pISSN 2302-1616, eISSN 2580-2909
Vol 11, No. 2, December 2023, pp. 215-225
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v11i2.38896

Ethanolic extract of Ling Zhi mushroom (*Ganoderma lucidum*) improves lipid profile, CRP and histopathological of liver in dislipidemia model rats

Jap Yulius Billy Soegianto[1], Pugud Samodro[2,3], Sindhu Wisesa[4], Hernayanti[5], Joko Setyono[6], Fitranto Arjadi[4*]

[1]Postgraduate Medical School, Faculty of Medical, Universitas Jenderal Soedirman
Jl. Profesor DR. HR Boenyamin No.708, North Purwokerto, Banyumas, Central Jawa, Indonesia. 53122
[2]Department of Internal Medicine, Faculty of Medical, Universitas Jenderal Soedirman
Jl. Profesor DR. HR Boenyamin No.708, North Purwokerto, Banyumas, Central Jawa, Indonesia. 53122
[3]RS Umum Daerah Prof. Dr. Margono Soekarjo
Jl. Dr Gumbreg No. 1 Purwokerto, Banyumas, Central Jawa, Indonesia. 53122
[4]Department of Anatomy, Faculty of Medical, Universitas Jenderal Soedirman
Jl. Profesor DR. HR Boenyamin No.708, North Purwokerto, Banyumas, Central Jawa, Indonesia. 53122
[5]Laboratory of Toxicology, Faculty of Biology, Universitas Jenderal Soedirman
Jl. Profesor DR. HR Boenyamin No.708, North Purwokerto, Banyumas, Central Jawa, Indonesia. 53122
[6]Department of Biochemistry, Faculty of Medical, Universitas Jenderal Soedirman
Jl. Profesor DR. HR Boenyamin No.708, North Purwokerto, Banyumas, Central Jawa, Indonesia. 53122
*Email: fitranto.arjadi@unsoed.ac.id

ABSTRACT. Dyslipidemia damages the liver characterized by increased inflammatory markers such as CRP *(C-reactive protein)* and histopathological alterations. Ling Zhi mushroom could improve the complications of dyslipidemia in the liver. The research objective was to determine the effect of ethanolic extract of Ling Zhi mushroom and to find the effective dose on the improvement of lipid profile, CRP and liver histopathology in dyslipidemia rats. The experiment methods were true experiments with post test only with control group design to 30 rats, were divided into 6 treatment groups, in example group 1 (healthy), group 2 (dyslipidemia), group 3 (dyslipidemia + simvastatin 0.18 mg/200 KgBW), group 4 (dyslipidemia + 200 mg/KgBW extract), group 5 (dyslipidemia + 400 mg/KgBW extract) and group 6 (dyslipidemia + 800 mg/KgBW extract). Examining lipid profiles using the principle of enzymatic reaction reagents such as cholesterol oxidase peroxidase aminoantypirin (CHOD-PAP), glyserol peroxidase phosphat acid (GPO-PAP), LDL direct and phosphotungstat reagent, CRP using ELISA method, and observing liver histopathology using an Olympus CX23 microscope with 100x and 400x magnification, computed using Scheuer scoring. The Brown Forsthe and Games Howell tests for CRP and LDL, as well as One Way ANOVA and Post hoc LSD testing for lipid profiles, were used for the statistical analysis. There were no significant differences (p values>0.05) between groups 4, 5, and 6, and the results indicated that the lipid profiles and CRP values in groups 4, 5, and 6 compared to group 2 exhibited significant (p values<0.05). The ethanolic extract of Ling Zhi mushrooms at a dose of 800 mg/KgBW and 200 mg/KgBB, respectively, improved the histopathological conditions in the portal and lobular areas, respectively, with an improvement in inflammation but no necrosis. It concluded that there was an effect on the improvement of lipid profile, CRP and liver histopathology from the dose of 200 mg/KgBW ethanolic extract of Ling Zhi mushroom.

Keywords: CRP; ELISA; Ling Zhi mushroom; lipid profile; liver histopathology

Article History: Received 31 January 2023; Received in revised form 18 March 2023; Accepted 18 May 2023; Available online 30 December 2023.
How to Cite This Article: Soegianto JYB, Samodro P, Wisesa S, Hernayanti H, Setyono J, Arjadi F. 2023. Ethanolic extract of Ling Zhi mushroom (*Ganoderma lucidum*) improves lipid profile, CRP and histopathological of liver in dislipidemia model rats. *Biogenesis: Jurnal Ilmiah Biologi*. vol 11(2): 215–225. doi: https://doi.org/10.24252/bio.v11i2.38896.

INTRODUCTION

Dyslipidemia is one of the chronic diseases in Indonesia and data from RISKERDAS 2018 shows that 54.4% of the population aged ≥15 years have abnormal cholesterol levels (Azqinar *et al*., 2022). The prevalence of dyslipidemia in Indonesia in the age group 25-34 years is 9.3% and increases with age to 15.5% in the age group 55-64 years (Lainsamputty & Gerungan, 2022). Unhealthy lifestyle conditions such as smoking, lack of physical activity and a high-fat diet led to dyslipidemia (Agung, 2021; Aman *et al*., 2022; Pappan and Rehman, 2022).

Continuous dyslipidemia conditions cause damage to the liver with the accumulation of lipid deposits in the form of droplets in hepatocytes. The composition of the droplets are triglycerides and

fatty acids as a result of unbalanced lipid metabolism. Steatosis is a condition where hepatocytes accumulate large amounts of fat (fatty liver). Factors that cause steatosis are an increase in free fatty acids from adipose tissue into the liver, increasing of lipogenesis in the liver and decreasing of oxidation process of fatty acids in hepatocytes and the imbalance of the releasing of triglycerides from hepatocytes derived from very low density lipoprotein (VLDL). The histological analysis of the lobular area, fibrosis, and portal area shows the type of injury. There are three types of injury that are evident: necrosis, fibrosis, and inflammation (Theise, 2013; Arvind *et al*, 2019). Damage to the liver causes hepatocytes to secrete an acute phase protein called *C-reactive protein* (CRP) with stimulation from the proinflammatory cytokine *interleukin-6* (IL-6) released by macrophages in hepatocytes (Badimon *et al*., 2018; Sproston and Ashworth, 2018). Increased levels of CRP in the form of *pentameric CRP* (pCRP) result in plaque in the blood vessels by activating nuclear factor kappa *B* (NF-κB) and increasing the expression of adhesion molecules such as E-Selectin and monocyte chemoattractant protein-1 (MCP-1) and inducing polarization of monocytes. On the other hand, CRP monomers (mCRP), which are activated by low-density lipoprotein (LDL) through oxidation, are present in blood vessels during the atherogenesis stage. Isomeric changes from the pCRP and mCRP forms are due to the process of apoptosis of leukocytes which will produce reactive oxygen species (ROS) and increase the chemotaxis of monocytes (Badimon *et al*., 2018).

