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The Effects of High Fat Diet on the Liver of the White Rat Model Obesity

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Abstract

BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease with the manifestation of over-accumulation of fat in the liver.

AIM: The purpose of this study was to assess the degree of occurrence of steatosis in rats induced by a standard diet, a high-fat diet, and a modified high-fat diet.

METHODS: This study used 18 white rats of the Wistar strain, divided into three groups, and fed for 9 weeks. Before feeding, all rats were measured their body weight, abdominal circumference, and body length. We measured body weight every week, while body length and waist circumference were measured every 2 weeks. After 9 weeks of diet, all rats were subjected to injection of Ketamine and examined for metabolic markers and histopathological examination of liver organs**.**

RESULT: There was an increase in body weight of rats in the three groups with the average percentage increase in body weight in the three groups of rats before and after being fed a diet for 9 weeks found in Group 1 29.19% 1 (187−264.40 g), Group 2 by 19.12% (219.33−275 g), and Group 3 24.53% (213.33−275 g). Steatosis in Group 1 was 57.50% of hepatocytes containing macrovesicular fat droplets and called Grade 2 (moderate). In contrast, with a high-fat diet, steatosis occurred around 93.33%−95% of hepatocytes containing macrovesicular fat droplets and called steatosis Grade 3 (severe).

CONCLUSION: The percentage of hepatocytes that had steatosis in obese rats induced by a high-fat diet was more significant than in obese models induced by a standard diet.

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease associated with metabolic disorders, such as type 2 diabetes mellitus, hypertension, dyslipidemia, and obesity [1], [2]. NAFLD exists on a spectrum from simple steatosis to steatohepatitis, characterized by steatosis, lobular inflammation, hepatocellular swelling, and liver injury resulting in necroinflammation with fibrosis [3], [4]. Several factors include the occurrence of steatosis, such as diet and lifestyle [5]. Thus, briefly, lipid accumulation leading to the fatty liver may be mediated by several mechanisms: Increased free fatty acids (FFA) from exacerbation of lipolysis or intake of high-fat content, decreased FFA oxidation, increased de novo hepatic lipogenesis, and decreased secretion of very low-density lipoprotein (VFA and VLDL) and triglycerides in the liver [6].

Diet plays a crucial role in developing the nonalcoholic fatty liver disease (NAFLD) [7], [8]. Dietary lipids, such as cholesterol and triglycerides (TGs), have been shown to exacerbate adipose tissue inflammation and non-alcoholic fatty liver disease in animal models [9], [10]. Histologically, the main feature of non-alcoholic fatty liver disease is benign steatosis, developing in 6%–55% of patients with nonalcoholic steatohepatitis (NASH) [11]. Apart from steatosis, NASH is mainly characterized by the presence of inflammatory cells in the liver parenchyma cells, activation of resident macrophages (Kupffer cells [KCs]), and the fibrotic process resulting in activation of hepatic stellate cells (HSCs) [12]. Based on previous research, obesity is closely linked with nonalcoholic fatty liver disease (NAFLD), and HFD can induce obesity and steatosis [13]. While a normal liver has oxidative stress-resistant, fatty liver is vulnerable to oxidative stress. As a result of obesity resulting in fatty liver, inflammation and also liver fibrosis, and steatohepatitis [14].

Our research aimed to determine the level of steatosis in Wistar rats given a standard diet, a high-fat diet, and a modified high-fat diet and evaluate weight gain and metabolic markers in the three groups of rats.

Materials and Methods

Animal models

This research was conducted with an accurate experimental study with a post-test control group design. The research was conducted at the Pharmacology Universitas Sumatera Utara, with the approval of Law No. 726/KEP/USU/2021 ethics committee of experimental animals. All animals were cared for by the principles and guidelines of animals. The experimental animal samples were white male rats of the Wistar strain, aged 10 weeks, weighing 150−250 g, kept in separate cages in the Pharmacology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. The treatment of rats was started by adapting (acclamation) rats for 7 days in a cage with a constant temperature, a standardized 12/12 h light/dark cycle with a laboratory atmosphere for observation of health and changes in behavior during the adaptation period given standard food, animal feed, and drinking-water ad libitum [15].

