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Effect of High Fat Diet on Histopathological Appearance of Pregnant Wistar Rat's Liver

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Abstract

Indonesia is facing increased consumption of calorie-dense foods, such as fast food, among its society. Changes in diet and physical activities towards unhealthy ones can result in an imbalanced lifestyle, leading to various consequences, including hyperlipidemia. Pregnant women experience significant increases in plasma concentrations of lipids, lipoproteins, and apolipoproteins. Excessive cholesterol levels in pregnant women will result in the formation of fat in the liver. The purpose of this study was to determine the effect of a high-fat diet to pregnant Wistar rats (*Rattus norvegicus*) on the histopathological features of the liver. A true experimental with post test only control group design was applied in this study, which was conducted from August 2020 to November 2021. Fourteen (14) pregnant female rats were divided into two groups. The first group of rats was fed with a high-fat diet, while the second group, which was the control group, was given a standard diet. The high-fat diet used was 3 mL/rat/day beef brain. After the rats delivered their litters, the rats' livers were retrieved, then examined histopathologically under a microscope. This research was conducted from No fatty liver was identified in the control group or the treatment group. Hence, a high-fat diet does not affect the liver histopathological features in pregnant Wistar rats.

Keywords: High-fat diet, hepar cells, pregnant

Introduction

Changes in people's lifestyles and eating habits that have an impact on a diet high in saturated fat, high carbohydrate, and low in fiber can cause hyperlipidemia. The modern diet contains a lot of triglycerides and cholesterol, accompanied by a high intensity of eating, consuming fewer vegetables and fruits, obesity, lack of exercise and smoking make cholesterol levels in the blood very difficult to control, this can lead to hypercholesterolemia conditions.[1] Hyperlipidemia is characterized by an increase in blood lipid levels (fats or fat-like compounds), mainly cholesterol and triglycerides.[2] Excessive cholesterol levels in the body will result in fat accumulation in the liver.[3]

In pregnant women, there is also a significant increase in the concentration of lipids, lipoproteins, and apolipoproteins in plasma. This fat storage occurs mainly in mid-pregnancy. This fat will later be distributed in the last trimester through the placenta when maximum fetal growth is in line with the need for essential fatty acids requirement. This energy storage mechanism could theoretically protect the mother and fetus during starvation or strenuous physical work.[4]

Hyperlipidemia in pregnant women is the most consistent and striking change that occurs in fat metabolism during pregnancy.[4] Consumption of high fat and cholesterol, during pregnancy, can increase the risk of gestational diabetes in pregnant women.[5] A high-fat diet and high carbohydrate intake affect enhancement of triglycerides, HDL, and LDL levels.[6] The study results reported that cases of severe dyslipidemia in Indonesia with total cholesterol levels of 240 mg/dl were primarily found in Jakarta and Padang (> 56%). At the same time, in other big cities such as Bandung and Yogyakarta, it reached 52.2% and 27.7%, respectively.[7]

Fatty liver is an excessive accumulation of fat in liver cells.[8] Fatty liver is caused by disorders of fat metabolism in the liver, abnormalities

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in hepatocytes, increased fat, fatty acids, or carbohydrates exceeding the liver's metabolic capacity.[9] Defined as non-alcoholic fatty liver if ≥5% steatosis is present in the absence of a secondary cause of fat accumulation in the liver.[10]

According to various studies, the exact prevalence of non-alcoholic fatty liver is unknown, but it ranges from 3% to 24%. Non-alcoholic fatty liver is a prevalent liver disease in the United States, affecting about 20% of adults. In other countries, the prevalence ranges from 10% to 24%. In the obese group, the prevalence increased from 57% to 74% and 25% to 75% in obese people with diabetes. This prevalence tends to increase over time due to the increasing prevalence of overweight and obesity.[11] In the research of Hasan et al. with a large enough sample, the prevalence of non-alcoholic fatty liver was 30.6%, with risk factors for obesity, diabetes mellitus, and hypertriglyceridemia. The cases tend to increase along with changes in diet and lifestyle.[12] This study aimed to determine the effect of giving a high-fat diet to pregnant Wistar rats (Rattus norvegicus) on the histopathological features of the liver.