Dyslipidemia-related organ damage can have catastrophic consequences, but medicinal herbs can be used for a long time with minimal danger of side effects and undesired drug interactions. Ganoderma lucidum, also known as Ling Zhi fungus, is one of the commonly utilized medicinal plants in Chinese medicine. Ethanolic extracts from Ling Zhi mushrooms are able to improve inflammatory conditions in the liver by increasing the status of mitocondrial antioxidants and suppressing the process of mitcondrial stress (Lin and Yang, 2019). Ling Zhi mushrooms contain bioactive compounds such as terpenoids, sterols, steroids, peptides and polysaccharides. Triterpenoid compounds such as ganoderic, lucidenic and ganodermic acids can be acts as antioxidants, antidislipidemia, antimicrobial and hepatoprotective. Amino acids such as histidine, lysine, phenylalanine and leucine act as antioxidants. Ganoderic acid is able to inhibit cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Terpenoid compounds are also able to inhibit the process of reducing the inflammatory response by reducing CRP values by inhibiting NF-κB and activator protein-1 (AP-1) in the nucleus (Chiu *et al*., 2017; Chan *et al*., 2021).

The goal of this study was to ascertain the optimal dosage of ethanolic Ling Zhi mushroom extract for reducing inflammation, enhancing dyslipidemia symptoms, and repairing liver damage.

MATERIALS AND METHODS

Study area. The research method using experimental method with post test only with control group design using dyslipidemia animal model. The study's exclusion criteria are if any of the white mice used in the experiment die, while the inclusion requirements are that the mice must be male, have a body weight of ± 200 grams, and be between 10 and 12 weeks old. The mice will be of the Wistar strain (*Rattus novergicus*). The number of animals using the Federer formula obtained 24 rats which were divided into 6 groups with each group of test animals amounting to 4 heads with an estimated drop out of 10% so that each rat treatment tested was 5. The number of experimental animals using the Federer formula is $(k-1)(n-1)\geq 15$, where k is the number of treatments. and n is the number of samples (Nelder and Federer, 1955). The treatment of the groups are group 1 (experimental animals given standard feed, group 2 (experimental animals given high fat diet/HFD), group 3 (experimental animals given high fat diet and simvastatin at a dose of 0.18 mg/200g BW), group 4 (experimental animals given a high-fat diet and Ling Zhi mushroom ethanolic extract 200 mg/KgBB), group 5 (experimental animals given a high-fat diet + Ling Zhi mushroom ethanolic extract at 400 mg/KgBB) and group 6 (experimental animals given a high-fat diet + Ling Zhi mushroom ethanolic extract at 800 mg/KgBB). High fat diet is made up of premade pellets for pigs (Pokphand 551), mice

fed 30 g/head/day of cat pellets (BOLT), and tubers like carrots and cassava fed three times a day. a week, one medium-sized fruit every three months each drum. This research held on Laboratory of Research, and Laboratory of Experimental Animal, Faculty of Medical, Universitas Jenderal Soedirman, Clinical Laboratory Medico Labora Purwokerto, and Laboratory of Anatomical Pathology Waskitha Yogyakarta and using ethical number No.003/KEPK/PE/XI/2022 from Ethic commission, Faculty of Medical, Universitas Jenderal Soedirman.

Extraction of Ling Zhi mushroom. The mushroom is collected from CV Asa Agro Corporation in Cianjur, West Java. Mushroom are determined in Laboratory of Mycology, Faculty of Medical, Universitas Jenderal Soedirman, by detrermination number No.001/01.MF/X/2022. Extraction held in Laboratory of Plant Physiology, Faculty of Biology, Universitas Jenderal Soedirman. The extraction process begins macerated of Ling Zhi mushroom body by the stepping powder from Ling Zhi mushrooms weighing 1000 g was dissolved with 2500 ml of 96% ethanol, stirred until all parts of the mushroom were evenly distributed and macerated at 60^{o}C for 72 h using a macerator. The extract was filtered using coarse filter paper, dried using a vaccum rotary evaporator at 40^{o}C and at a pressure of 100 mBar to remove the remaining liquid and obtain a thick ethanolic extract of Ling Zhi mushroom. The extract is poured into a cup and evaporated on a hot plate (Endah, 2017; Chairunnisa *et al*., 2019; Ratnaningtyas *et al*., 2018).

Dyslipidemia animal model. Animals were made dyslipidemic by giving *propylthiouracil* (PTU) at a dose of 12.5 mg/day in two divided doses so that each administration was 6.25 mg for 14 days. The dose for each administration was dissolved with distilled water 2 ml by dissolving 2 PTU tablets (100 mg PTU) dissolved with 64 ml of distilled water to obtain a PTU concentration of 3.125 mg PTU/ml and also giving duck egg yolk and quail egg yolk given as much as 2 ml every day. The administration of duck egg yolk and quail eggs is given orally once a day as much as 2 ml. PTU administration as an inducer of dyslipidemia because it can inhibit the production of thyroid hormones by inhibiting the deidonase enzyme that catalyzes the change from triiodothyronine (T4) to thyroxine (T3) in the thyroid gland and peripheral tissues, resulting in hyportiroidism. This condition has a direct effect on lipoprotein metabolism, namely an increase in cholesterol levels, especially LDL, caused by suppression of LDL receptor protein 1 (LRP1), the decreasing of the enzymes hepatic lipase like lipoprotein lipase (LPL) and cholesterol ester transfer protein (CETP) so that there is a decrease in LDL degradation and lipid metabolism is disrupted (Abdi *et al*, 2019; Untari and Pramukantoro, 2020; Wu *et al*., 2020).

