

Effect of Avocado Seed Extract through Milling on Reducing Triglyceride in Hyperlipidemic Rats

Rita Dewi¹, Jojor Lamsihar Manalu^{2*}, Yulia Tanti Narwati³, Maria Dara Novi¹, Alexander Yose Ardin Bagas Sulisty⁴

¹Department of Chemistry and Biochemistry, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, North Jakarta, Indonesia

²Department of Physiology, School Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, North Jakarta, Indonesia

³Department of Microbiology of School Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia

⁴School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, North Jakarta, Indonesia

*Corresponding author: Jojor Lamsihar Manalu, jojor.lamsihar@atmajaya.ac.id

Abstract

Background: Hypertriglyceridemia is a condition in which there is an increase in triglycerides in the blood. This increase is influenced by foods that contain extra calories. Hypertriglyceridemia can cause disturbances in the body in the form of cardiovascular disease.

Methods: The study used experimental methods on male Sprague Dawley rats. The research sample used fourteen rats which were divided into a control group and a treatment group. All experimental mice were induced to become hyperlipidemic by PTU14 gram/kg for 14 day. The control group was not given any treatment. The treatment group was given avocado seed extract 250 mg/kg for 14 day. Avocado seed extract is obtained through crushing and extraction processes. The statistic we used in this study is paired T-test.

Results: In the control group, there was no decrease in triglyceride levels with an average of 134.78 mg / dL. In the treatment group there was a decrease in the average triglyceride levels both after seven days of giving avocado seed extract on (day 28) 127.92 md / dL and after fourteen days of giving avocado seed extract on (day 35) 124.21 mg / dL.

Conclusion: Giving of avocado seed extract by grinding method for 30 minutes selama sekian hari had an effect in reducing triglyceride levels in hyperlipidemic rats.

Keywords: Avocado Seed Extract - Sprague Dawley Rats - Triglyceride Level.

INTRODUCTION

The pattern of consumption of foods in the modern era that contains high lipid & high carbohydrate and causes hyperlipidemia.¹

Hyperlipidemia is a pathological condition characterized by an increase in blood fats such as cholesterol and triglycerides. According to the Indonesian Cardiovascular Specialist Doctors Association,

Hyperlipidemia is a common health problem. The prevalence of hyperlipidemia in Indonesia reaches 39.8%.²

Triglycerides are one of the lipid profiles which when increased is not good for the health of the body. Increased triglycerides in the blood are also not good for the health of the body. This condition is called hypertriglyceridemia. Hypertriglyceridemia conditions can increase the risk of developing cardiovascular disease. Generally, triglycerides can be found in adipose tissue, but some triglycerides can be found in the bloodstream to provide energy for muscles at work.³ Based on research data taken from Basic Health Research (Riskesdas) in 2013, 13% of Indonesians aged ≥ 15 years have the number of abnormal triglyceride levels in the borderline high category and 11.9% in the very high category.⁴

Avocado seeds are waste that has not been widely used and it turns out that avocado seeds contain phenolic compounds which can affect the lipid profile, including reducing triacylglycerol levels.⁵ Reusing avocado seeds will be very useful for preserving the environment.

Researchers in Indonesia have tested the effect of avocado seed extract on total cholesterol levels in rats. Research conducted by Mufida et. al (2018)⁶ stated that avocado seed extract can reduce cholesterol levels in

mice. Research conducted by Rico Arlianto (2018)⁷ revealed that giving avocado seed extract can lower triglyceride levels in the blood. The two previous studies using avocado seed extract had no data regarding the 30 minutes of grinding time on the effectiveness of reducing triglyceride levels in the blood. Therefore, researchers are interested in examining the effectiveness of avocado seed extract by grinding for thirty minutes on reducing triglyceride levels in rats.

