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RESEARCH ARTICLE

Repeated Frying of Olive Oil has a Better Effect on Survival Rate Compared to Palm Oil through Glucose Energy Metabolism Increase in Mice

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ARTICLE HISTORY

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Abstract: ***Background*:** Vegetable oil is widely used for frying food, especially palm oil. Repeated use of cooking oil is often unavoidable. Frying is known to change cis unsaturated fatty acids into trans. However, the effects of repeated frying of olive oil compared to palm oil is still not widely known.

***Objective*:** The objective of this study is to determine the effect of repeated frying of palm oil and olive oil on survival rate and blood glucose pattern in mice.

***Methods*:** There were 39 adult male mice divided into 3 groups: control (n=12), palm (n=13), and olive (n=14). Control group was given oral gavage of water during research. Palm and olive oils were given in palm and olive groups. Before given to mice, palm and olive oils were used for frying sweet potatoes 5 times then given to mice for 4 weeks. At the beginning and after treatment, fasting blood glucose (FBG), the 2 hours postprandial oral glucose tolerance test (2-h OGTT) blood glucose level were measured and then area under the curve (AUC) was also calculated.

***Results*:** After 4 weeks of treatment, there were 9 mice that remained alive in the control group, 9 mice in the palm group, and 11 mice in the olive group. The survival rate was 75% in the control group, 69.23% in the palm group, and 78.57% in the olive group. Weight gain was significantly different between pre and post in the control and olive groups (p=0.009; p=0.004). FBG level increased significantly in the palm group compared to the control group (P<0.05). The 2-h OGTT glucose level increased significantly compared to pre in the control group (p=0.004) and olive group (p=0.000). AUC of the olive group increased significantly compared to the control group (p=0.0039).

***Conclusion*:** Consumption of olive oil which is fried repeatedly and given for 4 weeks shows a better survival rate than palm oil.

Keywords: Olive, palm, oil, glucose, *cis* fat, *trans* fat, mice, health.

1. INTRODUCTION

The use of vegetable cooking oil is increasing in Indonesia. Based on the data from the Health Research and Development Unit in Indonesia, in 2014, the majority of Indonesia's population (92.6%) consumed palm oil. The following are coconut and its processed products (29.4%), and other oils, including olive oil (7.1%) [1]. The olive oil has a predominant fatty acid in the type of oleic acid (18: 1, n-9), a monounsaturated fatty acid (Rice Bradley, 2018). It is also known that vegetable oil that is fried repeatedly can increase the formation of trans fatty acids (Sartika, 2009).

Some risk factors that can increase the incidence of metabolic diseases such as diabetes mellitus (DM) are obesity and the use of trans oil [2, 3]. Research conducted by Kavanagh *et al.* (2007) proved that trans fat can cause abdominal obesity and changes in insulin sensitivity in monkeys. Also, Risérus (2006), in his study, mentioned that increasing intake of trans fatty acids (TFA) can increase the risk of insulin resistance and type 2 diabetes mellitus (DM2). Therefore, an increase in fat consumption will lead to obesity, which triggers the occurrence of insulin resistance and further increases the risk of DM2. According to WHO (2018), the prevalence of Diabetes Mellitus in 1980 was 108 million and increased in 2014 to 422 million. The global prevalence of diabetes in adults over the age of 18 increased from 4.7% in 1980 to 8.5% in 2014 [4-6].

Therefore, the purpose of this study is to determine the effect of repeated frying of olive oil and palm oil on the survival rate and blood glucose levels pattern in mice. Blood glucose is the predominant energy source and fat can be changed to glucose *via* gluconeogenesis.

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2. MATERIALS AND METHODS

2.1. Animals

This experimental study used male mice (*Mus musculus*) Balb/C as a research animal with a pre- and post-test control group design.

This study used 39 mice of 8-week-old male mice weighing 15-30 grams at the beginning of the treatment. Acclimatization was carried out for 1 week. The mice were divided into 3 groups, namely the control group which was only given a standard diet and oral gavage of water, the palm group which was given a standard diet and oral gavage of palm cooking oil (0.1-0.3 mL), and the olive group which was given a standard diet and oral gavage of olive cooking oil (0.1 -0.3 mL). The duration of treatment was 4 weeks.

2.2. Blood Glucose

Fasting blood glucose and 2 hours post-prandial of oral glucose tolerance test (2-h OGTT) levels in mice were measured before and after treatment. Blood glucose examination was taken from the tail veins of mice. Glucose examination used the EasyTouch® GCU system with the EasyTouch® II Blood Glucose Test Strips by Bioptik Technology, Inc. Based on Abdelmotaleb *et al*. (2017) study; the EasyTouch® GCU system is an accurate measurement of glucose levels. The area under the curve (AUC) were calculated to describe the pattern.

2.3. Ethical Review

This research was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

2.4. Statistical Analysis

Data were analyzed using the Anova test (if the data distribution was normal) and followed by post hoc test. If the data distribution was not normal, the Kruskal-Wallis test was used and continued with the Mann-Whitney.

