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ORAL ADMINISTRATION OF THE HYPERCHOLESTEROL RAT FEED FORMULA TO MAKING THE ANIMAL DYSLIPIDEMIA MODEL ON SPRAGUE DAWLEY RATS

PEMBERIAN SECARA ORAL FORMULA PAKAN TIKUS HIPERKOLESTEROL TIKUS SPRAGUE DAWLEY UNTUK MEMBUAT HEWAN MODEL DISLIPIDEMIA

Fatchiyah Fatchiyah[1,2,3)*], Eko Suyanto[1,2,3)], Rista Nikmatu Rohmah[1,2,3)], Lidwina Faraline Triprisila[1,2,3)], Hazna Noor Meidinna[1,2,3)], Dewi Ratih Tirto Sari[1,2,3)], Iva Himmatul Aliyah[1,2,3)]

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Authors affiliation:

[1)]Dept. of Biology, Faculty of Math. and Natural Sciences, Universitas Brawijaya, Indonesia

[2)]Research Center of Smart Molecule of Natural Genetics Resources (SMONAGENES), Universitas Brawijaya, Indonesia

[3)]Institute of Biosains, Universitas Brawijaya, Malang

Correspondence email:

*fatchiya@ub.ac.id

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ABSTRACT

The aim of this study was to make animal dyslipidemia models in Sprague-Dawley strains induced by a high fat goat diet formula as hypercholesterol feed for two months. The experimental animal used in this study was 30 male rats (Rattus norvegicus strain Sprague Dawley) with an age of 2-3 months with an average body weight of 150g. Animal models are divided into two groups consisting of a control group without additional diet and dyslipidemia group given food consumption goat hypercholesterolemia with high-fat diet formula orally every day for two months. Physiological characteristics of dyslipidemia SD rats had higher body weight, increased food consumption and fecal weight, and decreased water intake and urine volume than the control group. Total cholesterol, triglyceride, and LDL-cholesterol levels increased, while HDL-cholesterol levels did not change in the dyslipidemia rats group compared to the control group. The conclusion of this study indicated that the hypercholesterol diet formula with a high composition of goat fat was successfully induced the SD rats to become dyslipidemia model rat with specific hypercholerol characteristics.

Keywords: *dyslipidemia, high fat diet goat, experimental animals, cholesterol, Sprague-Dawley rats*

ABSTRAK

Tujuan penelitian ini adalah untuk membuat hewan model dislipidimia pada tikus strain Sprague-Dawley yang diinduksi dengan formula diet tinggi lemak kambing sebagai pakan hiperkolesterol selama dua bulan. Hewan coba yang digunakan pada penelitian ini yaitu tikus jantan 30 ekor tikus (Rattus norvegicus strain Spargue Dawley) dengan usia antara 2-3 bulan dengan berat badan rata-rata 150g. Hewan coba dibagi dua kelompok terdiri dari kelompok kontrol tanpa diet tambahan dan kelompok dislipidimia yang diberi konsusmsi pakan hiperkolesterol dengan formula diet tinggi lemak kambing secara oral setiap hari selama dua bulan. Karakteristik fisiologi tikus SD dislipidimia memiliki berat badan lebih tinggi, konsumsi makanan meningkat, berat fecal naik, tetapi asupan air dan volume urin menurun. Kadar total kolestrol, trigliserida dan kolesterol-LDL meningkat, sedang kadar kolesterol-HDL tidak mengalami perubahan dibanding dengan kelompok tikus kontrol. Kesimpulan dari penelitian ini adalah formula diet hiperkolesterol dengan komposisi tinggi lemak kambing berhasil menginduksi tikus SD menjadi tikus model dislipidimia yang menunjukkan karakter spesifik hiperkolesterol.

Kata kunci: *dislipidimia, diet tinggi lemak kambing, hewan coba, kolesterol, tikus Sprague-Dawley*

INTRODUCTION

Animal models have contributed to scientific development in experimental research for many years to improve human knowledge and contribute in finding solutions to biological and biomedical problems [1]. The ideal animal model is an animal that has similarities in the process of being imitated, easy to maintain, capable of producing many offspring, low maintenance costs, one tail can provide blood and tissue samples, its genetic composition is known, and its disease status is known and can be explained.

Animal models serve as substitutes and not necessarily all models are identical to the subject being modeled. In general, experimental animal models are classified into two; there are spontaneously or genetically induced animal models and experimental or non-genetically induced animal models. Animal models of spontaneous or genetically normal animals that have phenotypic similarities with human or animal species abnormal caused by spontaneous mutations. Induced or non-genetic animal models are animals whose normal physiological status is

altered through surgery, genetic modification, and chemical application [2]. Non-genetic models are more frequently used than genetic models due to lower cost, more availability, methods of induction and maintenance easier [3].