METHODS

Research design

This study used an experimental research design. In this study, researchers looked at triglyceride levels in rats that experienced hyperlipidemia after being given an avocado seed extract which had been crushed for 30 minutes. The treatment was given to the sample group and the results were seen. The research was conducted by pretest and post-test on all research subjects.

Study subjects

This study subjects used white male rats *Rattus norvegicus* Sprague Dawley strain with inclusion criteria:

- White male *Rattus norvegicus* strain Sprague-Dawley with healthy condition (active and not disabled)

- White male Rattus norvegicus strain Sprague-Dawley with body weight > 140 grams
- White male Rattus norvegicus strain Sprague-Dawley with age \pm 8 weeks

Exclusion Criteria include rat that did not want to eat at the time of adaptation

Drop Out Criteria:

- Rat that was sick during the experiment
- Rat that died during the experiment
- Rat that did not develop hypertriglyceridemia after being induced by PTU

Sample size determination

The sample size for this study uses the following degree of freedom formula:

Range E : 10 -20

$E = \text{Total animal used} - \text{Total test groups}^8$

In this study, the sample used 12 rats with 2 dropouts. The samples were divided into two groups where the triglyceride levels before getting intervention and triglyceride levels after being given the intervention.

When entered into the formula, the following results are obtained:

$E = (14 \times 1) - 1$

$E = 14 - 1$

$E = 13$

Based on the results, E (degree of freedom) is within the normal limit, which is between 10 and 20, so the sample is considered representative.

Intervention

The rats will be past the acclimatization for one week.⁹ After one week of acclimatization rats will be induction with PTU 40 mg / KgBB and diluted with water with a ratio of 1 table: 12.5 ml of water for 14 days and provide standard feed orally to control the hypercholesterolemic state. Rats are said to be hyperlipidemic when they weigh more than 20% of the weight before treatment with total cholesterol levels >135 mg / dL, HDL < 35 mg/dL, LDL > 27 mg/dL, and TAG > 92 mg/dL. After PTU was given for 14 days and also rats were said to be in hyperlipidemia condition, in the treatment group, rats were also given avocado seed extract at a dose of 250 mg/Kg for 2 weeks along with the standard feed.

Measurements

Triglyceride levels were measured by taking blood samples from rats to be used as serum. Blood draws are made through the lateral vein or ventral artery of the tail. After the sample is taken, it is then centrifuged to take the serum. After that, serum was measured using a lipid pro meter to measuring triglyceride levels.

Statistical analysis

Data normality test was performed using the Saphiro-Wilk normality test, data on day 21 were obtained with a value of $p = 0.384$, data on day 28 with a value of $p = 0.346$, and data for day 35 with a value of $p = 0.248$. The three values are normally distributed based on p value > 0.05 . The paired T-test method was performed to find out whether there is a significant decrease in total cholesterol levels in the test group from days 21-28, 21-35, and 28-35. The test results in the test group on days 21-28 showed a significant decrease with a value of $p = 0.0000$ ($\alpha = 0.05$; $p < \alpha$), on days 21-35 showed a significant decrease with a value of $p = 0,0000$ ($\alpha = 0.05$; $p < \alpha$), on day 28-35, there was a significant decrease with a value of $p = 0.0034$ ($\alpha = 0.05$; $p < \alpha$)

Table 1. Paired T-test Results

Parameter	P value	Interpretation
Day 21-28	0,0000	Significant
Day 21-35	0,0000	Significant
Day 28-35	0,0034	Significant

RESULTS

Table 2. Triglyceride Levels in The Control Group

Control Group	Total triglyceride in the blood (mg/dL) Day 21
Rat 1	155
Rat 2	121
Rat 3	133
Rat 4	138
Rat 5	128

Control Group	Total triglyceride in the blood (mg/dL) Day 21
Rat 6	98
Rat 7	147
Rat 8	125
Rat 9	108
Rat 10	156
Rat 11	152
Rat 12	137
Rat 13	144
Rat 14	145
Average	134,78