Administration of simvastatin and ethanolic extract of Ling Zhi mushroom. Simvastatin 10 mg was dissolved into 100 ml of aquadest to obtain a simvastatin concentration of 0.1 mg/ml. Group 3 was given a simvastatin dose of 0.18 mg and the volume of simvastatin solution given was 2.0 ml. Simvastatin administration is given once a day for 21 days. The dose of group 4 was given extract in 200 mg/kgBB or 0.2 mg/gBB with the body weight of ± 200 grams then the calculation of dose of Ling Zhi mushroom as much as the Ling Zhi mushroom dose (mg/gBB) x rat body weight (0.2 mg / gBBx 200 grams) = 40 mg, at a dose of 400 mg/kgBB given 80 mg and at a dose of 800 mg/kgBB given 160 mg. Ling Zhi mushroom extract was taken orally once a day after being diluted in 2.0 ml of purified water for 21 days (Furi and Wahyuni, 2011).

Blood plasma collection. Blood was drawn by inserting a heparin microhematocrit capillary tube into the retro orbital vein of the right eye of the rat as much as 3 ml, then the blood was put into a purple tube containing *ethylenediaminetetraacetic acid* (EDTA) to prevent lysis of blood cells. (Nugroho, 2018). Blood samples were centrifuged at 6000 rpm for 10 minutes. In this study, the plasma part was taken at the top.

Lipid profile examination. Lipid profile examination (total cholesterol, LDL, HDL and triglycerides) uses enzymatic principles, for the examination of total cholesterol using cholesterol oxidase peroxidase aminoantypirin reagent (CHOD-PAP), triglycerides using glycerol peroxidase phosphat acid reagent (GPO-PAP) and LDL using LDL direct reagent and HDL using phosphotungstat reagent (Ministry of Health, 2010). The procedure for lipid profile examination was

carried out by taking 200 µL of plasma and adding 500 µL with each reagent for lipid profile examination. The sample was allowed to stand for 10 minutes, centrifuged for 10 minutes at 6000 rpm (Department of Health, 2010). After mixing, the next process is that the test tube is labeled with a standard blank and the test tube is purchased with a sample label, and adjusted to the number of samples examined. The mixture was homogenized with a vortex and incubated for 10 minutes at room temperature and measured the absorbance of standards and samples using a spectrophotometer with a wavelength of 546 nm (Ministry of Health, 2010).

CRP examination. The CRP examination was carried out using the ELISA method by taking 40 µl of rat blood plasma, putting it into the well and adding 10 µl of CRP biotin antibody. As a standard, CRP standards with concentrations of 75 ng/L, 150 ng/L, 300 ng/L, 600 ng/L and 120 ng/L were used. CRP samples and standards were added with horseradish peroxidase (HRP) of 50 µl each and incubated for 1 hour at 37^0C and then washed with wash buffer 3 times @ 350 ml using an ELISA washer and added chromogen A and B substrates of 50 µl each. After incubating in the dark for 15 minutes, 0.1 N HCl stopping solution @ 100 µl was added. The absorbance of samples and standards was measured on an ELISA reader with a wavelength of 450 nm and the results of the standard calibration curve were used to determine CRP levels in rat blood plasma (BT Laboratories, 2012).

Termination of animal experiment. After blood samples are taken, the experimental animals are anesthetized with ether and terminated by cervical dislocation, which is a holding the rat's neck with a strong enough holder or held using the thumb and other parts of the body with the procedure the tail is pulled as hard as possible so that the rat dies quickly. The advantage of using this method is that it does not affect the examination results, especially on organs (Abdillah *et al*., 2020; Leary *et al*., 2020).

Histopathological examination of liver. Liver was taken from the peritoneal cavity then cutted to smaller size and then put into a container containing 0.9% NaCl solution. Preparation of liver histological preparations by cutting using a microtome with a thickness of 3-5 mm. The stages of making hepatic organs are as follows fixation, trimming, dehydration, embedding, cutting, staining, and reading. Staining using hematoxylin-eosin (HE). The reading in this study was done using an Olympus cx23 microscope with magnification of 100x and 400x (Jusuf, 2009; Setyawati *et al*, 2016; Berata *et al*., 2018).

Statistical analyze. Lipid profile and CRP data were calculated for mean and standard deviation then tested for normality using Shapiro-Wilk test and data homogeneity test using Levenes's test. The parameters of total cholesterol, HDL, and triglycerides were examined using One Way ANOVA to see changes between treatments, and the Post Hoc LSD test was then used to determine whether there were significant differences between the groups. One Way ANOVA and Post Hoc LSD tests and Brown-Forsythe test and Games-Howell test if the data were normally distributed but not homogeneous. In liver histopathology, the degree of damage to the porta, lobular and fibrosis areas was calculated using the Scheuer scoring method.

RESULTS AND DISCUSSION

Effect of Ling Zhi mushroom ethanolic extract on lipid profile. Dyslipidemia is characterized by increased levels of total cholesterol, LDL, triglycerides and decreased HDL values caused by changes in lipid metabolic pathways. The cause of exogenous factors with a high-fat diet is an increase in the absorption of fatty acids, total cholesterol and triglycerides in the small intestine form chylomicrons whereas endogenous factors caused by lipid metabolism disorders such as changes in genes that regulate lipid metabolism. Liver r secretes VLDL and will be converted into LDL or HDL by *lipoprotein lipase* (LPL). Another type of LDL is lipoprotein a (Lp(a)) which is an LDL molecule with added Apo.A. and increased levels of Lp(a) are associated with the risk of arterosclerosis (Mosca *et al*., 2022). In dyslipidemia, cholesterol accumulation increases intercellular adhesion molecule-1 (ICAM-1) and E-Selectin which causes monocyte adhesion. Adhesion will cause monocytes to turn

into macrophages and produce (MCP-1), increase ox-LDL and increase *radical oxygen species* (ROS) which cause endothelial dysfunction resulting in inflammation (Bereda, 2022).

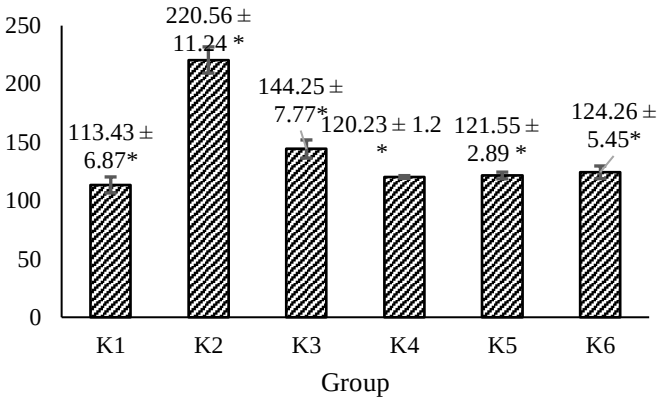

Fig. 1. Total cholesterol level after treatment, K1 (healthy group), K2 (dyslipidemia group), K3 (dyslipidemia group + Simvastatin 0.18 mg/200g BW, K4 (dyslipidemia group + mushroom extract 200 mg/KgBB, K5 (dyslipidemia group + mushroom extract 400 mg/KgBB) and K6 (dyslipidemia group + mushroom extract 800 mg/KgBB). Note: * = significant difference between K2 and K1, K3, K4, K5, K6 (Post Hoc LSD; p > 0.05).