Research on dyslipidemia, diabetes mellitus, and insulin resistance continues to be carried out to find the right treatment strategy to prevent and overcome its complications. Rodents are the animals most frequently used as an animal model in biomedical and behavioral research since mice have properties such as a short gestation period, a relatively short life span, benign and have health and genetic background is known. The genome of mice has a close homology with the human genome so that the manipulation of the mice genome can produce animal models similar to human disease phenotypes. Laboratory mice are commonly used is *Rattus norvegicus* which is the order of Rodentia and Muridae family [4].

One way of making experimental animal models based on certain experiments is through the intake of feed that enters the body (diet). Rat strains influence their biological characteristics and the type of feed. The selection of a particular strain for a particular study can have a significant effect on research results. [5]. The Sprague Dawley strain mice (SD rats) were more effective for an animal model of a high-fat diet than the other mouse strains. Longer diet duration will induce dysiplidemia[2]. The previous study also showed that the Spontaneously Diabetic Torii (SDT) rats are animal models which appropriate to evaluate dietary effects [6].

In in vivo research, one of the models of induced or non-genetic experimental animals is by paying attention to the food intake, including diabetes models given alloxan or streptozotocin in adult rats, and high-fat diet models for modeling hypercholesterolemia, type 2 diabetes, or obesity. Some time ago, Fatchiyah (2015) has developed a hypercholesterolemic diet formula with a high content of goat fat which has been registered as a patent [7]. This study focuses on making an animal model of dyslipidemia using SD rats given the high fat goat diet formula for a certain period of time and compared with control animals.

METHODS

Ethics. This study has been evaluated and approved by the Ethics Committee of Brawijaya University, Malang, East Java, Indonesia (No: KEP-679-UB).

Hypercholesterol diet formula. This hypercholesterol diet formula with a high content of goat fat has been registered with IPR with registration number P00201507845 as a patent [7].

Dyslipidemia animal model establishment. The experimental animals used in this study were male rats (*Rattus norvegicus* strain Sprague Dawley) with ages of 2-3 months. Rats were obtained from the UPT LPPT Gadjah Mada University with a minimum body weight of 150g. As many as 30 SD rats were acclimatized for one week. SD rats were divided into two large groups of control rats without any additional diet and dyslipidemic mice. SD rats treated with dyslipidemia were given a hypercholesterol diet formula with high goat fat content and the composition of cholic acid, duck egg yolk, goat fat, flour, and comfeed pars, given orally every day for two months. Control rats were fed only with comfeed pars. After that, once every two weeks, cholesterol levels were measured using a cholesterol stick. Treatment rats were be dyslipidemia if their cholesterol levels were more than 240 mg/dL [8]. Furthermore, after SD rats were two months treatment, in addition to measuring total cholesterol, cholesterol levels were also measured for *Low Density Lipoprotein* (LDL), *High Density Lipoprotein* (HDL), and triglycerides (mg/dL).

Measurement of physiological characteristics of experimental animals. The two groups of rats were measured daily for body weight, food intake, water intake, fecal weight, and urine volume. After that, the average value of these parameter values was calculated to be compared with the control group.

Statistical analysis. Statistical analysis used GraphPad Prims 9.0 software at a significance of $p < 0.05$.

RESULTS AND DISCUSSION

Animal models have been used in experimental research to enhance human knowledge and contribute to finding solutions to biological and biomedical problem [1]. The experimental animal model of dyslipidemia can be induced obesity by increasing high fat intake (40-60% of total calories). The daily calorie requirement for rats is 10-15 kcal/day and the standard feed is available with a composition of 65-70% carbohydrates, 20-25% protein and 5-12% fat and the total calories are 2900 kcal/kg [9]. In this study, the high fat diet formula of goat was tested with No. Patent: P00201507845 (Fatchiyah, 2015), The group of dyslipidemic rats was fed hypercholesterolemia with a composition of 45-50% comfeed pars, 20-30% wheat flour, 3-6% duck egg yolk, 10-20% goat fat, 2- coconut oil. 3%, and cholic acid 0.1-0.5% [7].

In this study in Figure 1 for physiological characterization of rat, there was an increase in body weight in the dyslipidemia SD group of rat

compared to the control group of SD rats. This is in accordance with the morphology of the dyslipidemia SD rat, which is greater than the control group as in Figure 3. Although in the dyslipidemic SD rat group the food intake is also higher, but the water intake is less than the control group. These data were followed by a decrease in urine volume and fecal weight in the dyslipidemia SD group of rat. Providing a diet with a high proportion of fat (<85% total calories), carbohydrates, salt, and cholesterol can induce animal models for research on obesity, insulin resistance, hypertension, atherosclerosis, and dyslipidemia [9].