Table 3. Triglyceride Levels in The Treatment Group

Treatment Group	Total triglyceride in the blood (mg/dL)		
	Day 21	Day 28	Day 35
Rat 1	155	149	138
Rat 2	121	118	110
Rat 3	133	125	121
Rat 4	138	128	126
Rat 5	128	123	119
Rat 6	98	90	97
Rat 7	147	136	133
Rat 8	125	117	112
Rat 9	108	97	93
Rat 10	156	152	147
Rat 11	152	143	139
Rat 12	137	134	131
Rat 13	144	138	134
Rat 14	145	141	139
Average	134,78	127,92	124,21

Table 2 stated the triglyceride levels in the control group. Table 3 stated the triglyceride levels of the treatment group on day 21, 28, and 35 of giving avocado seed extract. Average of triglyceride levels in rats on day 21 was 134.78 mg/dL. In the treatment group, after being given avocado seed extract, the triglyceride levels of the rats decreased where the average of triglyceride levels of the rats on

the 28th day became 127.92 mg/dL and on the 35th day decreased to 124.21 mg / dL.

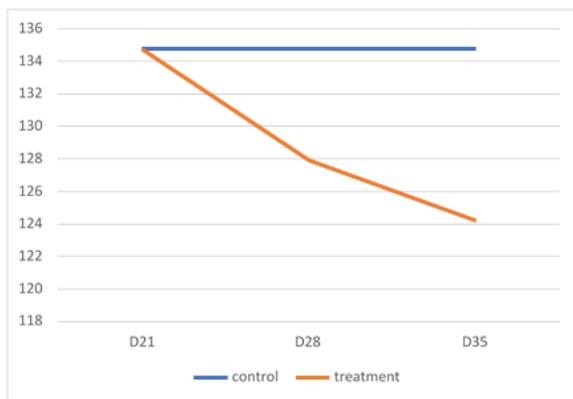


Figure 1. Average Triglyceride Levels of Control Group and Treatment Group

Figure 1 shows the comparison between the triglyceride levels of the control group and treatment group. There is no decrease in triglyceride levels in the control group. Meanwhile, in the treatment group, there was a decrease in triglyceride levels after being induced by avocado seed extract.

DISCUSSION

Before intervention, the rats in this study needed to be given an adaptation period of 7 days. After the adaptation period is complete, the mice will be induced by PTU for 14 days at a dose of 40 mg/kg. During 14 days of giving triglyceride levels an average of 134.78 mg/dL (Day 21). This data will be used as control group data in this study. Avocado seed extract lasted for 14 days. In the first 7 days, triglyceride levels decreased by an average of 127.92 mg/dL (Day 28). The

avocado seed extract was continued until the 14th day. On the 14th day, the triglyceride levels decreased from the previous 7 days with an average of 124.21 mg/dL. The data obtained were then performed with statistical tests. The statistical test begins by testing the normality of the data using the Shappiro-Wilk test. Shappiro-Wilk test on day 21 data with p-value = 0.384, data day 28 with p-value = 0.346, data day 35 with p-value = 0.248. From the three results, it is stated that the distribution is normal with a reference value of $p > 0.05$. Then the statistical test was continued with the Paired t-test. The results of the Paired t-test showed p value = 0.0000 on day 21 to day 28 and day 21 to day 35. On day 28 to day 35, the value was $p = 0.0034$. The three data state that the p-value is significant ($p < 0.05$) and H_0 can be rejected.

The decrease in triglyceride levels is influenced by the content of avocado seed extract, namely flavonoids. The working system of flavonoids is to inhibit cholesterol synthesis by inhibiting the activity of the 3-hydroxy-3-methyl-glutaril-CoA enzyme and increasing the activation of LPL (Lipoprotein lipase) and LCAT (Lecithin-cholesterol acyltransferase) plasma, thereby reducing total cholesterol, triglycerides, LDL-C, Apo-B Concentration and increase HDL.