The results of the lipid profile examination in figure 1, 2, 3, and 4 show that the average value of group 1, the values of LDL, HDL, triglycerides and total cholesterol are in the normal range, because in group 1 the experimental animals only given standard feed. The increasing in total cholesterol value was observed in group 2 in 94.44%, LDL by 156.75%, triglycerides by 100.98% and a decrease in HDL by 54.38%. The average of group 3 was decreased of total cholesterol, triglycerides, LDL and increased the value of HDL. The use of simvastatin as a dyslipidemia agent with statin mechanism inhibits the HMG-CoA reductase enzyme which is an enzyme that converts HMG-CoA into mevalonic acid and produces sterols to produce cholesterol in the liver. Another method for reducing LDL is to increase absorption at LDL receptors, which will cause a drop in plasma LDL levels (Zodda *et al*., 2018). The mean values of groups 4, 5, and 6 given Ling Zhi mushroom ethanolic extract at a dose of 200 mg/KgBB, 400 mg/KgBB and 800 mg/KgBB decreased in LDL, triglyceride and total cholesterol levels and increased HDL values. There was no significant difference with statistical calculations between groups 4, 5 and 6 (p value> 0.05) this indicates that starting from a dose of 200 mg/kgBB ethanolic extract of Ling Zhi mushroom is able to improve the condition of dyslipidemia.

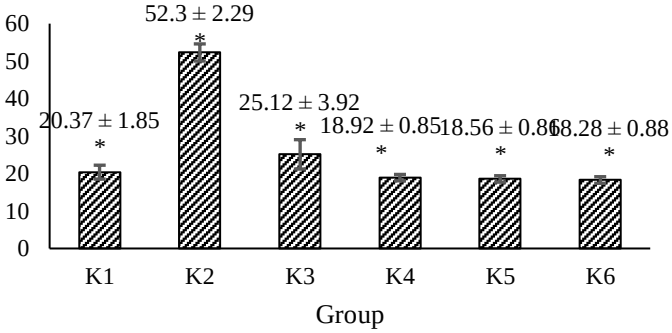

Fig. 2. Total LDL level after treatment, K1 (healthy group), K2 (dyslipidemia group), K3 (dyslipidemia group + Simvastatin 0.18 mg/200g BW, K4 (dyslipidemia group + mushroom extract 200 mg/KgBB, K5 (dyslipidemia group + mushroom extract 400 mg/KgBB) and K6 (dyslipidemia group + mushroom extract 800 mg/KgBB). Note: * = significant difference between K2 and K1, K3, K4, K5, K6 (Post Hoc Games Howell, p > 0.05)

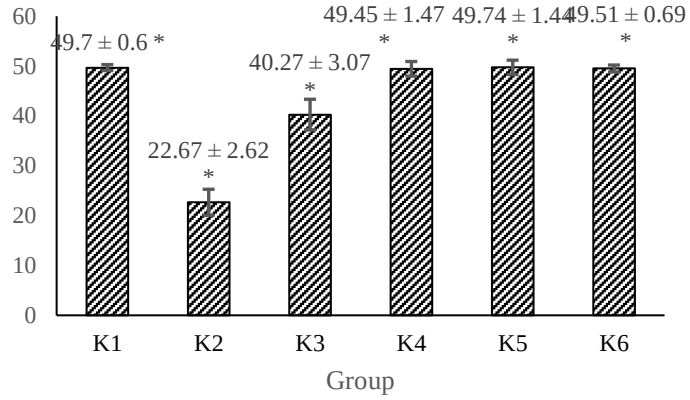

Fig. 3. Total HDL level after treatment, K1 (healthy group), K2 (dyslipidemia group), K3 (dyslipidemia group + Simvastatin 0.18 mg/200g BW, K4 (dyslipidemia group + mushroom extract 200 mg/KgBB, K5 (dyslipidemia group + mushroom extract 400 mg/KgBB) and K6 (dyslipidemia group + mushroom extract 800 mg/KgBB). Note: * = significant difference between K2 and K1, K3, K4, K5, K6 (Post Hoc LSD; p > 0.05)

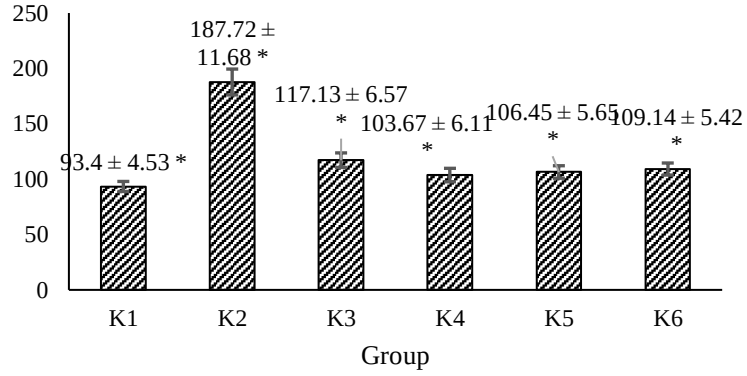

Fig. 4. Total triglyceride level after treatment, K1 (healthy group), K2 (dyslipidemia group), K3 (dyslipidemia group + Simvastatin 0.18 mg/200g BW, K4 (dyslipidemia group + mushroom extract 200 mg/KgBB, K5 (dyslipidemia group + mushroom extract 400 mg/KgBB) and K6 (dyslipidemia group + mushroom extract 800 mg/KgBB). (Note: * = significant difference between K2 and K1, K3, K4, K5, K6 (Post Hoc LSD; p > 0.05)

