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White Oyster Mushroom Effect Of Lowering Cholesterol Levels And Prevention Atherosclerosis In Wistar Male Rats Are Given High Cholesterol Feed.

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Abstract : *Atherosclerosis is a degenerative disease which onset can be accelerated by consuming foods high cholesterol. To prevent the formation of atherosclerosis is reduce of cholesterol levels.* ***Objective:*** *To determine the effect of oyster mushroom extract or residue in preventing the formation of atherosclerosis by lowering cholesterol and LDL levels.Preparation of the extract by maceration method using ethanol 96%. Animals using 24 male rats were divided into four groups, each group consisting of 6 animals. Group 1 (given regular feed), group 2 (fed with high cholesterol), group 3 (fed with high cholesterol, ethanol extract of oyster mushrooms 250 mg / kg bw once a day), group 4 (fed with high cholesterol, residue ethanol ekstract of oyster mushrooms 250 mg / kg bw once a day). Parameters measured were cholesterol, LDL, body weight, foam cells and the thickness of intima layer. Cholesterol levels, LDL is measured every week, foam cells and the thickness of the intima layer examined in histopathology at the end of the experiment. Analysis of data using one-way ANOVA. In the study showed that extract and residue of oyster mushrooms can prevent raising levels of cholesterol, LDL, foam cell formation, and thickening of the intima. The results indicate that extract ethanol of oyster mushrooms and the residue is useful for preventing atherosclerosis and also to lower cholesterol levels and LDL.*

Keywords**:** *Oyster Mushroom; cholesterol; LDL; foam cells; intima layer*

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I. Introduction

The exact cause of atherosclerosis until now unknown, several factors that cause, for example: metabolic syndrome, infectious disease, free radical formation by cigarette smoke, elevation of lipid levels in people who are obese (1), Cholesterol levels that are too high in the cells will someday lead to pathological states, especially in the cell walls of the arteries. Accumulation of cholesterol will lead to heart disease atherosclerosis (2).

The consequences that would arise in the event of hypercholesterolemia among others: vascular dysfunction, inflammation and oxidative stress, which in turn will accelerate the formation of atherosclerosis. The initial stage of the formation of atherosclerosis begins with vascular endothelial denudation processes and as a consequence is loss of normal function of the endothelium. namely: control of vascular tone, anti coagulants that are characteristic of the intima layer, and also serves as a defense against inflammatory mediators. The next stage is the formation of atherosclerotic plaque because of the accumulation of lipids in SMC (Smooth Muscle Cells) and macrophages, which are covered by a hood fibrinogen. The final stage is due to the release of the atherosclerotic plaque with stenosis or obstruction (1). Endothelial dysfunction is as basic initiation progressiveness of an atherosclerotic lesion (3). Next is the formation of atherosclerotic plaque because of the accumulation of lipids in SMC and macrophages, which are covered by a hood fibrinogen. The final stage is due to the release of the atherosclerotic plaque with stenosis or obstruction (1). Foam cells is a preliminary description of the atherosclerotic plaque formation. Genesis entry of monocytes into the intima layer of the arterial wall, will lead to the differentiation of monocytes into macrophages and will begin the process of gathering by arrest lipoprotein lipid modified resulting in the formation of foam cells. (3). According to (5), the formation of atherosclerotic foam cells through several stages: 1. Activation of the endothelium after the accumulation of modified lipoproteins in the intima layer. 2. Withdrawal of monocytes by chemoattractans and monocytes migrate into the intima. 3. Monocytes differentiate into macrophages and will catch lipoprotein

modified. 4. Accumulation of excessive lipid in macrorophages forming lipid-laden foam cells. 5. The foam cells die and remove its contents, it will attract more macrophages. Oxidized LDL (oxd-LDL) is the atherogenic lipoprotein particles. Initiation effect on atherosclerosis may be caused by the gathering of LDL in the sub-endothelial matrix. LDL will be more and more trapped in the sub-endothelial matrix. Atherosclerosis can not be cured but can be prevented by lowering plasma cholesterol levels. One way to prevent the increase in cholesterol is to eat mushrooms. In this study the fungus used is white oyster mushroom. Nutrient content of oyster mushroom consists of protein, minerals (Ca, P, Fe and Na) and some types of vitamins, vitamin C, B complex), (6). Antioxidants β-glucans also contained in the oyster mushroom (7), anti-oxidants serves capture free radicals in the body. Oyster mushrooms contain a substance that serves to lower cholesterol levels in the blood that is lovastatin. (8;9;10). Lovastatin is one potential drug used to lower blood cholesterol levels by means of competitive inhibition against the mechanism of action of the enzyme 3- hydroxy-3-methylglutaril Co enzyme A (HMG Co A) reductase.