Animal intervention

In this study, using 18 rats after acclimatization, rats were randomly selected (randomized) and then grouped into groups by feeding a standard diet (Group 1), a high-fat diet (Group 2), and a modified high-fat diet (Group 3). Each group of rat samples consisted of 6 rats/cage with a cage size of 50 × 40 cm = 200 cm^2. The composition of the feed given to each group of rats is as shown in the Table 1. We measured weight, body length, and abdominal circumference in all groups before diet administration, and every week. We measured the body weight. We measure the body length and abdominal circumference once every 2 weeks. We were feeding the diet for 9 weeks.

Table 1: Marker metabolic and the function of kidney

Parameter	Group 1		Group 2		Group 3	
	Mean	SD	Mean	SD	Mean	SD
Blood Sugar Level	216.27	119.02	255.80	46.46	284.58	65.04
Cholesterol	42.85	2154	67.17	8.67	68.35	16.72
Urea	41.66	21.35	22.45	4.62	21.99	5.49
Creatinine	2.76	1.35	3.32	0.11	3.34	0.09

Values are expressed as mean ± SD (n = 18)

Biomarker analysis

Examine biomarkers such as metabolic markers (BSL and cholesterol levels) and kidney function (urea and creatinine) by taking blood from the abdominal aorta after executing all groups of rats with an injection of Ketamine 75 mg/kg BW, i.p.

Histopathologic and immunohistochemistry analyses

The liver tissue, fixed in 10% (v/v) neutral-buffered formalin, was further embedded in paraffin at room temperature. Excision of liver tissue in the right lobe. Thin sections (4 mm) from paraffin blocks were processed for histology. The tissue was stained with hematoxylin-eosin according to the routine technique applied at the Department of Medical Pathology of Universitas Sumatera Utara. Histopathologists reviewed by utilizing the Nonalcoholic Steatohepatitis Clinical Research Network scoring system by observing the liver cells with 400 x magnification. The assessment carried out includes steatosis, necrosis, and whether the central vena cava is congested or not. The degree of steatosis was assessed irrespective of the experimental groups as previously described (16), based on the percentage of hepatocytes containing macrovesicular fat droplet, Grade 0, no steatosis or steatosis <5%, Grade 1 (mild), 5−33% of hepatocytes –containing macrovesicular fat droplet; Grade 2 (moderate), 33−66% of hepatocytes containing macrovesicular fat droplets; and Grade 3 (severe): >66% of hepatocytes containing macrovesicular fat droplets.

Statistical analysis

Before carrying out statistical tests, all sample variables were tested for normality the Shapiro Wilk test and found $p > 0.05$, meaning that the sample variables were typically distributed, tested by the ANOVA test and if not normally distributed the Kruskal–Wallis test. Values are expressed as the arithmetic mean ± standard error of the mean (SEM). One-way ANOVA was used to determine the main effects of diet (St vs. HF diet vs. HF modification diet) and their interaction. Tukey's multiple comparison test was used to determine differences between all experimental groups whenever identified a significant interaction. The difference was considered statistically significant when $p < 0.05$.

Results

In this study, there was an increase in body weight in three groups of rats every week. Still, the average increase in body weight in these three groups was not significantly different, where the average increase in body weight in the group of rats fed a diet before and after dieting for 9 weeks by 29.19% in the first group of rats (187−264.40 g), in the second group of 19.12% (219.33−275 g), and by 24.53% (213.33−275 g) in the third group for 9 weeks, Figure 1.