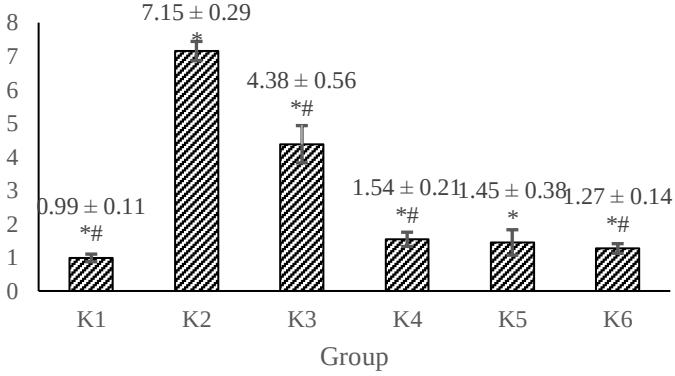

Fig. 5. Total CRP level after treatment, K1 (healthy group), K2 (dyslipidemia group), K3 (dyslipidemia group + Simvastatin 0.18 mg/200g BW, K4 (dyslipidemia group + mushroom extract 200 mg/KgBB, K5 (dyslipidemia group + mushroom extract 400 mg/KgBB) and K6 (dyslipidemia group + mushroom extract 800 mg/KgBB). (Note: * = significant difference between K2 and K1, K3, K4, K5, K6, # = significant difference between K3 and K1, K4, K5, K6 (Post Hoc LSD; p > 0.05)

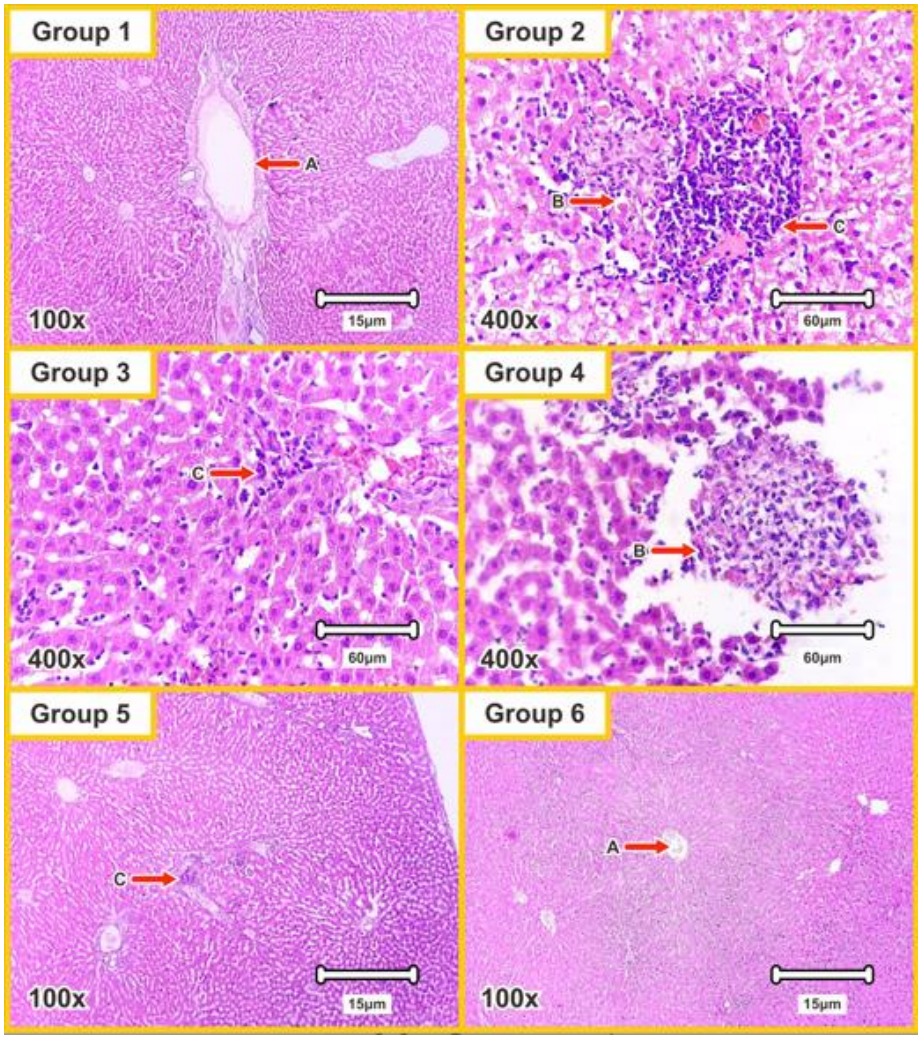

Fig. 6. Hepar histology of Rats after treatment. Observations at 100x and 400x magnification using an Olympus cx23 microscope. Staining using Hematoxylin-Eosin (HE). K1 (healthy group), K2 (dyslipidemia group), K3 (dyslipidemia group + Simvastatin 0.18 mg/200g BW, K4 (dyslipidemia group + mushroom extract 200 mg/KgBB, K5 (dyslipidemia group + mushroom extract 400 mg/KgBB) and K6 (dyslipidemia group + mushroom extract 800 mg/KgBB). Note: arrows A (Blood vessels), B (Necrosis characterized by cell lysis), C (Inflammation characterized by lymphocyte inflitration)

An additional mechanism contributing to the amelioration of dyslipidemia conditions is the upregulation of ABC transporters (ABC1 and ABCG1) in the heparin, which is essential for the creation of HDL (Lai *et al*, 2020). Alteration also occurs in lipid metabolism in the hepar, namely by lipid excretion through increased bile acids through feces through increased activity against the CYP7A1 enzyme (Lai *et al*, 2020).

Triterpenoid compounds also increase the expression of the liver X receptor alpha (LRXα) gene in the liver and small intestine (Lai *et al*., 2020). The LRXα gene contributes increasing of ABCA1 and ABCG5, where the function of ABCA1 plays a role in converting cholesterol into apolipoprotein A1 (APOA1), which is the formation of HDL whereas ABCG5 plays a role in changing from cholesterol to bile acids by increasing the function of RCT through an increase in CYP7A1 (Lai *et al*., 2020). Triterpenoid compounds also inhibit cholesterol absorption in the small intestine through the increasing of expression of ABCG5/8 (Lai *et al*., 2020). In the inflammatory process with the formation of foam cells in the blood vessels, triterpenoid compounds are able to increase the expression of LRXα in macrophages through an increase in ABCA1 and ABCG1 so that it will bring cholesterol back into the liver so that foam cells do not occur (Lai *et al*, 2020).

Effect of Ling Zhi mushroom ethanolic extract on CRP. A marker of inflammatory reaction is the measurement of CRP, an acute phase protein that characterizes tissue damage. Elevated levels

of CRP are risk factor for cardiovascular diseases such as myocardial infarction, stroke, and atherosclerosis because it induces vasoconstriction, proliferative, and inflammatory molecules. CRP production with activating of the NF-κB complex. NF-κB is a transcription factor involved in various biological processes such as in immune response, cell survival, and cell maturation. NF-κB transcription factor consists of five genes namely NFKB1, NFKB2, RELA, REL, and RELB which produce RelA (p65), RelB, c-Rel, p50, and p52 proteins respectively. Activation of NF-κB involves phosphorylation-dependent degradation of IκB protein IKKβ resulting in inflammation (Wynants *et al*., 2013).

