.38	215.25 ± 18.13	<0.001[b]
Levels of glucose II	24	71.88 ± 5.06	284.376 ± 28.67	155.63 ± 15.45	<0.001[b]
Levels of glucose III	24	72.50 ± 3.42	299.88 ± 20.54	144.75± 14.06	<0.001[b]

Note: [a]*One Way Anova;* [b] *Kruskal Wallis*

Based on the table above, the results of the analysis of glucose levels show no difference in the average analysis of initial glucose levels in each group of experimental animals. This can be proven by p values = 0.176 (p > 0.05). On the other hand, the other measurements have significant differences.

Examination of Malondialdehyde (MDA) Levels (nmol/mL)

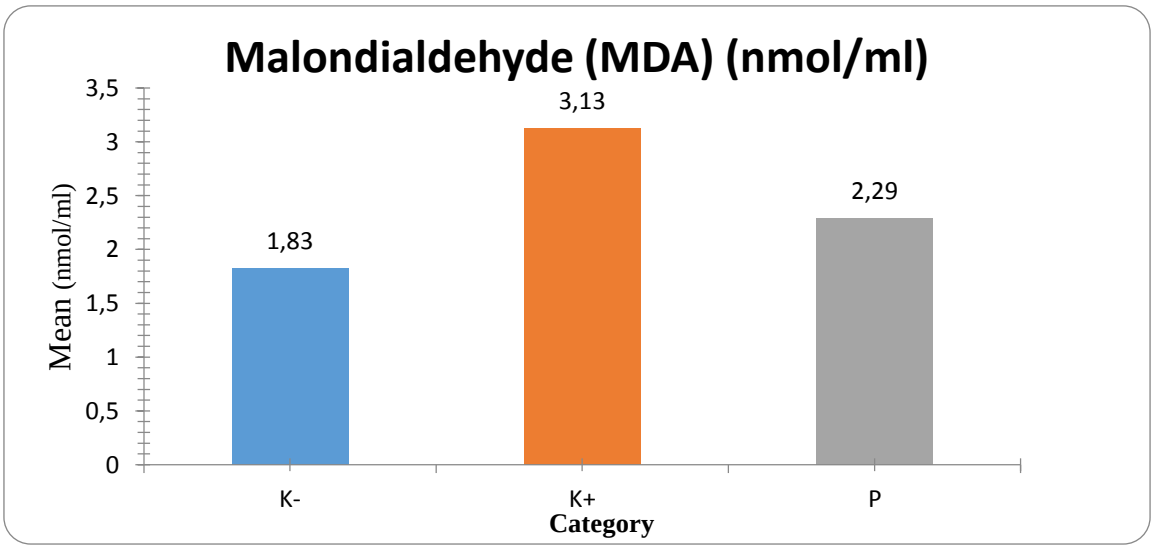

Figure 1. Results of Examination of Malondialdehyde (MDA) Levels

The mean MDA level of rats only induced by alloxan (K+) was higher, namely 3.13 nmol/mL, compared to K- and P. The group of rats that were only given standard feed or without alloxan induction and other interventions as negative controls (K-) had average MDA levels lower: 1.83 nmol/mL compared to K+ and P. Rats induced by alloxan and given olive oil at a dose of 0.45 mL/day, had lower MDA levels, namely 2.29 nmol/mL compared to the positive control grub.

There are differences in the mean MDA levels in each group of experimental animals. This can be proven by p-value = 0.002 (p ≤ 0.05).

Table 4. Post-Hoc Test for MDA Levels

Category	K-	K+	P
K-	-	0.020	0.006
K+	0.020	-	0.226
P	0.006	0.226	-

*uji *Man Whitney*

A significant difference in MDA levels was found in the positive control group (K+) and the negative control group (K-) with a p-value = 0.020. However, there was no significant difference in MDA levels between the positive control group (K+) and the treatment group (P), with a p-value = 0.226.

