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Modification of atherogenic diet causes atherosclerosis, increase total cholesterol, and showing hepar damage in mice as alternative animal model in atherosclerosis research

Dzul Fithria Mumtazah *, Hendri Busman, Gina Dania Pratami, Ahad Putra Dewantara and Faradhila Amanda

Department of Biology, Faculty of Mathematics & Natural Sciences, Universitas Lampung, Jalan Prof. Soemantri Brodjonegoro No. 1 Gedong Meneng, Lampung, Indonesia.

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Abstract

This study aims to determine the composition of the ideal atherogenic diet to increase the risk of atherosclerosis in the animal model. The research was used 15 male mice, acclimatized for 1-2 weeks to get the susceptible amount of diet to be given. The animal objects are divided into 3 groups; normal diet group (control), 4 weeks atherogenic diet groups, and 8 weeks atherogenic diet groups. The modified atherogenic diet consists of common broiler food, wheat flour, pork oil, quail egg yolks, and water, given 2 times a day of 30 g of food. Water for animal objects is given ad libitum. After 4 weeks and 8 weeks total cholesterol, the formation of foam cells, and hepatocyte degeneration. The results show that the total blood cholesterol of animal models in the group of 8 weeks has the highest level ($153, 66 \pm 6, 51$), compared to other groups (normal diet and 4 weeks). Animal models show that aortic cross-section formed foam cells in tunica intima and tunica media of endotel, also show the indication of hepar damage by hepatocyte degeneration.

Keywords: Modified atherogenic diet; Atherosclerosis; Foam cell; Total cholesterol; Mice; Hepar damage

1. Introduction

Atherosclerosis as a chronic inflammatory disease is a disease caused by abnormalities of lipid metabolism in the body [1]. Atherosclerosis is characterized by the accumulation of lipids and formation of foam cells caused by modified low-density lipoprotein (LDL) uptake. LDL modification takes place as a consequence of lipid oxidation and the catalytic action of a series of enzymes, one of which is secreted phospholipase A2 (sPLA2) [3]. sPLA2 is also a biomarker of the inflammatory process and plays an important role in atherosclerosis [4]. The use of animal models in atherosclerosis research is important as an object to determine the pathogenesis, causes and pathways of the disease. In this case the animals are often used are rodents [5], non-human primates, rabbits, dogs, pigs and other animals [6][7]. Although there are mice as animal models of atherosclerosis, they are not commonly used. Research on atherosclerosis in Indonesia uses a lot of rats that are easier to handle and have a larger heart and aorta size. However, rats (both the Wistar and Sprague-Dawley strains) were more difficult to obtain from mice, the availability is limited and harder to find for many researcher and students. Besides that, the price is more expensive. We strive to formulate an atherogenic diet modification suitable for use in atherosclerosis research using mice as a more affordable, inexpensive, and susceptible animal.

* Corresponding author: Dzul Fithria Mumtazah

Department of Biology, Faculty of Mathematics & Natural Sciences, Universitas Lampung, Jalan Prof. Soemantri Brodjonegoro No. 1 Gedong Meneng, Lampung, Indonesia.

2. Material and methods

A total of 15 male mice weighing 20-30 grams were acclimatized for one week from the experimental animal farms of the Lampung Veterinary Centre in the laboratory with standard feed and ad libitum water. Mice were divided into three (3) treatment groups, namely the control group, the modified atherogenic diet group for four (4) weeks and the modified atherogenic diet group for eight (8) weeks. The composition of the feed based on an atherogenic diet for each group is described in Table 1.

Table 1 Feed composition of atherogenic base diet in three groups.

Composition	Group		
	Control	4 Weeks	8 Weeks
Japfa Comfeed Broiler I	53%	50%	50%
Wheat Flour	24%	25%	25%
Pork Oil	0%	10%	10%
Quail Egg Yolks	0%	5%	5%
Water	24%	10%	10%

The control group, 4 weeks group, and 8 weeks group were fed with the composition as listed in Table 1 on an ad libitum basis with a dose of 30 grams per day. The dose of 30 grams per day is determined from the average amount of feed consumed by mice during the acclimatization period. Standard feed (comfeed) has been mixed with wheat flour for the entire treatment for eight weeks according to the predetermined percentage of feed. Furthermore, pork oil and quail egg yolk are added daily to the feed that will be given to the mice. Water is also given ad libitum.