In this study, it was found that the mean value of CRP values was highest in group 2 then decrease in groups 3, 4, 5, and 6 as shown in figure 5 and decreased in group 3 due to the administration of statin drugs can reduce LDL, triglyceride and total cholesterol levels and increase HDL levels. Dyslipidemia conditions will cause inflammatory conditions. With this condition, there will be a decrease in CRP as a response to the decrease in the inflammatory process. Statin drugs reduce CRP by inhibiting the mechanism of pro-inflammatory cytokine IL-6 which stimulates CRP production in the liver and by reducing LDL levels by statins will also reduce ox-LDL where if ox-LDL has high levels it will trigger arterosclerosis and increase CRP production from endothelial cells through suppression of adhesion molecules on endothelial cells (Kandelouei *et al*., 2022). The mean values in groups 4, 5 and 6 decreased CRP higher than group 3 but the decrease between groups 4, 5 and 6 showed that at a dose of 200 mg / kg BW the ethanolic extract of Ling Zhi mushrooms could significantly reduce CRP levels compared to the statin group, although not statistically significant. This is due to bioactive compounds, namely tritepenoids and sterols contained in Ling Zhi mushrooms, which inhibit the response of pro-inflammatory cytokines and inhibit NF-κB so that there is a decrease in the inflammatory response (Özden *et al*., 2022). Tritepenoid compounds which are derivatives of terpenoid compounds inhibit 10% cyclooxygenase-2 (COX2) and reduce iNOS. Another mechanism is related to its antioxidant properties by providing H+ ions to ONOO- thus neutralizing free radicals derived from lipid peroxidation (Tiyah *et al*., 2023).

Effect of ethanolic extract of Ling Zhi mushroom on hepatic histopathologic features. In this study no fibrosis condition was found in all groups of rats. In the porta area, no damage was found in group 1 but damage was found in groups 2, 3, 4 and 5 as shown in figure 6. The form of damage found was inflammation in the port area in all treatment groups 2, 3, 4 and 5 and the mild piecemal necrosis to zone 1 hepar in was found in samples 2.2 and 4.2 and in group 6 the liver histopatologycal are normal. Inflammatory conditions were also found in the lobular area in all treatment of groups 2, group 3 (3.1 and 3.2) and inflammatory conditions were found in group 4 (4.2). Damage to the liver organ as a result of dyslipidemia conditions is caused by changes in lipid metabolic pathways and inflammatory trigger factors (NF-κB) which will increase the number of kupffer cells and activation of hepatic stellate cells and the production of collagen matrix and cause inflammatory conditions in the hepatic organ (Byrne and Targher, 2015; Li *et al*, 2021). The necrosis condition is caused by damage to the cell membrane as a result of inflammation causing an increase in the concentration of Ca2+ ions into the cytoplasm (Byrne and Targher, 2015; Li *et al*, 2021). The increasing of Ca^{2+} ions result in depolarization of the mitochondria and a subsequent reduction in proton ions and ATP production resulting in an increase in the size of the mitochondria and damage to the outer membrane that will cause damage to cell organelles (Byrne and Targher, 2015; Li *et al*, 2021). Another mechanism related to the process of necrosis is the activation of NFκB and a decrease in caspases and an increase in receptor interacting protein-1 (RIP1) (Byrne & Targher, 2015; Li *et al*, 2021). This increase in RIP 1 will result in the formation of necrotic bodies and increase the occurrence of ROS and oxidative phosphorylation so that damage to cell membranes and organelles will occur (Byrne & Targher, 2015; Li *et al*, 2021).

Triterpenoid compounds in cases of damage complications of dyslipidemia reduce cholesterol and triglyceride levels by activating the mechanism of the action of the enzyme *acetyl-CoA carboxylase* (ACC) which affects suppression of fatty acid oxidation and reduction of ROS by giving

H+ ions from triterpenoid compounds to free radicals in the form of oxygen radicals which comes from the process of lipid peroxidation and also increasing superoxide dismutase (SOD) enzyme activity (Ratnaningtyas *et al*., 2018; Ahmad *et al*., 2023).

This study showed that at a concentration of 800 mg/KgBW of Ling Zhi mushroom ethanolic extract it was able to repair damage to the liver in the form of mild inflammation and piecemal necrosis in zone 1 of the liver in the portal area and at a dose of 200 mg/kgBW it was able to improve inflammatory conditions in the portal area which can be seen in the histopathological examination of the liver. This is because the triterpenoid compounds contained in the Ling Zhi mushroom suppress the expression of NF-κB, iNOS, COX-2 and IL-6 and reduce apoptotic cell death (Qiu *et al*, 2019; Zhao *et al*, 2019). Another repair mechanism is by increasing the endogenous antioxidant and decreasing the process of lipid peroxidation (Qiu *et al*, 2019; Zhao *et al*, 2019). Increasing SOD is able to reduce free radical levels so as to maintain the integrity of the cell membrane. Free radicals in dyslipidemic conditions are generated as a result of lipid peroxidation which produces the compound *malondialdehyde* (MDA) which is captured by SOD which acts as an antioxidant (Qiu *et al*, 2019; Zhao *et al*, 2019). Triterpenoid compounds are also able to inhibit the activity of the β-glucoronidase the enzyme as an indicator of liver damage by acting as an antioxidant mechanism and keeping Ca^{2+} ion levels in normal hepatocytes. Reduced hydropic degeneration in hepatocytes and a decrease in the quantity of cells exhibiting necrotic conditions are signs of necrotic conditions, which are caused by an excessive increase in Ca^{2+} ions from the endoplasmic reticulum as a result of inflammatory conditions. (Qiu *et al*, 2019; Zhao *et al*, 2019).

Future research is expected to observe the other organs that are affected by dyslipidemia such as the kidneys and heart by observing the biochemical parameters and histopathological examinations. Another suggestion is that it is necessary to examine the levels of β2-microglobulin in the kidneys and NT-proBNP in the heart because β2-microglobulin and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) are one of the parameters to see the damage of kidney and heart. The limitations in this study were not examining other organs affected by dyslipidemic conditions such as the kidneys and studying the expression of β2-microglobulin protein and histopathological features of the kidneys. The other limitation is the Federer formula which does not consider type I and type II errors that leads to increased bias in the research.