Methods

The type of this research is true experimental with a post-test only control group design, namely the design used to measure the treatment in the experimental group by comparing it with the treatment in the control group. This research was conducted at the Animal House Building, Faculty of Medicine, Andalas University to maintain and treat experimental animals and the Anatomical Pathology Laboratory, Faculty of Medicine, Andalas University for histopathological examination of the liver. This research was conducted from August 2020 to November 2021.

The population used was female rats with Wistar Albino Strain (Rattus Novergicus Strain Wistar Albino) obtained from the Integrated Laboratory of the Faculty of Medicine's experimental animal rearing unit at Andalas University. The sample used was female rats with Wistar Albino Strain (Rattus Novergicus Strain Wistar Albino) aged 2–3 months with 160–240 grams of body weight. Its normal range of rat's body weight.

Determination of the sample size based on the World Health Organization (WHO) criteria is five rats, the minimum number of samples for each group for experimental research on experimental animals. To prevent dropouts in the middle of the study because the rats died or were sick, the researchers took seven rats per group. This study consisted of 2 sample groups, namely a control group with standard feeding and a treatment group with a high-fat diet, so 14 rats were needed.

The data could not be analyzed statistically because the results of the study did not show any difference in the histopathological features of the liver between the treatment group and the control group. This research is part of the research of Dr. dr. Desmawati, M.Gizi entitle "The Effect of High Fat and Antioxidant Intake During Pregnancy on SOD Levels and Telomere Length in White Wistar Rats" has received approval from the Health Research Ethics Committee of Faculty of Medicine, University Andalas with number 354/UN.16.2/KEP-FK/2021

Results

Normal hepatocyte cells were found in the

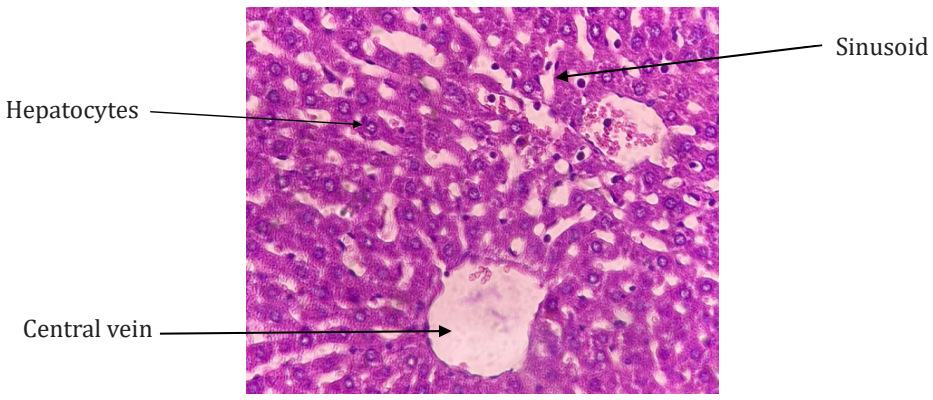

Figure 1 Histopathological Picture of Control Liver with 40x10 Magnification

Table Effect of High Fat Diet on Pregnant Wistar Rats (Rattus norvegicus) on Histopathological Appearance of the Liver

Group	No Sample	Percentage of Liver Cells That are Fat		
		Inflamation	Balloning	Steatosis
Control	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
High fat diet	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0

Percentage of liver cells that are fatty

histopathological picture of the control rats' livers. Hepatocyte cells appear with a clear nucleus and cytoplasm, no vacuolization, and a normochromic nucleus. The central vein and hepatic sinusoids appear white, indicating the absence of congestion.

On the histological picture of the liver of rats fed a high-fat diet in the form of the mashed beef brain at a dose of 3 mL/head/day during pregnancy until delivery, the hepatocyte cells were also normal. There is no fatty liver or inflammation in Figure 2.

Based on the results of the study in Table, it was found that there was no difference between the histopathological features of the liver in rats not fed a high-fat diet and rats fed a high-fat diet.

Discussion

Normal hepatocyte cells were found in the histopathological picture of the control rats' livers. Figure 1 shows hepatocyte cells with a clear nucleus, no vacuolization, clear cytoplasm, and normochromic nuclei. The central vein and hepatic sinusoids appear white, indicating the absence of congestion.