After administration of atherogenic diet modification based feed during the specified time, the animals were sacrificed. The control group was sacrificed After 8 weeks, the 4 weeks group was sacrificed after 4 weeks and the 8 weeks group was sacrificed after 8 weeks. A total of 1 ml of whole blood was taken from the cardiac sinus after terminal sacrifice using a 1 ml syringe, blood is stored in a vacutainer tube containing EDTA and preserved at -20 degrees Celsius before measuring total cholesterol levels. Besides the aorta were also collected from animal models to look for histopathological features of the aorta after several weeks of treatment. Total blood cholesterol in normal mice ranges from 26-82.4 mg / d [8] (1), total cholesterol above this range is considered high

1 ml of blood was used to calculate the total cholesterol, and was done at the Regional Health Laboratory Bandar Lampung, Lampung Indonesia. Total cholesterol was measured by the CHOD-PAP method. The aorta were collected, directly preserved using paraffin and tested histopathologically in the pathology laboratory of the Lampung Veterinary Center. The results of the histopathology test were carried out by counting the presence or absence of foam cells formed in the aorta as an early sign of atherosclerosis.

3. Results and discussion

3.1. Total cholesterol

Cholesterol as part of the composition of the diet of most of our daily generations cannot be denied that it has an important role in the body's metabolism. Not completely bad, cholesterol provides the insulation that supports the work of nerve cells and many other good functions. It is the types of dangerous cholesterol (LDL and triglycerides) whose concentration is higher than good cholesterol (HDL) in the body which is the problem of high metabolic syndrome and its inherited diseases, one of which is atherosclerosis. The following is an overview of the cholesterol profile of the tested animals after 4 weeks and 8 weeks of treatment. The data below are data on total cholesterol levels in the blood plasma of tested animals. In this data, total cholesterol levels in the 8 week group of treated animals had the highest levels. This could be due to long exposure to a high-fat and highly atherogenic diet in feed.

An atherogenic diet generally consists of cholesterol, cholic acid, and fat, in which the cholesterol and cholic acid components play an important and significant role in proatherogenic properties and gene expression that accompany them [9] (2). This study tried to determine the outcome of an atherogenic diet such as the composition shown in table

1, without the addition of cholic acid which is important in the induction of expression of genes involved in extracellular matrix deposition in hepatic fibrosis, and collagen accumulation which increases the risk of atherosclerotic plaque formation in blood vessels. The mean of total cholesterol in the eight-week treatment group had the highest number which showed that the composition of the feed was proven to be able to increase total cholesterol levels in animal models.

Table 2 Total cholesterol of animal models collected from blood (mg/dL).

No	Normal Diet	Modification Atherogenic Diet	
		4 Weeks	8 Weeks
1	77	182	147
2	139	104	154
3	101	120	160
Mean±SD	105,67 ± 31,26	135,33 ± 41,20	153,66 ± 6,51

The source of cholesterol in the formulated feed comes from quail egg yolks and pork oil. Based on studies, for the same weight of quail egg yolk and chicken egg yolk that are commonly consumed by the public, quail egg yolk has a higher total cholesterol [10] (3). Pork oil is mixed into the feed as an additional source of cholesterol for model animals. Pork oil has at least 97 mg of total cholesterol per 100 g [11] (4), making it a potential cholesterol source for increasing cholesterol levels in animal models for atherosclerosis and hypercholesterolemia research. This is in accordance with [12] (5) opinion that a diet high in saturated fat can significantly increase total cholesterol levels in the blood. This high saturated fat condition is found in egg yolks and pork oil, in addition the prices are cheap and the availability is high, which becomes the reason for choosing these ingredients as a feed mixture to form animal models with high total cholesterol as one of predictors in atherosclerotic conditions. This also shows that the duration of modified atherogenic diet administration also affects the average of total cholesterol levels in mice. The longer the atherogenic feed was given, the higher the average of total cholesterol level in animal models. These results also show that there is an association between cholesterol intake and serum cholesterol levels as in previous studies (6) [13].

Mice commonly highly resistant to diet-induced atherogenesis because of HDL level of total plasma cholesterol is almost 70% [14] (7), it causes the differences of total cholesterol of the three groups is not significant. Various studies on how to reduce the concentration of LDL and triglycerides in the blood have been carried out to date, one of which is by using the help of modified animal models to produce model animals that represent diseased organisms that have excess levels of LDL cholesterol and triglycerides and thus develop atherosclerosis on the walls of blood vessels.

3.2. Animal weight

The weight of the animal is considered as an additional contributing factor in the increase in cholesterol levels and its components in the blood. The treatment given is in the form of feeding with different compositions so that it is expected to produce animals with different atherogenic indexes. Overview increase in weight of the test animals can be seen in the following table.

It shows that the experimental animals have no difficulty eating and have a good appetite related to the given diet, as indicated by a positive increase in body weight. The 8-week diet group had a higher average body weight gain than the control because the contribution of cholesterol sources in the diet had an impact on greater body weight. All groups had a similar weight gain, this could be because the feed in all groups, both control, and treatment, was a feed with high carbohydrate content, where high carbohydrate diets were proven to have increased body weight [13] (6).