II. Methodology

2.1 Materials

The research subject male rat *Rattus Norvegicus* Wistar strain.

2.2 Procedure

Rats are adapted for 2 weeks, then divided into 4 groups. Each group consisted of six rats. P0 group (given regular feed and drink ad-libitum). P1 (fed with high cholesterol without any treatment). P2 (high cholesterol fed together with the provision EEJT, a dose of 250 mg / kg once daily). P3 were fed high-cholesterol together with the provision EEJT residue, a dose of 250 mg / kg once daily) for 20 weeks.

2.3 Determination of cholesterol and LDL levels

Every week examined levels of cholesterol, and LDL level using Spectrophotometer with a wavelength of 500 nm.

2.4 Determination of body weight

Every week examined levels of body weight using digital scales.

2.5 Determination foam cell number

After 20 weeks the rats turned off and taken aorta to see the formation of foam cells. Aortic made histopathological preparations stained with HE, and used digital microscope 400x magnification.

2.6 Determination of thickness of intima layer

After 20 weeks the rats turned off and taken aorta to see the thickness of intima layer. Aortic made histopathological preparations stained with HE, and used digital microscope 400x magnification. and to calculate the thickness of the intima layer used micrometer microscope**.**

Statistic: Test used one-way ANOVA with a significant level of 95%.(p velue 0,05), data used is the AUC (Area Under The Curve) value of cholesterol, LDL and body weight of each group, using SPSS 15. For normality test in this study used the Shapiro-Wilk test and variance test used the Levene's test.

III. Discussion

1.Cholesterol levels

Examination results cholesterol levels in rats fed residue or extract of *Oyster Mushroom* simultaneously by feeding high cholesterol showed on Graph 1

Post-hoc analysis using the Bonferroni method, the results are as follows:

1. P1 to P0, p value = 0.000
2. P1 to P2, p value = 0,000
3. P1 to P3, p value = 0,000

2. LDL Levels

The results of the examination LDL levels in rats fed extract or residue *Oyster Mushroom* showed on Graph 2

The result of Post-hoc analysis,

1. P1 to P0 p value = 0.000
2. P1 to P2 p value = 0.000
3. P1 to P3 p value = 0,000

3. Body Weight (bw)

The body weight after fed high cholesterol for 20 weeks with the administration EEJT for P2 and residue EEJT for P3 to be seen.

Post-hoc analysis of the results, as follows:

1. P1 to P0 p value = 0.000
2. P1 to P2 p value = 0.015

3. P1 to P3 p value = 0.04

4. Number of foam cells

Data on the number of foam cells.

Foam cells in theintima layer calculated under the endothelial layer, using a digital microscope brand Boeco (Germany) with 400 x magnification. Foam cells is calculated on ten field of view, and then averaged. Post-hoc analysis of the results.

1. P1 to P0, p value = 0,000
4. P1 to P2, p value = 0,000
5. P1 to P3, p value = 0,000

5. The thickness of the intima layer of the aorta

The thickness of the intima layer measured from the external elastika layer toward the lumen of blood vessels, a microscope is used to measure the thickness of the intima is the micrometer microscopes Primo Star, the brand Zeiss (Germany), with 400x magnification, the following results

To see the different groups significant then continued with Post-hoc analysis, the results of the Post-hoc analysis are:

1, P1 to P0 p value = 0.000
2. P1 to P2 p value = 0.000
3. P1 to P3 p value = 0.000

1.Cholesterol levels

After induction with high cholesterol feed for 20 weeks then the results are compared between P1 mean AUC cholesterol (2228.1 ± 64.1) with P0 mean of AUC cholesterol (1671.5 ± 20.6), P1 (2228.1 ± 64.1) with P2 mean of AUC (1444.1 ± 37.2), P1 (2228.1 ± 64.1) with P3 mean of AUC cholesterol (1433.2 ± 38.8), a significant number <0.05. AUC cholesterol comprehensive look smaller when compared with P1, the smaller area caused AUC cholesterol levels. This can happen because the P0 untreated except regular fed and drink ad-libitum. While at P1 fed with high cholesterol for 20 weeks and the graph showed increase in cholesterol levels in this group were not given any treatment, so that cholesterol levels increased. According to (11) that the liver and intestine accounted for 10% of all synthetic cholesterol levels in the total body. This condition causes a significant difference between P0 compared with P1. In the P2 group of the graph showed cholesterol levels do not increase, there is a possibility substances in extract ethanol of oyster mushrooms like statin, that inhibit the enzyme HMG Co-A reductase, this enzyme has a role in changing Acetyl Co-A into mevalonate which ended with a series of other processes will produce cholesterol (11; 12). By administer extract ethanol of oyster mushrooms the cholesterol not formed so that plasma cholesterol levels are not increased. That is extract ethanol of oyster mushrooms has the effect of lowering cholesterol levels. The results are consistent with the results of the study (9; 10; 13; 14; 15). In the group P3, the graph showed that the cholesterol levels of P3 tend to settle. This can occur because of the possibility of the residues extract ethanol of oyster mushrooms contain fiber will hinder the absorption of cholesterol from food, consequently cholesterol levels in plasma will also decrease. The results are consistent with the results of the study (16). No significant difference was seen between P2 compared to P3, significant numbers <0.05 means there is no difference in effectiveness between extract ethanol of oyster mushrooms and residues extract ethanol of oyster mushrooms in terms of preventing raising cholesterol levels.