CONCLUSION

Ling Zhi mushroom ethanolic extract at doses of 200 mg/Kg BW, 400 mg/Kg BW and 800 mg/Kg BW was able to reduce LDL levels, total cholesterol, triglycerides and CRP and increase HDL levels and improve liver histopathological conditions in dilipidemic rat models. The effective dose of ling zhi mushroom ethanolic extract to improve lipid profile, CRP and improve liver histopathological condition is 200 mg/KgBW in dyslipidemia rat model.

ACKNOWLEDGEMENTS

The authors would like to express the gratitude to Dean of Faculty of Medical, Universitas Jenderal Soedirman, Head of Biomedical Postgraduate School and all of those who facilitating the research, collecting and analyzing the data.

REFERENCES

Abdi H, Amouzegar A, Azizi F. 2019. Antithyroid Drugs. Iranian Journal of Pharmaceutical Research (2019), 18 (Special Issue): 1-12. Doi: 10.22037/ijpr.2020.112892.14005.

Abdillah R, Permatasari D, Badriyya E, Rachmaini F, Lailatulrahmi. 2020. Penuntun Praktikum Farmakologi. Fakultas Farmasi universitas Andalas.Tidak Dipublikasikan.

Agung LR. 2021. Pengaruh Daun Salam (Syzygium polyanthum) Terhadap Kadar Trigliserida dan Kolesterol Total Darah Pada Penderita Dislipidemia. Jurnal Ilmiah Kesehatan Sandi Husada.10 (2):2354- 6093. doi: 10.35816/jiskh.Vi0i2.617.

Ahmad MF, Ahmad FA, Zeyaullah M, Alsayegh AA, Mahmood SE, Shahrani AM, Khan MS, Shama E, Hamouda A, Elbendary EY, Attia KAHI. 2023. Ganoderma lucidum: Novel Insight into Hepatoprotective Potential with Mechanisms of Action. Nutrients 2023:15(8). https://doi.org/10.3390/nu15081874.

Aman AM, Soelistijo SA, Arsana PM, Wismandari, Zufry H, Rosandi R, Walemangko OC, Epriliawati M .2022. Pedoman Pengelolaan Dislipidemia di Indonesia. Jakarta: PB PERKENI.Pp 1-2.

Arvind A, Osganian SA, Cohen DE, Corey KE. 2019. Lipid and Lipoprotein Metabolism in Liver Disease.https://www.ncbi.nlm.nih.gov/books/NBK326742/.

Azqinar TC, Anggraini DI, Kania S. 2022. Penatalaksanaan Holistik Pada Wanita Usia 60 Tahun ke Atas Dengan Dislipidemia Melalui Pendekatan Kedokteran Keluarga. Jurnal Penelitian Perawat Profesional (11):1. http://jurnal.globalhealthsciencegroup.com/index.php/JPPP.

Badimon L, Pena E, Arderiu G, Padro T, Slevin M, Vilahur G. 2018. C-Reactive Protein In Atherothrombosis and Angiogenesis. Frontiner in Immunology. 9. doi: 10.3389/fimmu.2018.00430.

Berata KI, Arjana AAD, Sudira IW, Merdana IM, Budiasa IK, Oka IBM. 2010. Studi patologi kejadian cysticercosis pada tikus putih. *Jurnal Veteriner* 11(4):232-237. https://ojs.unud.ac.id/index.php/jvet/article/view/3450

Bereda G. 2022. Pathophysiology and Management of Dyslipidaemia. Biomed J Sci & Tech Res (43):2. doi: 10.26717/BJSTR.2022.43.006869.

BT Laboratories, 2012. Rat High Sensitive C-Reactive Protein (hsCRP) Elisa Assay. BT Laboratories Shanghai Chin. Tidak Dipublikasikan.

Byrne CD, Targher G. 2015. NAFLD: A Multisystem Disease. Journal of Hepatology (62): S47-S64.https://doi.org/10.1016/j.jhep.2014.12.012.

Chan SW, Tomlinsonb B, Chanc P, Lam C. 2021. The Beneficial Effects of Ganoderma Lucidum on Cardiovascular and Metabolic Disease Risk. PharmaceuticalBiology. 59(1): 1161-1171. https://doi.org/10.1080/13880209.2021.1969413.

Chairunnisa S, Wartini NM, Suhendra L. 2019. Pengaruh suhu dan waktu maserasi terhadap karakteristik ekstrak daun bidara (Ziziphus mauritiana L.) sebagai sumber saponin. Jurnal Rekayasa dan Manajemen Agroindustri 7(4): 551-560.

Chiu HF, Fu HY, Lu YY, Han YC, Shen YC, Venkatakrishnan K, Oksana Golovinskaia O, Wang CK. 2017. Triterpenoids and Polysaccharide Peptides-Enriched Ganoderma Lucidum: A Randomized, Double-Blind Placebo-Controlled Crossover Study of Its Antioxidation and Hepatoprotective Efficacy in Healthy Volunteers. Pharm Biol.5 (1): 1041-1046. doi: 10.1080/13880209.2017.1288750.

Departemen Kesehatan Republik Indonesia. 2010. Pedoman Pemeriksaan Kimia Klinik. Keputusan Menteri Kesehatan Republik Indonesia Nomor 1792/MENKES/SK/XII/2010.

Endah SRN. 2017. Pembuatan ekstrak etanol dan penapisan fitokimia ekstrak etanol kulit batang sintok (Cinnamon sintoc BL.). Jurnal Hexagro 1(2). ISSN 2459-2691.

Furi P, Wahyuni AS. 2011, Pengaruh Ekstrak Etanol Jamur Ling Zhi (ganoderma lucidum) Terhadap Kadar HDL (High Density Lipoprotein) Pada Tikus Dislipidemia. Pharmacon. 12(1):1-8. ISSN: 1411-4283.

Jusuf AA. 2009. Histoteknik dasar. Bagian Histologi Fakultas Kedokteran Universitas Indonesia. Tidak Dipublikasikan.

Kandelouei T, Abbasifard M, Imani D, Aslani S, Razi B , Fasihi M, Shafiekhani S,Mohammadi K,Jamialahmadi T,Reiner Z,Sahebkar A. 2020. Effect of Statins on Serum level of hs-CRP and CRP in Patients with Cardiovascular Diseases: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Hindawi 2022 (20). doi: 10.1155/2022/8732360.