Feeding diet for 9 weeks in the three groups of rats increased abdominal circumference. Still, the addition of abdominal circumference in the three groups of the rats showed no significant difference in the 2nd week. Still, in the 4th week, there was a significant difference in the addition of abdominal circumference in Group 1 and Group 3 ($p < 0.05$). In contrast, in group 1 and group 2,

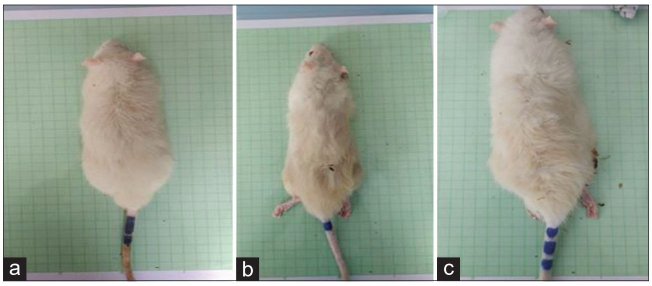


Figure 1: (a) Group 1, (b) Group 2, (c) Group 3

there was no significant difference (p > 0.05); at the sixth and 8th weeks, there was no significant difference in the addition of abdominal circumference in the rat Group 1, Group 2, and Group 3 (p > 0.05).

The increase in body length (BL) in the rat group occurred in the 2nd week, and there was a significant increase in BL in Group 1 and Group 3 (p < 0.05), but there was no significant difference in body length gain in Group 2 (p > 0.05). In the 4th, 6th, and 8th weeks of body length increase, there was a significant difference in Group 1 with Group 2 and Group 3 (p < 0.05), but there was no increase in body length. There was no significant difference in Group 2 and Group 3 (p > 0.05)

We examined metabolic markers by measuring blood sugar level (BSL)-and cholesterol levels and examining kidney function such as urea creatinine. BSL examination with the GOD/PAP method and cholesterol examination with the CHOD/PAP method. And with the ANOVA test, there was no significant difference in BSL in the three groups of rat p > 0.05. However, there was a significant difference in cholesterol levels in Group 1 with Groups 2 and 3 p < 0.005, while in Group 2 and Group 3, there was no significant difference (p > 0.05). On examination of the kidney function, there was a significant difference in urea levels in Group 1 and Group 3 (p < 0.05), but there was no significant difference in urea levels in Groups 1 and 2 (p > 0.05), and there was no difference in creatinine levels in the three groups of rats (p > 0.05), we can see the Table 1.

Table 2: The weight of the liver of the group (gram)

	Mean	SD
Group 1	8.68	4.26
Group 2	8.94	1.54
Group 3	10.01	1.63

In the three groups of rats, the average weight of the liver in Group 3 was heavier than Group 1 or Group 2, as shown in the Table 2. Our study showed that the adipose layer of the abdomen has a higher average weight in rat Group 2 and Group 3 than rat Group 1, we can see the Table 3 and Figure 2.

Table 3: Abdominal adipose tissue of the groups (gram)

	Mean	SD
Group 1	6 06	0.80
Group 2	9.53	0.90
Group 3	9.54	0.91

In this study, there was a significant difference in the occurrence of steatosis in groups 1 with groups 2 and 3 (p < 0.05), but there was no significant difference in the process of steatosis in groups 2 and 3 (p > 0.05). The average steatosis process in group 1 was around 57.50%, while in group 2, it was 93.33% and in group 3 was around 95%. In the process of steatosis groups, 2 and 3 followed by process of congestive and necrosis compared to group 1 (Table 4).

Table 4: Microscopic state of the liver of obese model rat

Group	Steatosis (%) Mean ± SD
Group 1	57.50 ± 29.28
Group 2	93.33 ± 2.58
Group 3	95 ± 3.16

Discussion

In this study, the three groups of rats experienced weight gain every week until the 9th week. The process of steatosis in Group 1 experienced steatosis of 57.50%, which was moderate grade steatosis. In contrast, Groups 2 and 3 had severe grade steatosis; another study in animals given High carbohydrates for 24 weeks indicates obesity with severe hepatic steatosis [16], [17], [18]. The occurrence of fatty liver (steatosis) in this study showed a significant difference in the group of rats fed a high-fat diet compared to the group of rats fed a standard diet wherein the group of rats fed a standard diet, hepatocyte cells were still partially normal. Other researchers say that steatosis is caused by consuming a high-fat diet [19].

This study showed that the average weight gain during dieting in the first group of rats was 29.19% (187−264.40 g), more significant than in the other two groups, namely, the second group of 19.12% (219.33−275 g) and 24.53 g in the third group. % (213.33−275 g), this is in line with other studies that found that a high-fat diet did not cause weight gain but caused a more significant increase in liver fat than controls [20].