DISCUSSION

Effect of Antioxidant Olive Oil on Malondialdehyde (MDA) in Hyperglycemic Rats

This study showed differences in the mean levels of Malondialdehyde (MDA) in all groups of mice using the Kruskal-Wallis test (p-value = 0,002). Based on the table, the negative control group (K-) rats had the lowest average MDA level of 1.83 ± 0.20 nmol/mL, then the treatment group (P) had a lower average MDA level of 2.29 ± 0.28 nmol/mL compared to the positive control group (K+) with an average MDA level of 3.13 ± 1.28 nmol/mL. In this research procedure, pure negative control (K-) rats were not given any treatment, then the positive control group (K+) rats were rats that were given alloxan at a dose of 100 mg/kg BW, waited 3-7 days to reach Hyperglycemia without giving olive oil, while The rats in the treatment group (P) were rats that had been given alloxan induction at a dose of 100 mg/kg BW followed by 25mL/day of olive oil for 14 days.

In the positive control group (K+), rats that were given alloxan damaged pancreatic β cells, resulting in decreased insulin secretion. This increased blood sugar until it reached Hyperglycemia, with the result that the average blood sugar at the beginning of the measurement was 65.38 mg/dl, increasing to 299.88 mg/dl at the end.

Hyperglycemia causes an increase in Reactive Oxygen Species, resulting in oxidative stress. Oxidative stress is defined as an imbalance between the production of reactive oxygen species and the rate of their degradation by the endogenous antioxidant defense system, which is composed of various enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and heme oxygenase-1 (HO). -1). Overproduction of ROS is associated with several molecular mechanisms, including alteration of the mitochondrial electron transport chain, formation of advanced glycation end products, and activation of specific ROS-producing enzymes such as NADPH oxidase 4 (Nox4). During hyperglycemia-mediated oxidative stress, high levels of ROS cause endothelial dysfunction by damaging DNA, proteins, and lipids and by deregulating the production of NO and the redox-sensitive transcription factor, erythroid-associated nuclear factor 2 factor 2 (Nrf2), which regulates the expression of genes encoding redox enzymes.[26]

Meanwhile, the rats in the treatment group (P) were also induced by alloxan until they reached Hyperglycemia with an initial blood sugar average of 70.00 mg/dl to 144.75 mg/dl. After

achieving Hyperglycemia, olive oil was given at a 25 mL/day dose to suppress free radicals caused by alloxan administration.

The analysis results show that the group (P) experienced a decrease in the average MDA compared to the (K+) group, which was not given olive oil. These can happen because oil contains polyphenols, which can capture free radicals to help reduce oxidative stress so that, it affects MDA levels. Recently, it was recognized that antioxidants have a direct inhibitory effect on lipid peroxidation.[27] The antioxidants in olive oil protect cells from oxidative damage caused by free radicals. These results align with previous studies where the treated rats had lower MDA levels than the control group.[16]

Another study found that the combination treatment of olive oil and fish oil gave better results in reducing MDA levels than the treatment of olive oil alone. Another experimental study on rats treated with olive oil combined with andaliman showed significant results for lowering MDA levels because it has good antioxidant content. So, it can be concluded from the findings of this study that the smaller amount of antioxidants can explain the increased susceptibility to oxidation, which causes oxidative stress.[28] Another study found that the combination treatment of olive oil and fish oil gave better results in reducing MDA levels than the treatment of olive oil alone. This is due to one of the components of olive oil, namely tocopherol, which consists of alpha, beta, gamma, and delta tocopherols. The alpha-tocopherol type is the highest concentration in olive oil, reaching ninety percent, and is ideal as an antioxidant. On the other hand, fish oil contains astaxanthin, which can protect cells from oxidation by neutralizing free radicals and preventing oxidation reactions. The content in fish oil requires tocopherol compounds to work effectively as antioxidants.[16]

