The Effect of Cloud Ear Fungus (*Auricularia polytricha*) on Serum Total Cholesterol, LDL And HDL Levels on Wistar Rats Induced by Reused Cooking Oil

Ratih Budinastiti [1], *Henna* Rya Sunoko [1,2,*], *Nyoman* Suci Widiastiti [1]

[1] Faculty of Medicine. Diponegoro University, Semarang - Indonesia
[2] Doctoral Program of Environmental Science, School of Postgraduate Studies, Diponegoro University, Semarang - Indonesia

Abstract. The usage of reused cooking oil affects the increase of serum total cholesterol (TC) and LDL, also the decrease of serum HDL. This condition escalates the risk of atherosclerosis, which could lead to the incidence of cardiovascular disease. Cloud ear fungus is a natural antioxidant that contains polysaccharides, flavonoids, niacin, and vitamin C, which can improve the lipid profiles. Objective of this research is to analyze the impact of water from boiled cloud ear fungus on total cholesterol, LDL, and HDL level of Wistar rats that have been given reused cooking oil. This study is a true experimental research with post test only control group design, using 12 weeks-aged male Wistar rats (n = 24) that were randomly divided into 4 groups. K1 as the negative control, K2 was given reused cooking oil and standard diet, K3 was given water from boiled cloud ear fungus and standard diet, and K4 was given reused cooking oil, water from boiled cloud ear fungus and standard diet. Serum total cholesterol, LDL, and HDL levels were measured by the CHOD-PAP method after 28 days treatment. The study showed that TC mean value of K1 (80.2217 ± 3.61 mg / dL), K2 (195.8483 ± 5.47 mg / dL), K3 (75.5800 ± 4.02 mg / dL), and K4 (110.8683 ± 5.82 mg / dL); p = 0.000. LDL mean value of K1 (29.9200 ± 1.53 mg / dL), K2 (78.4167 ± 1.77 mg / dL), K3 (24.3167 ± 1.77 mg / dL), and K4 (40, 1617 ± 2.84 mg / dL); p = 0.000. HDL mean value of K1 (65.8950 ± 1.99 mg / dL), K2 (24.3233 ± 1.44 mg / dL), K3 (73.2300 ± 1.92 mg / dL), and K4 (54 , 9550 ± 2.04 mg / dL); p= 0.000. **Conclusion:** Water from boiled cloud ear fungus decreases the serum total cholesterol and LDL, and increases serum HDL levels of Wistar rats that has been given reused cooking oil.

1 Introduction

The practice of using heated cooking oil repeatedly or usually called *jelantah* oil to cook was done by people frequently to save the expense. Heating the cooking oil repeatedly caused composition alteration such as increased saturated fatty acids and trans fatty acids content and reactive oxygen compound appeared. It caused the total amount of cholesterol, LDL, TG, free fatty acids, phospholipids and cerebrosides content increased and HDL content decreased [1,2, 3]

The increased total amount of cholesterol, trigliserida and LDL content and the decreased amount of HDL content indicated a state called dyslipidemia. Dyslipidemia increased the risk of complex pathology occurred which was called atherosclerosis. If the cholesterol content in the blood managed to be controlled, the development of atherosclerosis would be resisted [4]. Preventing and taking medical treatment were something than can be done. Preventing actions included improving nutrition, controlling blood pressure and doing physical exercises. On the other hand, taking medical treatment could be done by having synthetic and traditional medicine, and one of them was cloud ear fungus [6].

Edible mushrooms were widespread used in order to prevent degenerative diseases popped out. It was because edible mushroom, mainly cloud ear fungus had been proven contained antioxidants. [6]Cloud ear fungus were one of foodstuffs containing polysaccharides which could be used to lower cholesterol and prevent platelet aggregation [7, 8]. Besides, it also contained natural antioxidant which could be used as an alternative in resisting oxidation process. A new research showed that the use of synthetic antioxidant was limited as it showed potential danger [9, 10].

2 Method

This study was an experimental study with post-test only group design. Branches of science covered in this study were pharmacy and pharmacology, biochemistry, and

* Corresponding author: hennarsunoko@gmail.com

also environmental health. This study was done for 35 days, starting from March 22nd until April 27th 2016 in Inter-University Study Centre Laboratory for Food and Nutrition (*PAU*) Gadjah Mada University. The subjects of this study was 12 week male galur Wistar white mouses weighing 150-220 grams and had no anatomical disorder. The free variable of this study was the supply of cloud ear fungus broth dosed 3.6 gram/200gramBB and *jelantah* oil dosed 3ml/200gramBB each day for 28 days, while the dependent variable used was total cholesterol, LDL, and HDL serum content.