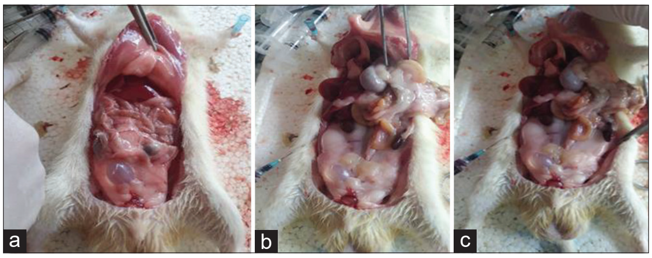
In addition to, hepatocyte cells experiencing steatosis, central venous congestion was also found in this study, and there were also necrotic cells. This process could be distinguished in rats with a regular and high-fat diet. Fatty liver is characterized by excessive accumulation of lipids due to excessive consumption of fat or carbohydrates [21]. Many studies have been conducted on obese rat models with various dietary compositions such as Feeding high fat and high cholesterol diet for 10 weeks showed an increased significance of the weight, epididymal fat, and steatosis. The study showed that diet with sphingomyelin attenuates hepatic steatosis and adipose tissue inflammation in high-fat-diet-induced obese mice [22]. In this study, we also found that the average liver weight was higher in Group 3 (10.01 g) compared to Groups 1 and 2, where a high-fat diet caused a more considerable liver weight than a regular diet; this is by the other study in the results of the study found that liver weight was more significant in the HFD group compared to the Low Fat Diet. And according to the other study, giving HFD to Sprague–Dawley rats for 12 weeks showed hepatocyte cells in liver tissue of different sizes of lipid droplets in the cytoplasm compared to controls, which did not have any characteristics associated with steatosis [23]. Administration with HFD for 12 weeks resulted in steatosis with a 6.5-fold increase in steatosis score compared to the CD group, which showed normal liver morphology [24].

Another study established a rat model of nonalcoholic fatty liver disease in Sprague–Dawley rats by giving a high fat diet for 10 weeks, and this study also stated that Animal models of NAFLD can be divided into two types: Those caused by genetic mutations and those induced by dietary or pharmacological modifications [23]. Another study suggested that the degree of steatosis was exacerbated by the induction of dietary HFD in combination with ethanol, indicating the involvement of ethanol in the development of steatosis. This study by Souza also showed that oxidative stress could also worsen the state of steatosis [25].