The avocado seed extract was given for 14 days because it was quoted from a study conducted by Abraham T. Suhendra et. Al¹⁰

states that giving avocado seed extract at a dose of 250 mg/KgBB for 14 days is effective in reducing cholesterol levels in the blood. In addition, research conducted by Rico Arlianto⁷ also revealed the same thing as previous research. However, there is a slight difference in the lipid profile test of the two studies. In the research of Abraham T. Suhendra et al.¹⁰ the lipid profile tested was total cholesterol. Research conducted by Rico Arlianto tested the lipid profile is triglycerides. This research is in line with this research. However, in this study, the triglyceride levels did not reach normal levels. This situation is thought to be caused by differences in metabolism between mice, avocado seed extract that is stored for too long, the measurement of lipid levels in mice before being induced with PTU so there is no picture of lipid levels, and a decrease in rat triglyceride levels to normal values takes more than 14 days.

CONCLUSION

Giving avocado seed extract by grinding method for 30 minutes had an effect in reducing triglyceride levels in Sprague Dawley strain *Rattus norvegicus* rat.

CONFLICT OF INTEREST

No relevant disclosures.

REFERENCES

1. Erwinanto, Santoso A, Putranto J.N.E, Tedjasukmana P, Sukmawan R, Suryawan R, Sodikur R, Sutomo K. Pedoman Tatalaksana Dislipidemia. 1rd rev. ed. Jakarta: Centra Communications, 2013. 20 p.
2. Erwinanto, Santoso A, Putranto J.N.E, Tedjasukmana P, Sukmawan R, Suryawan R, Sodikur R, Sutomo K. Pedoman Tatalaksana Dislipidemia. 1rd rev. ed. Jakarta: Centra Communications, 2013. 1 p.
3. Expert Panel On Integrated Guidelines For Cardiovascular Health And Risk Reduction In Children And Adolescents. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report. PEDIATRICS. 2011 Dec 1;128(Supplement):S213-56.
4. Hasil Riskesdas 2013.pdf [Internet]. [cited 2019 May 4]. Available from: <http://www.depkes.go.id/resources/download/general/Hasil%20Riskesdas%202013>
5. Pahua-Ramos M, Ortiz-Moreno A, Chamorro-Cevallos G, Hernández-Navarro M, Garduño-Siciliano L, Necochea-Mondragón H et al. Hypolipidemic Effect of Avocado (*Persea americana* Mill) Seed in a

Hypercholesterolemic Mouse Model. Plant Foods for Human Nutrition. 2012;67(1):10-16

6. Mufida, Rahman N, Supriadi. Efek ekstrak daun alpukat (*Persea Americana* Mill) dalam menurunkan kadar kolesterol darah pada mencit (*Mus musculus*). J. Akademika Kim. August 2018; 7(1):18-11.
7. Rico A.S, usmo. Uji Efektivitas Antikolesterol Ekstrak Biji Alpukat Pada Tikus Jantan Galur Wistar Secara In Vivo Beserta Skrining Fitokimia. 2018 [cited January 13 2021]. Available from: <http://eprints.ums.ac.id/62684/14/NASKAH%20PUBLIKASI-2.pdf>.
8. Ilyas MN, Adzim MKR, Simbak NB and Atif AB, Sample Size Calculation for Animal Studies Using Degree of Freedom (E); an Easy and Statistically Defined Approach for Metabolomics and Genetic Research. Current Trends in Biomedical Engineering & Biosciences. 2017;10
9. Arts J, Oosterhuis N, Kramer K, Ohl F. Effects of Transfer from Breeding to Research Facility on the Welfare of Rats. Animals. 2014 Dec 3;4(4):712-28.
10. Suhendra A.T, Awaloei H, Wuisan J. Uji efek ekstrak biji alpukat (*Persea americana* Mill.) terhadap kadar kolesterol total pada tikus wistar. J. eBm. Januari-Juni 2016; 4(1):5p.