The number of samples referred to the WHO standard which were minimum 5 mouses. This study used 6 mouses for each group. The total number of mouses used in this study was 4 mouses which were divided randomly into 4 groups. All mouses were adapted by individual denned and given standard diet for 7 days. After adaptation process, they were divided into 4 groups randomly. Group K1 was given standard diet and aquades. Group K2 was given standard diet, aquades and *jelantah* oil per sonde 3ml/200gramBB. Group K3 was given standard diet and cloud ear fungus broth dosed 3.6gram/200gramBB per sonde as much as 2 ml/200gramBB. Group K4 was given standard diet, *jelantah* oil per sonde 3ml/200gramBB. After 28 day treatment, the researcher took their blood sample as much as 3 ml using hematocrit pipet from the mouse's plexus retroorbita and measured mouse's total cholesterol, LDL, and HDL serum content using CHOD-PAP approach in spectrophotometry manner and stated in mg/dL.

The process of making *jelantah* oil used 5 time heated bulk oil which had been used to fry sweet potatoes. The process started by taking the cooking oil to the pan as much as ± 2500mL, and it was heated until 180ºC. A 1 kilogram sweet potato which was bought in Kopeng market was fried for 10 minutes. Then, the oil was put aside until it got cooler and then the researcher continued to re-heat the oil until the 5th heating. The oil used was the same (there was no replacing or adding new oil volume). The procedure of making cloud ear fungus broth required 85 gram dried cloud ear fungus which had been cleaned, cut and boiled in 1800ml water until the volume was 600ml left. After that, it was strained using flanel material and steamed at 90º until the volume was 50 ml left.

The data showed in this study was a primary data measured in laboratories in a form of: total cholesterol, LDL, and HDL content. It was analyzed in descriptive manner which was presented into tables after its normality was measured using *Shapiro-Wilk* test. In order to differentiate the content of each experimental group, it was analyzed using *One WayAnova* and then proceed to *Post Hoc Bonferoni* statistic test using *SPSS 21.0 for Windows*.

3 Result and discussion

3.1 Result

3.1.1. Sample Analysis

Based on the research, out of 24 mouses from adaptation stage to after experimental stage, there was no mouses which were found dead. So, the total amount of mouses whose blood were taken to be measured for its total cholesterol, LDL, and HDL serum content was still 6 mouses for each group.

Weight measurement was done each week to determine both *jelantah* oil and cloud ear fungus broth doses. The mouses' weight measurement result was shown in Table 1.

Weight	Experimental Group			
	K1	K2	K3	K4
Before Experiment	193,83 ± 4,53	196,33 ± 2,66	193,33 ± 3,72	194,00 ± 5,02
After Experiment	218,17 ± 4,58	233,00 ± 2,37	217,50 ± 3,51	226,33 ± 5,28

Table 1. Mouses' Weight Description (g)

The result of statistic analysis indicated that there was no significant differences from the weight average between experimental groups before experiment showed by *p=0,597* (p>0,05) through *One Way Anova* test. It could be concluded that each experimental group had no significant different variations or characteristics.

3.1.2. Total Cholesterol Content

The average of mouses' total cholesterol serum content examination from each group was presented in Table 2.

Table 2. Average of Total Cholesterol Serum Content

Group	Average (mg/dL) ± SB
K1 (n=6)	80,22±3,61
K2 (n=6)	95,85 ±5,48
K3 (n=6)	75,58±4,02
K4 (n=6)	110,87 ±5,82

The result of statistics test showed that the data was in normal distribution and had homogen variation and then proceeded to *One Way Anova* parametric test. *One Way Anova* test of total cholesterol serum content showed that at least there was a significant difference of total cholesterol content from 2 groups (p=0,000). In order to

know which groups showing a significant difference of total cholesterol serum content, the process was continued by *Post-hoc Bonferoni* analysis. It was found that there were significant differences between group K1 and group K2 (p=0,000), group K2 and group K4 (p=0,000), and group K3 and group K4 (p=0,000). However, there was no significant difference between group K1 and group K3 (p=0,667).

3.1.3. LDL Cholesterol Content

The average of mouses' LDL cholesterol serum content examination from each group was presented in Table 3.