2. LDL Levels

The significant difference when P0 mean of AUC LDL (753.9 ± 14.6) compared with P1 mean of AUC LDL (1190 ± 27.6), P1 (1190 ± 27.6) with P2 mean of AUC LDL (727 ± 20.7), P1 (1190 ± 27.6) with P3 mean of AUC LDL (738.8 ± 20.7) significant number <0.05. From the graph of LDL seen rising levels of LDL in P1 while at P0 LDL levels not seen improvement. By calculated the mean AUC LDL to P0, P2, and P3 is smaller when compared with the AUC LDL P1, this situation showsed the levels of LDL P1 higher than P0, P2, and P3. Larger the area AUC then the higher LDL levels. This can happen because the P0 not fed with high cholesterol and do not receive any treatment, at P0 only given regular feed and drink ad-libitum. While P1 of the graph shows broad LDL AUC is larger, this can happen because the P1 fed with high cholesterol for 20 weeks without any treatment, so that from the graph to see increasing LDL levels. Cholesterol comes from food will be partially absorbed by the intestine, especially in the jejunum and with some of the cholesterol in the process of boxed and tied with chylomicrons, in the liver chylomicrons are removing cholesterol back to the plasma as a component of plasma lipoproteins, LDL is one of the plasma lipoproteins. In the graph showed increasing cholesterol levels in the group P1, with increased levels of cholesterol, the LDL levels will also increase, this is because the LDL receptor is not formed in conditions of hypercholesterolemia. Plasma LDL levels depend on many factors including cholesterol in food, saturated fat and speed of production and elimination of LDL. The

group given the extract ethanol of oyster mushrooms (P2) visible area of the AUC LDL smaller shows that levels of LDL lower, this situation occurs because in extract ethanol of oyster mushrooms contained a substance that resembled statins which serves to lower cholesterol levels, are expected to decrease cholesterol levels will stimulate the formation of LDL receptors , LDL receptor will bind to LDL, thus LDL form a complex and bind to LDLR cholesterol will be degraded, thus there will be a decrease in levels of LDL, it is consistent with studies of (15). Besides allegedly contains a substance that resembled statins, was in extract ethanol of oyster mushrooms there are also anti-oxidants such as plavonoid. Anti-oxidants serves as a binder of free radicals. Anti-oxidants will serve as free radicals scafenger receptor, according to (15), which would bind free radicals, particularly free radicals that oxd-LDL lipid. Since free radicals are bound with anti-oxidants, this will cause the amount of LDL is reduced. The results are consistent with the results of the study (9, 10: 13; 14: 15). In the P3 group by the residue of extract ethanol of oyster mushrooms the graph shows LDL levels decrease, it does show that residues also play a role in lowering LDL levels. Residue is the fibers of oyster mushrooms, these fibers function blocking absorption of cholesterol from the intestine, consequently cholesterol bit is in the blood, other than that the residue contained anti-oxidant group flavonoida that serves as scafenger receptor (SR) against free radicals, thus radically smoking will be bound to the SR, with a free radical bound to the formation oxd SR-LDL will decline. The results are consistent with the results of the study (13; 14; 16). No significant difference seen in the P2 area mean AUC LDL (727 ± 20.7) when compared with P3 AUC LDL (738.8 ± 20.7), a significant number <0.05, meaning there is no difference in effectiveness between extract ethanol of oyster mushrooms with residue extract ethanol of oyster mushrooms in lowering LDL levels.