Lai P, Cao X, Xua Q, Liu Y, Li R, Zhang J, Zhang M. 2020. Ganoderma Lucidum Spore Ethanol Extract Attenuates Atherosclerosis by Regulating Lipid Metabolism Via Upregulation of Liver X Receptor Alpha. Pharmaceutical Biology 2020. Vol. 58, No. 1, 760–770. https://doi.org/10.1080/13880209.2020.179847.

Lainsamputy F, Gerungan N. 2022. Korelasi Gaya Hidup dan Stres pada Penderita Hiperkolesterolemia. Jurnal Ilmiah Kesehatan Sandi Husada (11):1.doi : 10.35816/jiskh.v11i1.719.

Leary S, Underwood W, Anthony R, Cartener S, Corey D, Grandin T, Greenacre C, Brant SG, Crackin M, Meyer B, Miller D, Shearer J, Yanong R. 2020. AVMA Guidelines for The Euthanasia of Animals: 2020 Edition. Pp 16,44.

Li P, Dong XR, Zhang B, Zhang XT, Liu JZ, Ma DS, Ma L. 2021. Molecular Mechanism and Therapeutic Targeting of Necrosis, Apoptosis, Pyroptosis, and Autophagy in Cardiovascular Disease. Chin Med J (Engl). 2021;134(22): 2647–2655. doi: 10.1097/CM9.0000000000001772.

Lin Z, Yang B. 2019. Ganoderma and Health Pharmacology and Clinical Application. Springers.1182.Pp: 225-226. https://doi.org/10.1007/978-981-32-9421-9.

Mosca A, Araújo G, Costa V, Correia J, Bandeira A, Martins E, Mansilha H, Tavares M, Coelho MP. 2022. Dyslipidemia Diagnosis and Treatment: Risk Stratification in Children and Adolescents. Journal of Nutrition and Metabolism. doi: 10.1155/2022/4782344.

Nelder JA, & Federer WT. 1956. Experimental Design, Theory and Application. MacMillan. Amerika Serikat

Nugroho RA. 2018. Mengenal Mencit Sebagai Hewan Laboratorium. Mulawarman University Press. Tidak Dipublikasikan.

Özden H, Şahin Y, Kilitçi A, Karaca G, Gömeç M, Yildiz A, Uçar C. 2021. Comparison of The Healing Effects of Mesazaline and Ganoderma Lucidum in Acetic Acid-Induced Colitis in Rats. Annals of Surgical Treatment and Research 2022 102(1):29-35. https://doi.org/10.4174/astr.2022.102.1.29.

Pappan N, Rehman A. 2022. Dyslipidemia. Stats Pearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK560891/.

Ratnaningtyas NI, Hernayanti, Andarwanti S, Ekowati N, Purwanti ES, Sukmawati D. 2018. Effects of Ganoderma Lucidum Extract On Diabetic Rats.Biosaintifika 10 (3) 642-647. doi:http://dx.doi.org/10.15294/biosaintifika.v10i3.15356.

Qiu Z, Zhong D, Yang B. 2019. Preventive and Therapeutic Effect of Ganoderma (Lingzhi) on Liver Injury. Adv Exp Med Biol. 2019;1182:217-242. doi: 10.1007/978-981-32-9421-9_9.

Ratnaningtyas NI, Hernayanti, Andarwanti S, Ekowati N, Purwanti ES, Sukmawati D. 2018. Effects of Ganoderma Lucidum Extract On Diabetic Rats.Biosaintifika 10 (3) 642-647. doi:http://dx.doi.org/10.15294/biosaintifika.v10i3.15356.

Setyawati I, Putra IGNAD, Wiratmini NI, Sudarti NW. 2016. Liver histology of female rat (Rattus norvegicus) fed diet containing Calliandra leaf and Pineapple peel during gestation. International Journal of Advances In Pharmacy, Biology and Chemistry 5(1). ISSN: 2277 – 4688.

Sproston NR, Ashworth JJ. 2018. Role of C-Reactive Protein At Sites of Inflammation and Infection. Front Immunol.9. doi: 10.3389/fimmu.2018.00754.

Tiyah SW, Ratnaningtyas NI, Wibowo ES, Mumpuni A, Ekowati N, Hikam AH. 2023. Ganoderma lucidum as Anti-Inflammatory Agent on The Level of Albumin and Globulin in Rat (Rattus Norvegicus) Rheumatoid Arthritis (RA) Model. The 5th International Conference on Multidisciplinary Approaches for Sustainable Rural Development. Proceeding ICMA-SURE – 2023.

Theise ND. 2013. Histopathology Of Alcoholic Liver Disease. Clinical Liver Disease. 2 (2): 64-67. https://doi.org/10.1002/cld.172.

Wu TY, Wang CH, Tien N, Lin CL, Chu FY,Chang HY, Lim YP. 2020. A Population -Based Cohort Study on the Association of Hyperthyroidism With The Risk of Hyperlipidemia and the Effect of Anti-thyroid Drugs on Hepatic Gene Expression. Frontiners in Medicines (7) 228. Doi: 10.3389/fmed.2020. 00228

Wynants M, Vengethasamy L, Ronisz A, Meyns B,Delcroix M, Quarck R. 2013. NF-κB Pathway is Involved in CRP-Induced Effects on Pulmonary Arterialendothelial Cells in Chronic Thromboembolic Pulmonary Hypertension. J Physiol Lung Cell Mol Physiol.305: 934–942. doi:10.1152/ajplung.00034.2013.

Untari MK, Pramukantoro GE.2020. Aktivitas Antihiperkolesterolemia Ekstrak Etanol Daun Stevia Rebaudiana Bertoni Pada Tikus Putih Jantan. Journal Syifa Sciences and Clinical Research 2(1).http://ejurnal.ung.ac.id/index.php/jsscr

Zhao C, Fan J, Liua Y, Guoa W, Cao H,Xiao J, Wang Y, Liu B. 2019. Hepatoprotective Activity of Ganoderma lucidum Triterpenoids in Alcohol-Induced Liver Injury in Mice, an iTRAQ-Based Proteomic Analysis. Food Chemistry 271(2019)148-156. doi: 10.1016/j.foodchem.2018.07.115

Zodda D, Giammona R, Schifilliti S. 2018. Treatment Strategy for Dyslipidemia inCardiovascular Disease Prevention: Focus on Old and New Drugs. Pharmacy (Basel) 6(1). doi: 10.3390/pharmacy6010010.