Table 3. Average of LDL Serum Content

Group	Average (mg/dL) ± SB
K1 (n=6)	29,92 ± 1,53
K2 (n=6)	78,42± 1,77
K3 (n=6)	24,32 ± 1,77
K4 (n=6)	40,16± 2,84

The result of statistic test showed that the data was in normal distribution and had homogen variation and then proceeded to *One Way Anova* parametric test. On the LDL serum cholesterol content data, the *One Way Anova* test indicated that at least there was a significant difference of LDL cholesterol content between two groups (p=0,000). In order to know which groups showing a significant difference of LDL cholesterol content, the process was continued by *Post-hoc Bonferoni* analysis. It was found that there were significant differences between group K1 and group K2 (p=0,000), group K1 and group K3 (p=0,001), group K2 and group K4 (p=0,000), and group K3 and group K4 (p=0,000).

3.1.4. HDL Cholesterol Content

The average of mouses' HDL cholesterol serum content examination from each group was presented in Table 4.

Table 4. Average of HDL Serum Content

Group	Average (mg/dL) ± SB
K1 (n=6)	65,89 ± 1,99
K2 (n=6)	24,32 ± 1,44
K3 (n=6)	73,23 ± 1,92
K4 (n=6)	54,95 ± 2,04

The result of statistic test showed that the data was in normal distribution and had homogen variation and then proceeded to *One Way Anova* parametric test. The HDL serum cholesterol content data was analyzed using *One Way Anova* test and showed a result where p= 0,000. Therefore, it could be concluded that at least there was a significant difference of HDL cholesterol content between two groups (p<0,05). Then, *Post-hoc Bonferoni* analysis test was done in order to know which groups showing a significant difference of HDL cholesterol content. It was found that there were significant differences between group K1 and group K2 (p=0,000), group K1 and group K3 (p=0,000), group K2 and group K4 (p=0,000), and group K3 and group K4 (p=0,000).

3.2 Discussion

This study reported that giving *jelantah* oil as much as 3 ml/200gram BB for 28 days causing the total cholesterol content decreased. This result supported the previous studies about giving *jelantah* oil. Based on several studies, giving *jelantah* oil caused the total cholesterol content on the examined animals decreased. Experimental studies on examined animals which were given repeatedly heated palm oil diet stated that there were total cholesterol, trigliserida, LDL cholesterol content addition and HDL cholesterol reduction on examined animals which had been given repeatedly heated palm oil diet (*jelantah* oil). [2] The other result indicated that on a group of animals which was given refined palm oil, there was also total cholesterol, trigilserida, and LDL cholesterol content addition and HDL cholesterol content reduction [11].

Giving cloud ear fungus broth could lower the toal cholesterol content as much as 43.39% and LDL cholesterol as much as 49.78% and increased the HDL cholesterol content as much as 55.73% on mouses which had been induced by *jelantah* oil. However, compared to the group which was given *jelantah* ol without cloud ear fungus broth, the reduction was statistically significant (p<0,05). The result of this present study supported the previous studies where cloud ear fungus extract had antihypercholesterolemia effect. This was in line with the previous study in 2014 which reported that there was a significant reduction on hipokolesterolemia mouses' total cholesterol content which were given straw mushroom compared to controlled group [12].

By having significant differences between a group of mouses which had been given *jelantah* oil and cloud ear fungus broth (K4) and a group of mouses which had only been given *jelantah* oil and aquades (K2), so it could be concluded that cloud ear fungus broth dosing 3.6 gram/200gramBB was potential to help preventing oxidative stress as the result of giving *jelantah* oil that it could lower the total cholesterol, LDL cholesterol content and increase the HDL cholesterol content of mouses' serum.

The reduction of total cholesterol, LDL cholesterol content and the addition of HDL cholesterol was happened because of several cloud ear fungus's nutrient compound components which influenced the changing of blood lipid profile. Some of the compounds were polysaccharides, arkobat acid, niasin, dan flavonoid.[7, 8, 13, 14] The previous studies mentioned that cloud ear fungus (*Auricularia polytricha*) contained soluble polysaccharides compound which had antihypercholesterol effect. [8] Polisakarida was used as

the fiber source which could be used to decrease cholesterol content. β-glucan polysaccharide was one of the fiber in cloud ear fungus which considered as a strong inhibitor to impede gatrointestinal lipase enzyme so that it could lower blood cholesterol content. [14] One of the roles of fiber toward fat metabolism was to impede gastrointestinal lipase enzyme reaction such as pancreatic lipase enzyme. Besides, the food fiber would block enterohepatic cycle (gall reabsorbance in the intestines to liver) with its ability and viscosity trapping micelles containing gall acid in the intestines and freed them from the bundle by luminal membrane of the intestinal epithelium transporter. The process reduced fat absorbance and reabsorbance including cholesterol and fat acid that it would increase the feces excretion.