3. RESULTS

Characteristics of body weight (BW) of mice at the beginning and the end of the treatment can be seen in Fig. (**1**). The results showed that significant weight gain during the study occurred in the control group (p=0.009) and the olive group (p=0.004).

At the beginning of the treatment, there were 12 mice in the control group, 13 mice in the palm group, and 14 mice in the olive group. During 4 weeks of treatment, the number of living mice was 9 mice or 75% in the control group, 9 mice or 69% in the palm group, and 11 mice or 78.5% in the olive group (Table **1** and Fig. **2**).

Fasting blood glucose (FBG) level after treatment showed a significant increase in the olive group compared to other groups. FBG before and after treatment in the olive group also showed a significant increase (p = 0.004) (Fig. **3**).

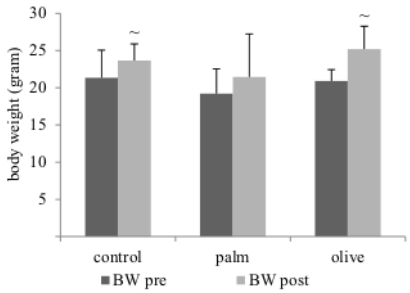

Fig. (1). Body weight (gram) before (BW pre) and after (BW post) treatment. Statistical analysis was done using Kruskal-Wallis and Mann-Whitney test. ~: significantly different (p<0.05) compared with BW pre. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

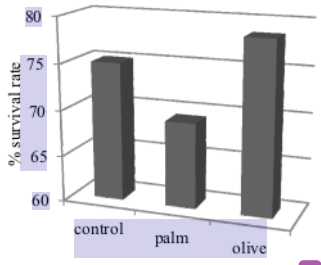

Fig. (2). Mice survival rate after treatment (%). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Mice survival rate after treatment.

	n (pre)	n (post)	survival (%)	dead (%)
control	12	9	75.00	25.00
palm	13	9	69.23	30.77
olive	14	11	78.57	21.43

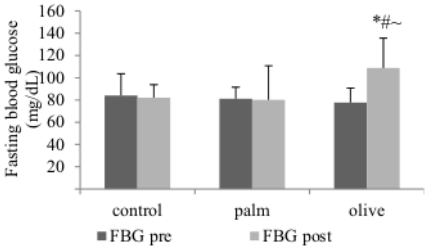

Fig. (3). Fasting blood glucose (mg/dL) before (FBG pre) and after (FBG post) treatment. Statistical analysis was done using Anova, posthoc LSD, and paired T test. (*) significantly different (p<0.05) compared with control; (#) significantly different (p<0.05) compared with palm (~) significantly different (p<0.05) compared with FBG pre. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In the measurement of 2 hours postprandial of OGTT (2-h OGTT), there were no significant differences between groups (Fig. **1**). However, in the control and olive groups, there was a significant increase in the OGTT after treatment (OGTT post) compared to before treatment (OGTT pre) (p=0.004; p=0.000) (Fig. **4**).

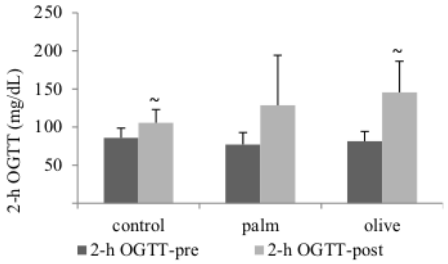

Fig. (4). The 2 hours postprandial (2-h OGTT) blood glucose level (mg/dL) before (2-h OGTT-pre) and after (2-h OGTT-post) treatment. Statistical analysis was done using Kruskal Wallis and Mann Whitney test. (6 significantly different ($p<0.05$) compared with 2-h OGTT-pre. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

An illustration of patterns of changes in blood glucose levels based on time and the results of the calculation of the area under curve (AUC) can be seen in Fig. (**5**). The results showed that the AUC after treatment (AUC post) of the olive group increased significantly when compared to the control group (p=0.0039). Also, the AUC post of the olive group increased significantly compared to AUC pre (p=0,000).

Based on the results above, when compared with the other groups, the olive group had many changes in the variables such as BW, FBG, 2-h OGTT, and AUC.

4. DISCUSSION

The growth of mice in this study was represented by weight gain. All groups showed an increase in body weight. The olive group gained the most increasing of weight, followed by the control group, and the least was the palm group. In the control and olive groups, body weight before and after treatment increased significantly. The olive group increase was greater than the control group because there were additional calories in the form of olive oil in the olive group. Meanwhile in the palm group, although there was a gain in weight, the increase was slight. This can be interpreted that there is a factor that inhibits growth in the administration of palm oil that was used for cooking repeatedly. Based on the addition of the type of calorie diet, there were different responses in the body weight because calories were given in the form of fat addition (in treatment group) or carbohydrates dominant or standard diet (in control group). The previous research stated giving additional carbohydrates (glucose) for 8 weeks shown weight loss [7]. Another study that also used olive oil for 12 weeks gave a different result from this study; it was a weight loss [8]. This difference can be caused by the given olive oil that was not in the form of fried oil.