On the histological picture of the liver of rats fed a high-fat diet in the form of the mashed beef brain at a dose of 3 mL/head/day during pregnancy until delivery, the hepatocyte cells

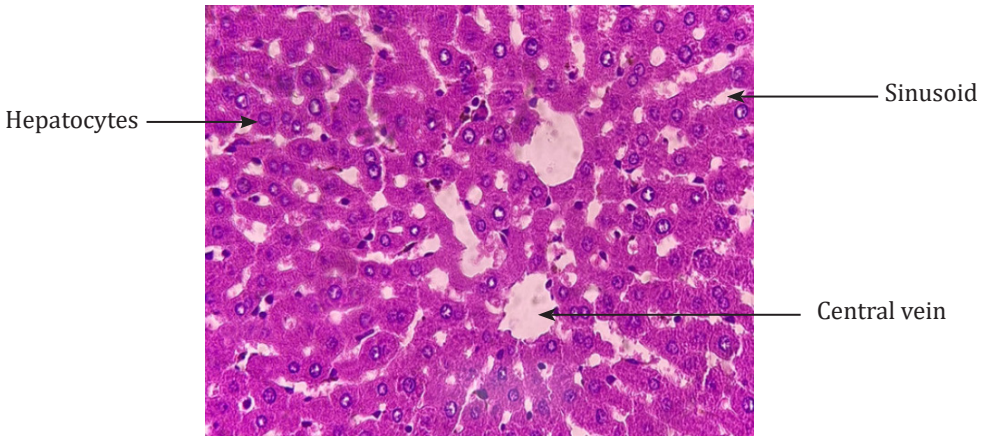

Figure 2 Histopathological Picture of the Liver Fed a High-Fat Diet with a Magnification of 40x10

were also found to be expected. There is no fatty liver or inflammation in Figure 2.

The absence of liver fat formation can be influenced by the dose of the cow's brain and the length of time it is given. It was given a cow's brain as much as 3 mL/rat/day is insufficient to cause fatty liver in pregnant rats. This dose is obtained by calculating the conversion of a high-fat diet in humans. When viewed from the mother's needs during pregnancy in humans, there is an increase in the need, which is around 180 kcal/day in the first trimester and 300 kcal/day in the second and third trimesters. Thus, the need for protein, fat, vitamins, and minerals will also increase during pregnancy.[13] Giving a high-fat diet during pregnancy with a span of 21 to 28 days has not been able to cause fatty liver. This is presumably because fatty liver disease is a chronic disease with an intermediate course of the disease.

Based on the results of study, it was found that there was no difference between the histopathological features of the liver in control and treatment group rats. In this study, there was an increase in body weight in rats that were not given a high-fat diet or in rats that were given a high-fat diet. Another result of this study, which measured total cholesterol levels, showed an increase in total cholesterol in rats fed a high-fat diet with an average of 142.57 mg/dL compared to the control group, which had an average total cholesterol level of 80.43 mg/dL. Unfortunately, in this study, it was not known what the baseline cholesterol level was in rats before pregnancy. This is in line with Priambudi's, which induced hypercholesterolemia by using cow brains as much as 2 mL/rat/day for 28 days.[14]

In Wahyuni study, it was found that there was no relationship between total cholesterol levels and body mass index. Increased total cholesterol levels can be caused by the consumption of foods that contain lots of cholesterol. This means that high cholesterol is not always influenced by obesity, but is caused by other factors such as diet. In addition, the increase in total cholesterol levels in the blood is also influenced by smoking, consumption of drugs, and exercise. Total cholesterol measured in blood circulation, related to hyperactivity of apolipoprotein synthesis, obesity-related with increased fat tissue (triglycerides deposition). [16]

The increase in total cholesterol in the blood that occurs in rats has not induced fatty liver in rats. It can be influenced by various things, such as the dose and duration of administration. The administration of a high-fat diet in this study while the rats were pregnant or 21 days had not been able to affect hepatocytes. One of the reasons for this is the long life span of hepatocytes, which is 200–400 days in rats and mice. For this reason, a longer intervention is needed to see the effect of a high-fat diet on the histological picture of hepatocytes.