Effect of Antioxidant Olive Oil on Blood Glucose in Hyperglycemic Rats

This research showed differences in the average initial blood glucose levels in all test groups seen from 3 measurements after alloxan administration and treatment. The initial measurement found that the average blood glucose level in the alloxan-induced group was higher by 65.38 mg/dL compared to the K- group of 72.75 mg/dL and the P group of 70.00 mg/dL. Then, in the first measurement, the average blood glucose result in the K+ group increased by 254.36 mg/dL as well as the K- and P groups with a p-value <0.001 (<0.05) or there was a significant difference. In the second blood glucose measurement, there was an increase in blood glucose levels in the K+ group of 284.376 mg/dL and a decrease in the K- group of 71.88 mg/dL and the P group from 215.25 mg/dL to 155.63 mg/dL. In the 3rd measurement of blood glucose levels, there was an increase in blood glucose levels in the K+ group of 299.88 mg/dL and the K- group of 72.50 mg/dL. However, there was a decrease in blood glucose levels in the P group to 144.75 mg/dL.

Administration of alloxan at a dose of 100 mg/kg BW causes damage to pancreatic beta cells so that the secreted insulin is unable to regulate glucose in the blood and makes significant changes to increasing blood glucose levels in alloxan-induced rats.[29] Alloxan in the body will undergo oxidation-reduction metabolism, which produces free radicals. These radicals cause damage to pancreatic β cells.[30] Alloxan is toxic and selective to pancreatic β cells that produce insulin because alloxan accumulates through the glucose transporter, GLUT2. Alloxan accumulates in pancreatic β cells because alloxan has properties analogous to glucose, so it is accepted by GLUT2. It can enter the plasma membrane of the lipid bilayer of pancreatic β cells.

The result of the entry of alloxan into pancreatic β cells is damage to insulin-producing granules[31], and increased blood glucose levels.[32]

Some phenolic compounds from olives have antioxidants. Oleuropein and hydroxytyrosol can inhibit leukotriene B4, a pro-inflammatory cytokine. Several other polyphenolic compounds can also inhibit platelet aggregation, lipooxygenase, and eicosanoid pathways.[33] This study showed that giving olive oil extract for 14 days significantly reduced blood sugar levels in rats. This study's results align with the research by SH Bintari & K. Nugraheni (2012), which states that giving olive oil to Sprague Dawley rats can reduce blood glucose levels. The average blood sugar level in the control group given distilled water was 138.14 mg/dl. Provision of olive oil as much as 0.5 g / day, 0.7 g/day, and 0.9 g/day, respectively, showed a decrease in blood glucose of 40.43 mg/dl, 57.30 mg/dl, and 62.23 mg/dl compared to the control group.[34] The decrease in blood glucose in rats was caused by the role of olive oil in triggering the production of the hormone GLP-1 (Glucagon-Like Peptide1).[35] GLP-1 plays a role in slowing gastric emptying and stimulating insulin secretion. That is why the consumption of antioxidants from olive oil in this study has been shown to reduce blood glucose levels. A recent review reported that high phenolic olive oil does not modify the lipid profile compared with its low phenolic counterpart[36] though other studies have reported that extra virgin olive oil decreases LDL-C directly measured as concentrations of apoB-100 and the total number of LDL particles as assessed by NMR spectroscopy.[37,38] We expected coconut oil to raise LDL-C compared with olive oil. Still, in the current study, we observed no evidence of an overall average increase in LDL-C in individuals allocated to olive oil intervention. LDL = Cholesterol - HDL – (Triglycerides/2.2).[39]

CONCLUSION

From the results of this study, it was found that giving the olive oil effectively lowered blood glucose levels, reducing levels of Malondialdehyde in hyperglycemic rats.

It is hoped that further research can be carried out with extended treatment time intervals, which may be why the results of the decrease in MDA levels are not statistically significant. Other researchers can also continue with other variables related to the effect of giving olive oil antioxidants to hyperglycemic rats that have not been analyzed in this study, such as SOD (SuperOxide Dismutase) activity, lipid profile, and its effect on pancreatic β-cell histology.

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AUTHORS CONTRIBUTION

AZO : Penulis, pengumpul data, penyusunan draf naskah. EY : Penulis, meninjau dan menyetujui final naskah. R : Penulis, meninjau dan menyetujui final naskah. A : Penulis, meninjau dan menyetujui final naskah.

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CONFLICT OF INTEREST

Competing interests: No relevant disclosures".

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