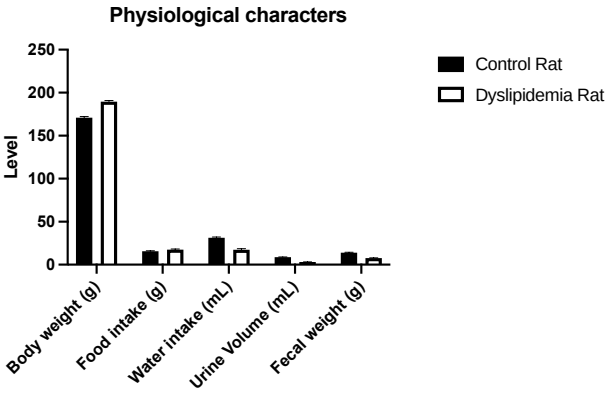

Figure 1. Physiological characteristics of control and dyslipidemia rats. Significance analysis with GraphPad Prims 9.0, p <0.05.

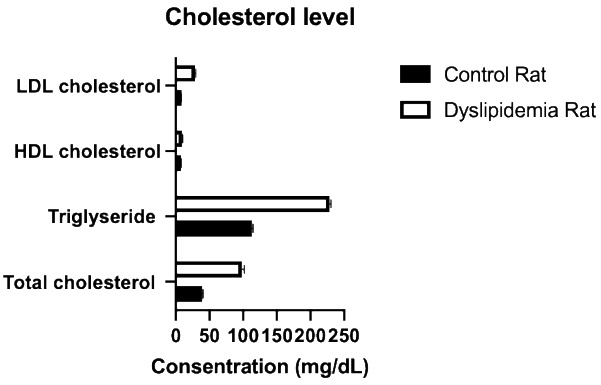

Figure 2. Cholesterol levels in control and dyslipidemia rats. Significance analysis with GraphPad Prims 9.0, p <0.05.

Dyslipidemia is a condition of high cholesterol levels in the blood, a form of autosomal dominant genetic disorder that causes the accumulation of plasma low-density lipoprotein (LDL) cholesterol in sufferers. [10]. Cholesterol level analysis data in Figure 2 shows an increase in total cholesterol and triglyceride levels in the dyslipidemic SD rats group which was significantly different at p <0.05 compared to the control SD group of rat. Meanwhile, LDL cholesterol levels in dyslipidemia SD rats also increased but not significantly different. HDL cholesterol levels in the dyslipidemic SD rat were almost the same in control SD rat. According to Chatzistefanidis et al., the main lipid fraction abnormality in dyslipidemia or hypercholesterolemic patients is an increase in total cholesterol, LDL-cholesterol, and triglycerides and a decrease in HDL-cholesterol levels [10]. Based on the results in Figure 2, all cholesterol levels in dyslipidemia rats obtained are in accordance with cholesterol levels in dyslipidemic rats, but in these dyslipidemic rats, HDL cholesterol levels did not decrease.

Cholesterol is synthesized in most body tissues from acetyl-CoA in the microsomal and cytosolic components of cells. More than 23 enzymes are involved in the formation of cholesterol from acetyl-CoA, one of which is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [11]. Based on previous research, 1 in 250 individuals sustain hypercholesterolemia. More than 50% of men and 30% of women who experience this disease will develop coronary heart disease at the age of 50 and 60 years. Hypercholesterolemia can be inherited in an autosomal dominant manner, so that a person can experience it at the age of under 18 years. This can lead to early coronary heart disease [12].

A person is declared to have hypercholesterolemia if the LDL-C content in the blood is more than 240 mg/dL [13]. The accumulation of LDL cholesterol can occur because of a disturbance when the LDL in the blood will be cleaned so that the LDL cleaning process becomes obstructed. Two known forms of hypercholesterolemia are autosomal dominant and autosomal recessive. Most of the hypercholesterolemic sufferers have an autosomal dominant inheritance pattern, which is as much as 90%. There are three genes that can cause autosomal dominant hypercholesterolemia, namely low-density lipoprotein receptors (LDLR), apolipoprotein B (APOB), and proprotein convertase subtilisin / kexin type 9 (PCSK9). Apart from these three genes, hypercholesterolemia also causes mutations in LDLRAP (low-density lipoprotein receptor protein adapter) which is also known as autosomal recessive [14].

Figure 3. Morphological Sprague Dawley rat of normal and dyslipidemia

CONCLUSION

The making of dyslipidemia animal model on Sprague Dawley rat has been successful after given a high fat goat diet formula according to body weighing of morphological dyslipidemia rat and hypercholesterol sufferers with increased levels of total cholesterol, triglycerides, and LDL compared to control rat.

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