3. Body Weight (bw)

Post-hoc analysis of it can be seen that the average comprehensive P0 AUC bw (4649.5 ± 71) compared to the average P1 AUC bw (5184.5 ± 184.9), P1 (5184.5 ± 184.9) compared with the P2 area mean AUC bw (4870.2 ± 186.3), P1 (5184.5 ± 184.9) compared to P3 mean AUC bw (4880.2 ± 246.3), significant number <0.05 significant changes , this shows that the vast AUC bw to P1 wider than P0. This may occur because the P0 ret did not receive any treatment unless the provision of regular feed and drink ad-libitum, while the P1ret fed with high cholesterol for 20 weeks without being given extract ethanol of oyster mushrooms or residue extract ethanol of oyster mushrooms will cause cholesterol levels to rise, conditions of hypercholesterolemia will trigger obesity. Excessive cholesterol will be stored in adipose cells, will cause the size of enlarged adipose cells (18). P1 AUC bw greater than the P2, means in this study weight gain P2 is smaller when compared to weight gain P1. This may occur because the P1 fed with high cholesterol without being given any treatment, in contrast to P2 in addition to high feed cholesterol in this group were also given extract ethanol of oyster mushrooms simultaneously. And the graph shows also weight gain in P2 with the increase in cholesterol levels, although not as big as weight gain in P1 this happens because extract ethanol of oyster mushrooms contain substances that can lower cholesterol levels, so in P2 no increases cholesterol levels, resulting in weight gain in P2 not as big at P0. The results are consistent with studies (17). P1 AUC bw greater when compared with P3. This happens because P1 ret were fed high-cholesterol without any treatment, cholesterol from the graph showed that cholesterol levels increased with duration of breast feeding high cholesterol. Body Weight can be triggered by conditions of hypercholesterolemia. Mean while, in the P3 residue extract ethanol of oyster mushrooms given simultaneously by feeding high cholesterol and seen from the graph that the weight gain of body weight in P1, it is likely due to the residue extract ethanol of oyster mushrooms contained anti-oxidant flavonoids which would bind free radicals formed due to auto especially on lipid oxidation, in addition to the residue extract ethanol of oyster mushrooms, also contain fiber which will hinder the absorption of cholesterol in the intestine that comes from food, consequently cholesterol levels in plasma will also decrease. Decrease in cholesterol levels will reduce the enlargement of adipose cells, so the weight gain is not grossly inflated. This study is consistent with research (16). The comparison between P2 wide mean AUC body weight (4870 ± 184.9) and P4 wide mean AUC body weight (4880.2 ± 186.3) does not seem significant, meaning there is no significant difference effectiveness extract ethanol of oyster mushrooms with residue extract ethanol of oyster mushrooms in weight loss.

4. Number of foam cells

The significant differences in terms of foam cell formation can be seen from the analysis of Post-Hoc, that at P0 with a mean form foam cells (2.00 ± 0.89) when compared with the average P1 to form foam cells (8.00 ± 0.89) statistically significantly different, P1 compared with P2 mean that form foam cells (4.17 ± 0.75). P1 compared with P3 the average number of foam cells (3.67 ± 1.03) significant figures <0.05. This happens because at P0 were not given any treatment, rats were given regular feed and drink ad-libitum, while it was in the P1 foam cell formation more because in this group were fed high-cholesterol without any medication, from the graph shows an increase in cholesterol levels. With increasing levels of cholesterol, the LDL receptor is not formed, it will increase the levels of LDL, while the state of hypercholesterolemia causes a decrease in

endothelial function of blood vessels, resulting in decreased bioavailability of NO and leading to inhibition of platelet aggregation, the adhesion of leukocytes, the proliferation of vascular smooth muscle (VSM) , this situation will lead to remodeling of the cell wall and macrophage derived monocyt start capturing and stockpiling LDL, in addition to the cholesterol levels will also trigger the release of free radicals will oxidize lipids, especially LDL becomes oxd-LDL, LDL oxidized later will turn into foam cells , according to the study (18). P2 group foam cell growth less when compared with P1, meaning that there is an influence on the growth of foam cells extract ethanol of oyster mushrooms significant. This happens because extract ethanol of oyster mushrooms contains a substance that resembled statins which serves to lower cholesterol levels, by decreasing the levels of LDL cholesterol levels will also decrease. Besides, it would also reduce the state of oxidative stress on the environment so it does not happen auto chiefly LDL lipid oxidation, because if oxidized LDL to trigger the formation of foam cells. In the group of P3 by residues extract ethanol of oyster mushrooms, cholesterol and LDL is not too high, it is because residues extract ethanol of oyster mushrooms contains fiber and anti-oxidants that will lower cholesterol and anti-oxidants will hinder the process of oxidation of LDL becomes oxd-LDL, resulting in the formation of foam cells will also be reduced. No significant difference can be seen in P2 (give extract ethanol of oyster mushrooms) the average number of foam cells (4.17 ± 0.75) when compared to P3 (residue extract ethanol of oyster mushrooms) the average number of foam cells (3.67 ± 1.03) means that there is no difference in effectiveness significant in terms of preventing the formation of foam cells between extract ethanol of oyster mushrooms with residue of extract ethanol of oyster mushrooms.