As compensation after losing cholesterol and gall acid stock in the liver, there would be some addition of cholesterol which converted into gall acid by liver. It would result in free cholesterol reduction in the liver due to endogenous cholesterol synthesis and caused 7*α-hydroxilase* dan *HMG CoA reductase* activitiy increased. This condition would trigger hephatic LDL cholesterol receptor regulation to store LDL cholesterol serum spare. Fiber also caused longer feeling of satiety which would result in controlled calorie and cholesterol consumption [15].

Another possibility that would influence blood cholesterol, LDL and HDL content was the amount of antioxidant in cloud ear fungus [10]. Flavonoid was one of the antioxidants. It reduced LDL serum content through VLDL blocking mechanism. LDL was formed from VLDL which had been gone through lipid separation cascade so that the LDL content would be determined by VLDL content. The VLDL's component composers were just the same as LDL's which were trigliserida, ester cholesterol and apolipoprotein B, and also some other lipid components. VLDL content reduction by flavonoid was done through MTP protein transfer and ACAT enzyme blocking. MTP (*microsomal triglyceride transfer protein*) was a transfer protein which was responsible in combining trigliserida, ester cholesterol and Apo0-B process, while ACAT enzyme (*Acyl Co-A Cholesterol Acyl transferase*) was an intraselular enzyme whose role was to catalyze ester cholesterol from cholesterol and facilitate Apo B translocation acrossing endoplasmic reticulum membrane, from cytoplasm heading to lumen. MTP and ACAT blocking would reduce VLDL content and lower LDL serum and total cholesterol content [16, 17].

The reduction of bloods' LDL cholesterol content influenced bloods' HDL cholesterol content to decrease. The previous studies mentioned that fiber helped to bind gall acid which was then excreted along with the feces. To synthesize gall acid, liver needed cholesterol, and if the supply was empty, the liver would send a message to the brain and it would respon by sending signal to HDL to take cholesterol lied in the tissue to be brought back to the liver.

Other component which was presumed to be influential was ascorbic acid (vitamin C). The result of experimental study on animals which tried to see the effect of giving vitamin C to lipid profile reported that there was a significant reduction on total cholesterol content, and LDL cholesterol in white mouses after being given vitamin C. Besides, there was also trigliserida content reduction and HDL cholesterol which was increased though it was not statistically significant. [20] An explanation which might seem to be quite logic in this case mentioned that it was the result of 7α-*hydroxylase* enzyme activation by ascorbic acid which increased the conversion of plasma cholesterol into gall acid and further caused the total cholesterol serum content decreased. Another mechanism to decrease total cholesterol serum content was bby hydroxylate steroid hormone synthesis which needed cholesterol as its precursors [20, 21].

Niasin or nicotinic acid was a part of vitamin B complex which also known as vitamin B3. Niasin could affect lipoprotein containing apo-B such as VLDL and LDL, and it could also increase lipoprotein containing Apo-A 1 such as HDL. The newest invention showed that niasin inhibited *Diacylglycerol Acyltransferase-2 (DGAT-2)* enzyme activity directyly whereas it was the most important enzyme in triglycerides synthesis. Inhibiting TG synthesis by niasin would result in apo-B intraseluler degradation and decrease VLDL and LDL secretion. Another mechanism was that niasin controlled TG content by inhibitting lipolysis on adipocytes so that it would decrease TG content in plasma. To influence HDL cholesterol content, niasin played a role as the inhibitor of HDL cholesterol and Apo-A 1 absorbance and transference [22, 23].

Cloud ear fungus broth had a way to prevent oxidative stress by being exogen antioxidant which would decrease the amount of free radicals formed after consuming *jelantah* oil. Therefore, the more antioxidants, the more active antioxidants which could neutralize free radicals formed after being given *jelantah* oil.

4. Conclusion and Suggestion

Based on the result, we could conclude that cloud ear fungus broth played a role to prevent total cholesterol and LDL content increased and HDL cholesterol serum content decreased. It was necessary to do further study by using various doses of cloud ear fungus broth to see the most effective doses to improve blood lipid profile. Besides, it was also necessary to do further analysis on active compound components on cloud ear fungus broth.