Table 3 Overview increase in average animal weight (g) from week to week, the weight of the animals in the control group decreased at the end of treatment

Time	Control	4 Weeks	8 Weeks
Week 1	33,6	33	37,6
Week 2	36,75	35,4	36
Week 3	39,25	35,6	39,4
Week 4	40	35,2	37,8
Week 5	39,75		38,2
Week 6	40,75		36,8
Week 7	39,25		38,2
Week 8	38,25		40,4

3.3. Aortic and hepar histopathology

In the group of mice with modified diet for eight weeks, microscopic view of the aorta showed the presence of foam cells (arrows) visible on the tunica intima and tunica media (Fig.1).

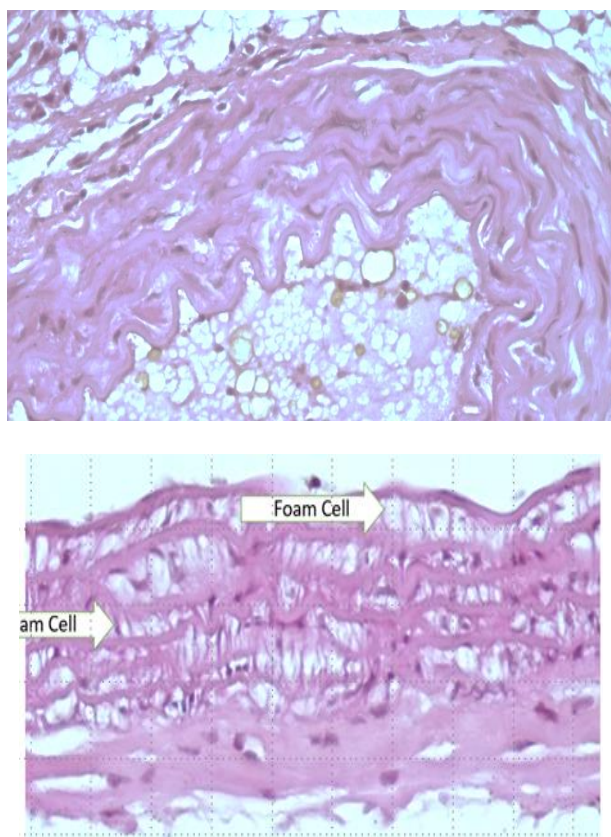


Figure 1 Aortic cross-section showing the formation of foam cells in the tunica intima and media

Foam cell is a part of atherosclerotic plaque in blood vessels that actively plays a role in cholesterol accumulation and can worsen due to excessive intake of LDL cholesterol in the blood [15] (8). These cells originated from monocytes or macrophages that function in scavenging oxidized LDL cholesterol that enters blood vessels via LDL receptors or other pathways. The high level of total cholesterol in animal models shown in Table 2 above can be one of the causes of high cholesterol uptake by monocytes and macrophages in the blood and contribute to the number of foam cells present in the aorta. Atherosclerotic progression in the present and in the future is closely related to the formation of foam cells,

it causes many atherosclerotic studies use this indicator to determine the severity of plaque formation. So that further study of cell foam will be a great help in developing therapeutic intervention in atherosclerosis [16] (9).

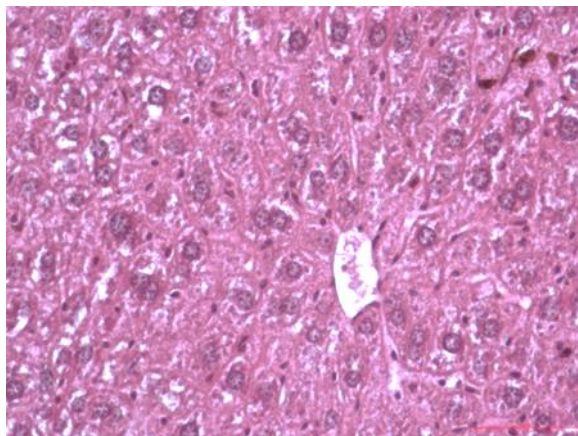


Figure 2 Hepar cross-section showing the hepatocytes, showing hydropic degeneration and necrosis

Apart from aortic histopathology, liver cross section of experimental animals were also observed under a microscope, shows the presence of damage to many hepatocyte cells, which may be due to the early stages of fatty liver cases due to modified diets consumed by mice containing high cholesterol (Fig. 2).

4. Conclusion

The modified atherogenic diet consist of 50% common broiler feed , 25% wheat flour, 10% pork oil, 5% quails egg yolks and 10% water could be one of alternatives to induce atherosclerosis, increase total cholesterol and causing hepar damage in mice.

Compliance with ethical standards

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Disclosure of conflict of interest

There's no conflict of interest in this research

Statement of ethical approval

This study has met the standard of treatment for laboratory animals and meets the research code of ethics.

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