5.The thickness of intima layer of the aorta

By looking at the results of a post-hoc analysis of different groups of significant note. There are three different groups were statistically significant, the group is the average thickness of the intima layer P0 (18.00 ± 0.89) compared with the mean thickness of the intima layer P1 (33.50 ± 1.24) significant figures <0.05. Here we can see the thickness of the intima layer P1 is thicker when compared P0, P1 for the fed high cholesterol without any treatment, resulting in increased LDL cholesterol levels and as shown in the graph and LDL cholesterol. Thickening of the diameter of the intima layer occurs due to accumulation of foam cells sub-endothelial layer of the intima, which is caused by the entrapment of oxidized LDL, Oxd-LDL increased in conditions of hypercholesterolemia, more cells will accumulate more and thicker foam layer intimanya. Cell foam in P1 more than foam cells formed in the P0, P1 consequently the thickness of the intima layer thicker than P0.

A significant difference in the mean thickness of the intima layer P1 (33.50 ± 1.24) compared with P2 (extract of oyster mushrooms) the average thickness of the intima (24.75 ± 0.87) with a significant level of <0.05. This situation occurs because the P1 rats fed with high cholesterol for 20 weeks, the graph shows an increase in cholesterol levels and LDL cholesterol, as well as the formation of foam cells more when compared with the P2, because the foam cells more formed, the thickness of intima layer also increased, in P1 compared to P2, this indicates that extract ethanol of oyster mushrooms can lower LDL cholesterol levels and thus can be prevented formation of foam cells.

Similarly, if the average thickness of the intima layer P1 (33.50 ± 1.24) compared with P3 (residue of oyster mushrooms) with a mean thickness of the intima (27.21 ± 0.86) with a significant level of <0.05. This situation occurs because the P1 rats fed with high cholesterol for 20 weeks, the graph shows an increase in cholesterol levels and LDL cholesterol, as well as the formation of foam cells more when compared with a P3, because the foam cells more formed, the thickness of intima layer also increased in P1 compared to P3, P3 From the graph looks the increase of cholesterol in cholesterol but did not reach the state of cholesterol and LDL, so that the foam cells are formed not much, because not a lot of foam cells formed, the thickening occurs in the intima layer also not great. The cause of the condition was due to the residue Extract Ethanol of oyster mushrooms contain which flavonoids anti-oxidants will scavenge free radicals so that no auto oxidation will oxidize lipids and become foam cells, with the formation of foam cells will lead to thickening of the intima layer of the aortic wall. Residues extract ethanol of oyster mushrooms also contain fiber that will inhibit the absorption of cholesterol in the intestine, this will lead to decreased cholesterol levels as well as LDL. This study is consistent with research (15). No significant difference seen in P2 (give extract ethanol of oyster mushrooms) with a mean thickness of the intima (24.75 ± 0.87) when compared to P3 (give residue of extract ethanol of oyster mushrooms) with a mean thickness of the intima (27.21 ± 0.86) In this study there was no significant difference between extract ethanol of oyster mushrooms foam cell formation and the residue extract ethanol of oyster mushrooms and this caused no significant difference was also on the diameter of the intima layer of the two groups either using residue extract ethanol of oyster mushrooms or extract ethanol of oyster mushrooms.

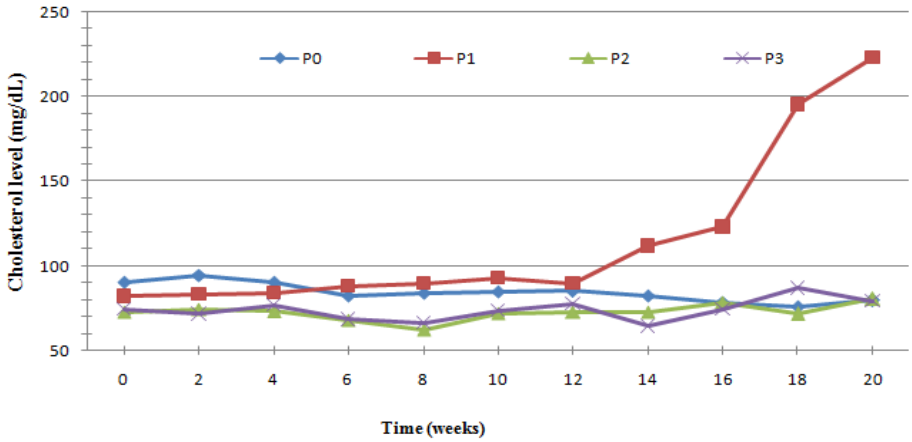
IV. Conclusion

Effect of oyster mushroom as follows:

a. Cholesterol levels, white oyster mushrooms in the form extract ethanol or residue was able to prevent an increase of cholesterol levels in male Wistar rats. There is no statistically significant difference between extract ethanol and residue.
b. Levels of LDL, both white oyster mushroom in the form extract ethanol or residue was able to prevent raising the levels of LDL in male rats. There is no statistically significant difference between extract ethanol and residue.
c. Body weight, white oyster mushroom in the form extract ethanol or residue can prevent in increase of body weight in male rats. There is no statistically significant difference between extract ethanol and residue
d. White oyster mushrooms in the form extract ethanol or in the form residue , can inhibit the formation of foam cells. There is no statistically significant difference between extract ethanol and residue.
e. Thickening of the intimal layer of the aorta turns can be limited both by the extract ethanol white oyster mushroom or residue good shape, the smaller form foam cells, the thickening of the intima layer little too. The oyster mushroom in the form extract ethanol or residue can prevent the formation of atherosclerosis in rat fed a high cholester

GRAPHIC

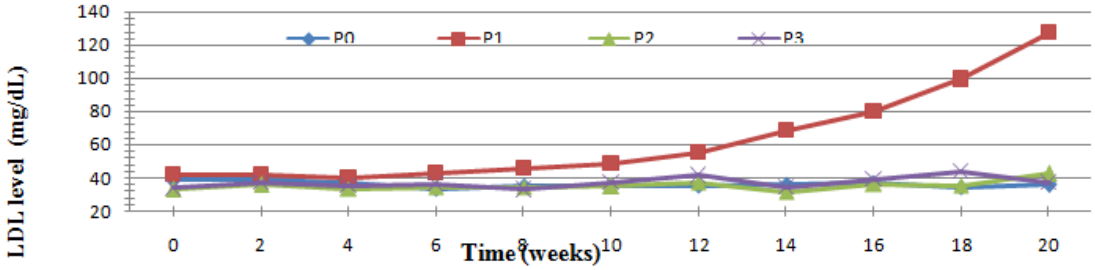
1. Cholesterol level



Graph 1 Relationship cholesterol levels against time on P0, P1, P2 and P3

Description: P0: given regular feed
P1: fed with high cholesterol
P2: fed high cholesterol and given extract ethanol of oyster mushrooms
P3: fed high cholesterol and given residue extract ethanol of oyster mushrooms

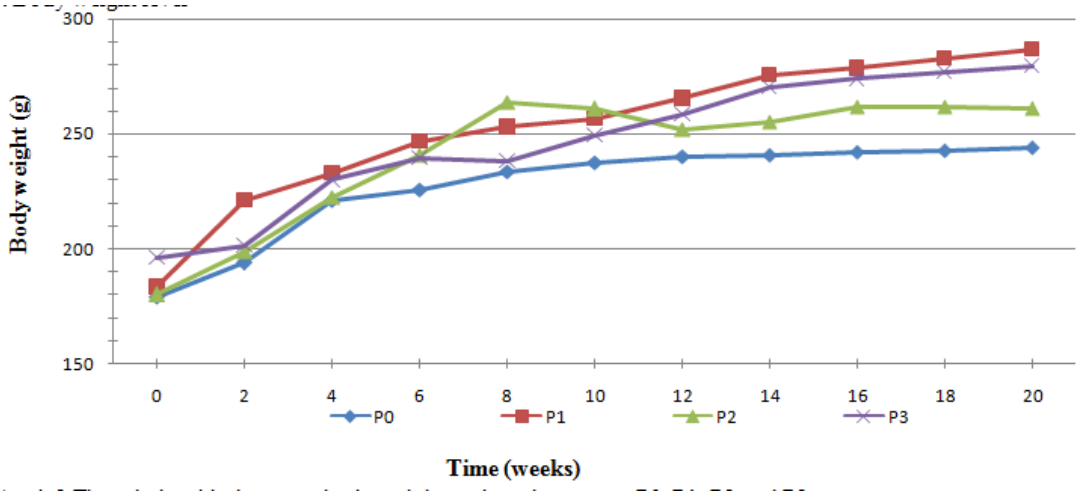
2. LDL level



Graph 2 Relationship LDL levels against time on P0, P1, P2 and P3

Description: P0: given regular feed
P1: fed with high cholesterol
P2: fed high cholesterol and given extract ethanol of oyster mushrooms
P3: fed high cholesterol and given residue extract ethanol of oyster mushrooms