Cholesterol from food will be absorbed from the intestine and, together with other lipids, including cholesterol synthesized in the intestine, will be incorporated into chylomicrons and VLDL.[17]

Furthermore, chylomicrons will release triglycerides in adipose tissue. The remaining chylomicrons will carry cholesterol to the liver, which is excreted in the free form or as bile acids into the bile. The remaining cholesterol will become one with VLDL. VLDL will transport cholesterol into the bloodstream to maintain cholesterol balance in lipoproteins and membranes. VLDL-containing cholesterol is metabolized into IDL and LDL. LDL is a mediator of the entry of cholesterol esters into the tissue. HDL will move cholesterol from the tissues to the liver to be converted into bile acids, which is known as the reverse cholesterol transport process.[17] Some HDL cholesterol can be transferred to VLDL and chylomicrons and then reprocessed. All cholesterol excreted from the body must enter the liver and be excreted in the bile as cholesterol or as cholic acid in bile salts.[17]

Dwinanda et al. conducted a study on male Wistar rats induced by a hyper cholesterol diet made from two ml of pork oil and one gram of crushed boiled quail egg yolk. The diet was given by induction using a gastric probe for 14 days. On histopathological examination of the liver, it was found that the liver cells near the central vein were swollen due to fat degeneration and the sinusoids looked irregular.[3]

In Hahn's research in Heriansyah, giving a high-fat diet to rats caused an increase in total cholesterol levels. It caused a hypercholesterolemic condition after being given a high-fat diet for five weeks, but there was no decrease in HDL levels. In Heriansyah's research, there was a rapid decrease in HDL levels because of the addition of cholic acid-induced it. The addition of cholic acid to a high-fat diet can alter the appearance of plasma lipoproteins by increasing plasma LDL levels and decreasing plasma HDL levels. Thus cholic acid is considered a lowering of HDL cholesterol levels.[6]

The first hit's pathogenesis of fatty liver occurs due to obesity, a high-fat diet, and insulin resistance (IR) seem to be responsible

for the deposition of triglycerides (TG) in the hepatocytes, a pre-requisite for hepatocyte injury.[18] The increase in triglycerides in diabetic people due to the increase in free fatty acids from adipose tissue also causes triglycerides to be hydrolyzed. Triglycerides are synthesized from the esterification of glycerol with three fatty acid molecules. The amount of triglyceride synthesis from fatty acids depends on the availability of fatty acids. Fatty acids derived from lipolysis of fatty acids, diet, and de novo lipogenesis (DNL) are then used to synthesize triglycerides.[19] In fact, the entry point of fatty liver is excess energy, not only from fat or cholesterol food sources but also from excess carbohydrates. A high intake of carbohydrates causes insulin resistance, then decrease control of HPL dan LPL activity for maintaining homeostasis. In this study, after measuring total cholesterol levels, there was an increase, but the levels of triglycerides, LDL, and HDL were not measured in this study.

Fatty liver occurs due to an increase in the content of fatty acids and triglycerides in the liver and disturbances in beta-oxidation of fatty acids in hepatocyte cells. A liver with excess fat content will be more susceptible to stressors such as ROS, adipokines, and cytokines than a normal liver. The ability of fatty liver cells will be disrupted.[20]

The limitation of this study is that the dose of a high-fat diet given to experimental rats only had one dose variant, namely 3 mL/head/day. Giving various doses can see how the dose affects the histopathological picture of the liver. In this study, the rats' triglyceride, HDL, and LDL levels were not measured. Knowing triglyceride levels can further strengthen the results of the study because fatty liver begins with an increase in the fatty acids and triglycerides in the liver. In this study, it was also possible to examine MDA levels to determine how lipid peroxidation in the liver is. The increase in MDA is a marker of an increase in lipid peroxidation in the liver which can continue to damage cells and even lead to irreversible cirrhosis.

In conclusion, no histopathological differences in rats in the control group and the high-fat diet group. Further studies are needed regarding the length of time and dose of a high-fat diet in pregnant rats on the microscopic picture of liver cells.

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