The survival rate in the olive group had the highest percentage, followed by the control group, and the lowest was the palm group. This showed that during the treatment, mice experienced stress, including the control group. The stress can come from the oral gavage process or could be due to the environment at the time of the study. However, the environment of this study was the same for all groups. It is known that during stress, the body needs a lot of energy. The addition of fried olive oil administration seems to meet these needs. Nevertheless, strangely, fried palm oil which is also an additional calorie, showed a worse survival rate than the control group. This finding requires further study to reveal the underlying mechanism.

Throughout stress, the body increased its metabolism to provide energy. One of the hormones that stimulates this process is cortisol. The effect of metabolism that is affected by cortisol is an increase in blood glucose level for stress anticipation. On the other hand, excessive cortisol also has a negative effect because it can interfere with growth and decrease the immune system [9]. The addition of calorie is one of the efforts to cope with stress. However, cortisol level was not measured, which is one of the limitations of this study.

Fasting blood glucose (FBG) level in the treatment groups showed an increase, but the increase in the olive group was the most significant. Meanwhile, the control group showed a slight decrease that was not significant. The increase of FBG level in the palm group was not significant compared to the control group. It can be assumed that the mechanism of stress regulation was not optimal, referring to a lower survival rate. This is different from the research conducted by Larasati, Wirjatmadi, & Adriani (2016) which showed that palm oil heated repeatedly resulted in a significant increase in FBG [10-12].

The blood glucose level of two hours post prandial of oral glucose tolerance test (2-h OGTT) showed a significant increase between pre- and post-treatment in control and olive groups. In contrast, the increase in the palm group was not significant. In terms of survival rate, it seems that the increase in the glucose level has become one of the mechanisms to manage stress and inhibit the decrease of body immunity. There was no similar thing that occurred in the palm group, so further research is needed to uncover this phenomenon, such as measurement of insulin and glucagon level, and immunity performance.

Research by Abunab, Dator, & Hawamdeh (2017) presented that olive leaf extract improved fat levels and decreased blood glucose levels in DM mice. Another study conducted by Jamal & Ibrahim (2011) on subjects with DM given 30 mL of olive oil also showed a decrease in fasting blood glucose and lipid profile levels. Previous studies concluded that consumption of trans fatty acids, which are transitional forms of cis fatty acids due to frying or heating, does not contribute to the risk of diabetes and insulin resist-

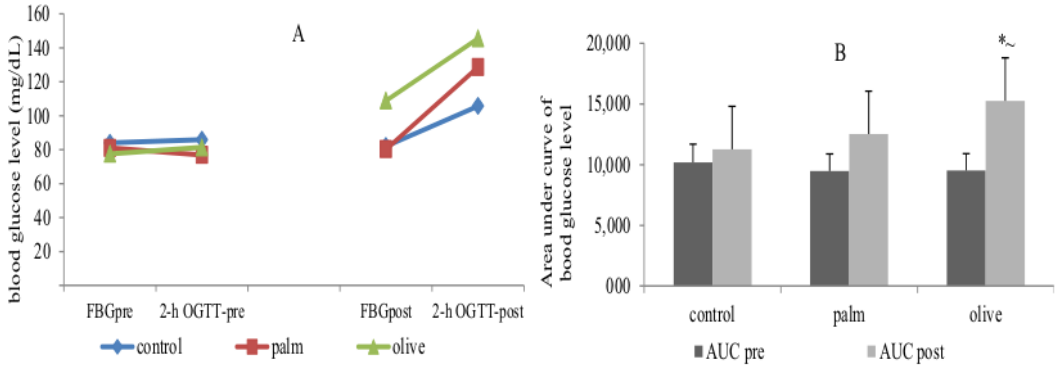

Fig. (5). A: Changes in blood glucose levels based on time using oral glucose tolerance test and to show the area under curve. **B:** AUC before (AUC pre) and after (AUC post) treatment. (*) significantly different ($p < 0.05$) compared with control; (~) significantly different ($p < 0.05$) compared with AUC pre. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

ance [13, 14]. However, studies in Finnish men suggest that an increase in total serum fasting monounsaturated fatty acids and oleic acid (18: 1, n-9) can predict the risk of developing DM2 [15].

In this study, we used olive oil that was fried repeatedly and the study was carried out in subjects with normal conditions, therefore, the results could be the difference, although in general, olive oil (frying/or not) shows a better effect for health, which has a higher survival rate, compared to palm oil.

CONCLUSION

Consumption of olive oil, which is fried repeatedly for 4 weeks administration, shows a better survival rate than consumption of palm fried oil. This can be due to the mechanism of the body compensation in dealing with stressors given by fried oil that is through the regulation of glucose metabolism. The regulation mechanism leads to an increased level of fasting and postprandial blood glucose levels. This is useful as a source of energy for diminish stress. However, further studies need to be performed to find out the mechanism behind this.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All animal research procedures were followed in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals (published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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