3. Body weight level



Graph 3 The relationship between body weight against time group P0, P1, P2 and P3

Description: P0: given regular feed
P1: fed with high cholesterol
P2: fed high cholesterol and given extract ethanol of oyster mushrooms
P3: fed high cholesterol and given residue extract ethanol of oyster mushrooms

4. Number of foam cells

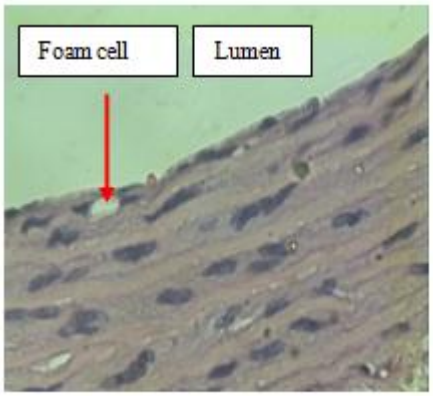


Fig 1 The foam cells of P0

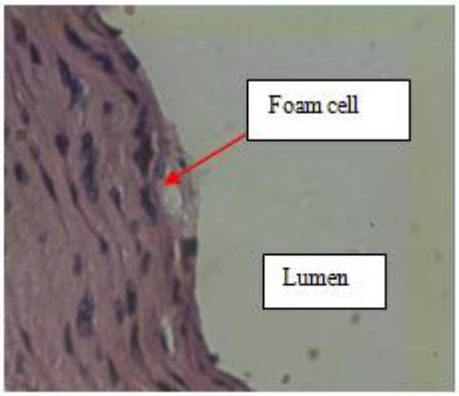


Fig 2. The foam cells of P1

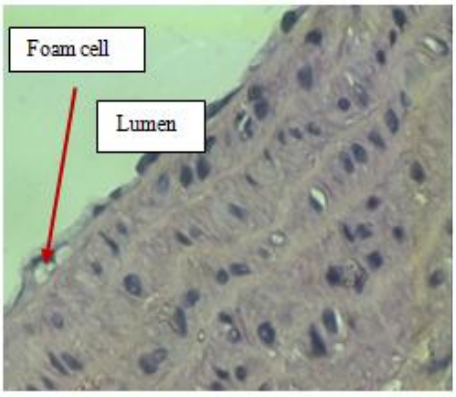


Fig 3 The foam cells of P2,

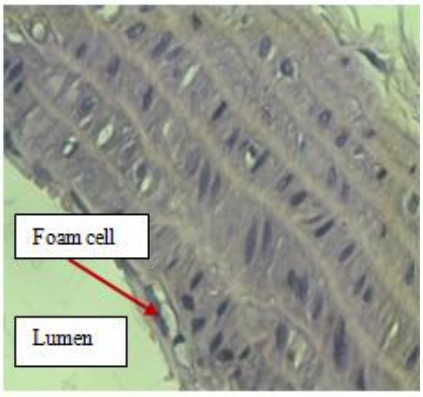


Fig 4. The foam cells of P3

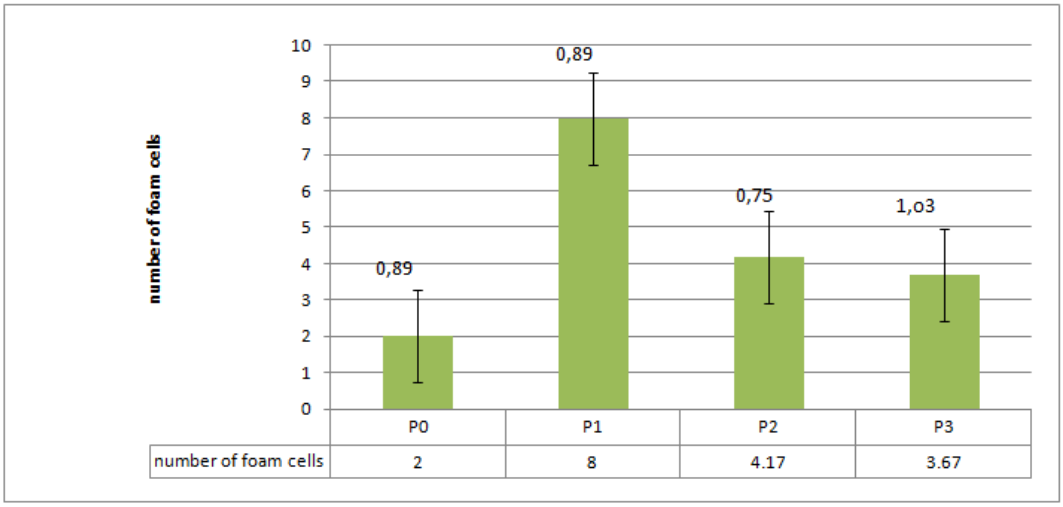


Table 1. The number of foam cells group P0, P1, P2 and P3
Description: P0: given regular feed
P1: fed with high cholesterol
P2: fed high cholesterol and given extract ethanol of oyster mushrooms
P3: fed high cholesterol and given residue extract ethanol of oyster mushrooms