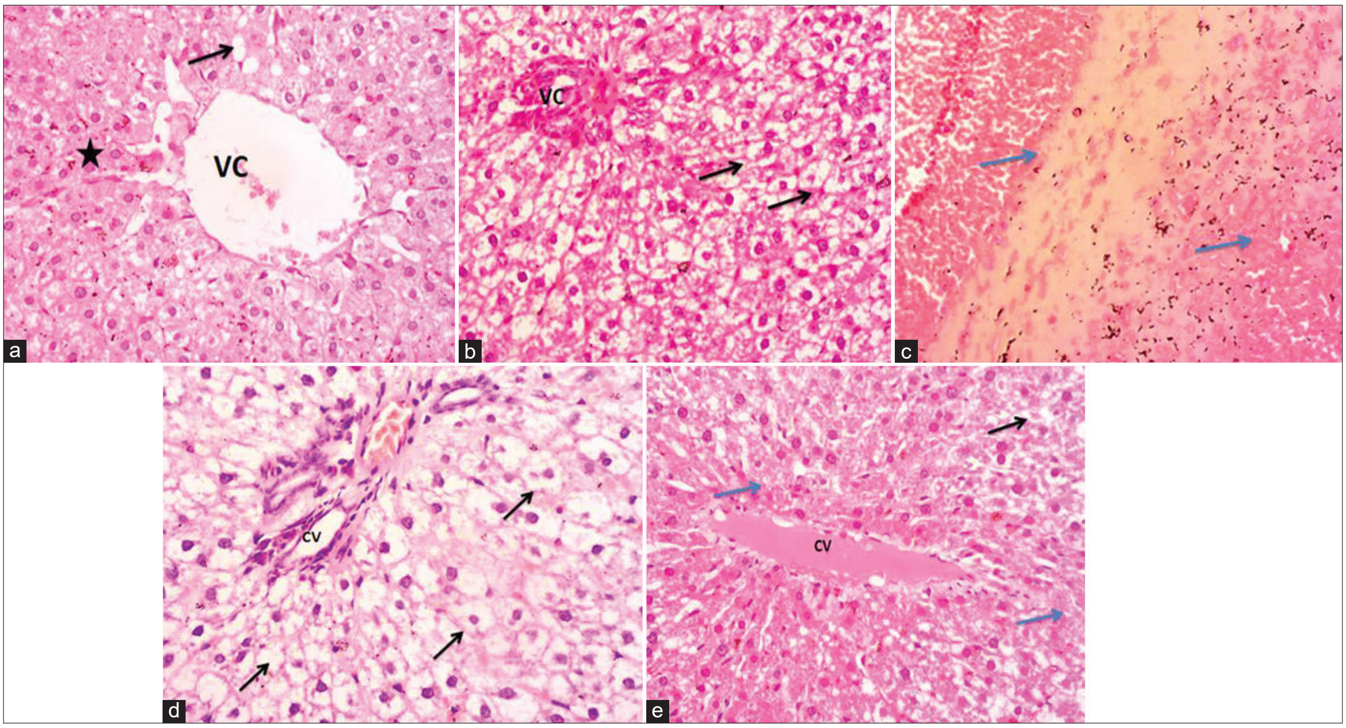
In the study, we conducted on mice induced with a high-fat diet and a modified high-fat diet, in addition to the occurrence of severe grade steatosis, it also resulted in necrosis of hepatocytes and also central vein congestion, in contrast to mice induced with a standard diet, which still showed some hepatocyte cells were still normal, this is by the study that SD rats with regular diet showed that liver tissue from mice with normal diet showed standard structure, without degeneration and necrosis of liver cells. However, the liver tissue of animals from a group of mice fed a high-fat diet showed swollen liver cells, scattered cytoplasm, and large fat droplets were visualized, showing accumulation of liver fat and signs of vacuolar degeneration and necrosis [26].

In another study, giving HFD to male rats with a composition of 60% energy from fat (lard), 20% from carbohydrates, and 20% protein for 10 weeks caused steatohepatitis [27]. And feeding high-fat–high-fructose (HFHFR) diet at male Wistar developed mildly overweight, associated with increased adipose tissue weight, hepatic steatosis, hyperglycemia, and hyperinsulinemia after 8 weeks of HFHFR diet [28].

In our study that we conducted on three groups of mice where steatosis occurred in these three groups



AQ3 *Figure 2: Abdominal adipose tissue in ND rats (a), HFD (b), and HFD modification (c)*



AQ3 *Figure 3: Histopathological examinations of liver tissues with oil red O staining. (a-e) Represent oil red O-stained liver sections of rats in the groups ND (a), HFD (b and c) and HFD modification (d and e). Some hepatocytes are still normal (black star) and some have steatosis (black arrow). VC=venous centralis (a), hepatocyte cells mostly undergo steatosis (fatty liver, black arrow) and necrosis (blue arrow). Central Vein (CV) some are congested (b, c), hepatocyte cells have steatosis (fatty liver, black arrow), some are also necrotic (blue arrow). Central Vein (CV) some have congestion (d and e). HE, image was taken 400× magnification*

but based on the degree of steatosis based on the non-alcoholic steatohepatitis clinical research network scoring system by Kleiner *et al*. 2005, the occurrence of steatosis in group 1 on average occurs in Grade 2 or grade moderate which the occurrence of steatosis in liver cells is around 34−66% while in Groups 2 and 3 the occurrence of steatosis in Grade 3 or severe grade occurs steatosis in liver cells > 66%. This showed that a high-fat diet causes a greater degree of steatosis in liver cells than a diet containing less fat (Figure 3).

Conclusion

A consumed high-fat diet does not fully increase body weight but results in a higher percentage of hepatocyte cells experiencing steatosis than a standard diet.

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