References

1. N. Chun-Yi, Yusof Kamisah, Othman Faizah, Zakiah Jubri, HjMohd Saad Qodriyah, Kamsiah Jaarin. International Journal of Vascular Medicine (2012).
2. SK. Adam, Ima Nirwana Soelaiman, Nor Aini Umar, Norhayati Mokhtar, Norazlina Mohamed, Kamsiah Jaarin. McGill Journal of Medicine, **11**,146-151. (2008)

3. N. Chun-Yi , Xin-Fang Leong, Norliana Masbah, Siti Khadijah Adam, Yusof Kamisah, Kamsiah Jaarin. Vascular Pharmacology **61**:1-9 (2014)
4. H. Istiadi, Endang Sri S. Media Muda Medika **4**:1-10 (2010)
5. E, Braunwald, *Approach to the patient with cardiovascular disease*, **16th ed**. In: Kasper D, et al., editors. *Harrison's principles internal medicine*. New York: McGraw-Hill Medical Publishing Division, 1301-4 (2005)
6. Hung PV, Nhi NNY. Research Journal **19**:611-615 (2012)
7. H. Puspitasari, Sri Peni Fitrianingsih, Lanny Mulqie. *Pengaruh Pemberian Ekstrak Jamur Kuping Hitam terhadap Penurunan Kadar Kolesterol Mencit Swiss Webster Jantan*. FMIPA. Bandung: Universitas Islam Bandung, (2015).
8. S, Zhao, Chengbo Ronga, Yu Liua, *et al*. Carbohydrate Polymers **122**:39-45 (2015)
9. Septiyana. *Uji Toksisitas Akut Air Rebusan Jamur Kuping Hitam (Auricularia polytricha (Mout) Sacc.) Serta Gambaran Histopatologi Organ Hepar dan Bobot Limpa pada Mencit Putih (Mus musculus) Jantan Galur BALB/C. Farmasi*. Semarang: Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi, (2010).
10. Sun Y-X, Ji-Cheng Liua, John F. Kennedy. Carbohydrate Polymers **82**:299-304 (2010).
11. SL. Wansi, *Et al*. Internationl Journal of Pharmacrutical, Chemical, and Biological Sciences (IJPCBS) **3**:627-634 (2013).
12. AE. Damayanty, *Pengaruh Pemberian Ekstrak Jamur Merang (Volvariella volvacea) Terhadap Kadar Kolesterol Total, Enzim LpPLA2 dan MDA Darah*. Magister Ilmu Gizi. Semarang: Universitas Diponegoro, (2015).
13. S. Falakh, *Aktivitas Antioksidan Ekstrak Jamur Kuping Hitam (Auricularia polytricha)*. Fakultas Matematika dan Ilmu Pengetahuan Alam. Bogor: Institut Pertanian Bogor, (2008).
14. Smith, Rowan, Sullivan. *Medicinal Mushrooms: Their therapeutic properties and current medical usage with special amphasis on cancer treatments*, (2002).
15. E. Theuwissen, P. Ronald, Mensink. Physiology & Behavior **94**:285-292 (2008).
16. A. Pramono, UK. Solikah, HT. Nurul, AY. Rahma Mutiara Medika **11**:130-143 (2011).
17. PA. Mayes, Botham KM. *Metabolisme asilgliserol dan sfingolipid*. Biokimia Harper. Jakarta: EGC, (2009).
18. I, Zaki, *Pengaruh Pemberian Jus Mangga Terhadap Profil Lipid dan Malondialdehyde pada Tikus yang Diberi Minyak Jelantah*. Program Studi Magister Ilmu Gizi. Semarang: Universitas Diponegoro, (2014).
19. B. Hartoyo, I. Irawan, N. Iriyanti, Animal Production **7**:27-33 (2005).
20. MU. Eteng, HA. Ibekwe, TE. Amatey, BJ. Bassey, FU. Uboh, DU. Owu. Journal Of Physiological Sciences **21**:15-19 (2006).
21. Z. Prakoso. *Pengaruh Pemberian Vitamin C terhadap Kadar Kolesterol LDL dan HDL Serum Tikus Wistar Jantan Hiperlidemia Setelah Perlakuan Jus Lidah Buaya (Aloe vera Linn)*. Fakultas Kedokteran. Semarang: Universitas Diponegoro, (2006).
22. VS. Kamanna, ML. Kashyap. American Journal of Cardiology **101**:20B-26B (2008)
23. J. Haseeb, HK. Richard, TK. Jeffrey. Clinical Lipidology **4**:565-57 (2009).