5. The thickness of the intima layer of the a

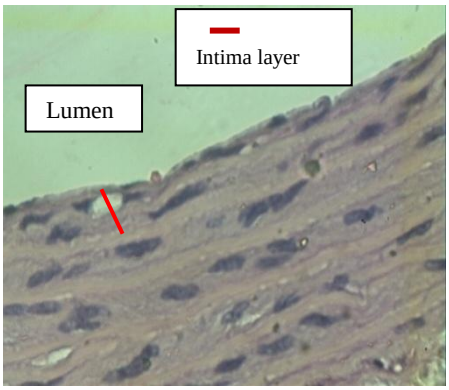


Fig 5 intima layer thickness of P0, the thickness of intima layer 18,38 µm

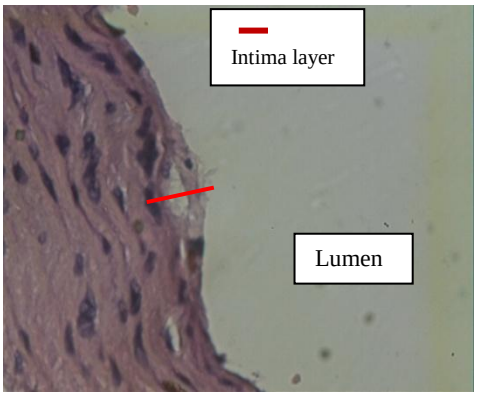


Fig 6. intima layer thickness of P1, the thickness of intima layer 34,36µm

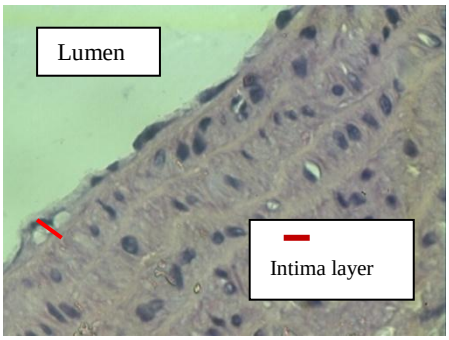


Fig 7 intima layer thickness of P2, the thickness of intima layer 45,11µm

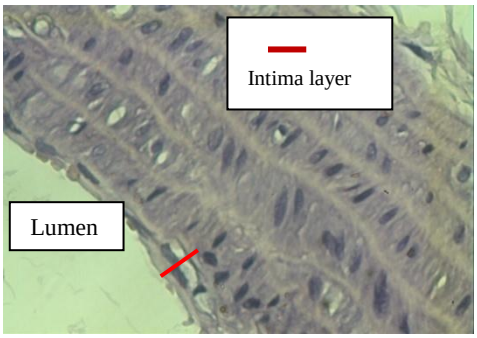


Fig 8 intima layer thickness of P3, the thickness of intima layer 34,36µm

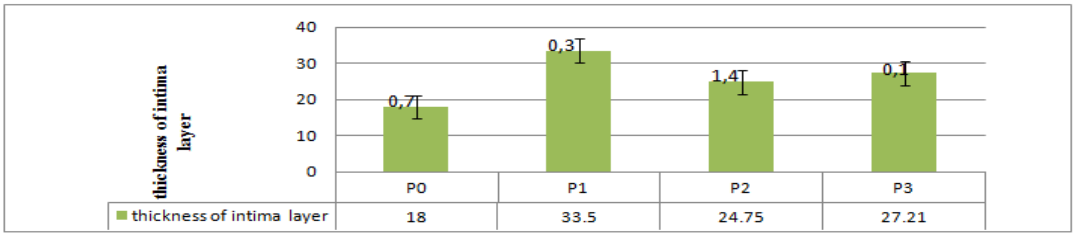


Table 2. The thickness of intima layer group P0, P1, P2 and P3

Description: P0: given regular feed
P1: fed with high cholesterol
P2: fed high cholesterol and given extract ethanol of oyster mushrooms
P3: fed high cholesterol and given residue extract ethanol of oyster mushrooms

References

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