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The Effects of High-Fat Diet and CCl_4 Administration on Liver Function and Lipid Profile in Non-Alcoholic Fatty Liver Disease Rat Model

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ABSTRACT
Non-alcoholic fatty liver disease (NAFLD) is often encountered in the field of hepatology. The disease has a broad spectrum ranging from non-inflammatory fat-accumulating macrovesicles (simple steatosis) and develops to fibrous and cirrhosis. The rate of NAFLD is 15-30% in western countries and around 5-18% in Asia. This prevalence continues to grow worldwide following the trends in unhealthy lifestyles and diets. A high-fat diet (HFD) can cause fat accumulation in hepatocytes and adipocytes. NAFLD is predicted to be the main cause of morbidity and mortality associated with liver disease. Therefore, it is necessary to conduct studies that can provide solutions to this problem, either using experimental animal models that reflect the pathogenesis of NAFLD or through clinical studies in humans. NAFLD animal models can be induced in several ways, such as through HFD treatment and the use of carbon tetrachloride (CCl_4). This study was focused to figure out the influence of combined HFD intervention and CCL_4 administration on liver function (aspartate aminotransferase/AST and alanine aminotransferase/ALT levels) and lipid profile (cholesterol and triglycerides levels) as the metabolic parameters in the NAFLD rat model. Twenty-eight Sprague Dawley rats (body weight between 160-210 grams, 8-10 weeks old,) were split within three groups, namely (I) control group, rats were given a standard food (RatBio®); (II) the HFD group, rats were given a high-fat diet (self-made pellets with a composition of 30% fat, 11% protein, 9% fiber, and 14% water) intervention; and (III) the HFDCCl_4 group, rats were given HFD intervention and administering intraperitoneal CCl_4 (0.5 mg/ml/kg body weight two times a week). Both diet and chemical interventions were carried out for 12 weeks. Rats were weighed every week. Blood samples were taken to measure the liver function parameters (AST and ALT levels) and the lipid profiles (cholesterol and triglycerides levels) pre- (week 0) and post- intervention (week 12). All groups displayed an increase in body weight after the intervention (p=0.000). However, the average weight gain between the three groups did not differ (166.88 + 33.87 g, 188.44 + 30.39 g, and 173.30 + 30.75 g, respectively). The pre-intervention and post-intervention AST and ALT levels in all groups were still within normal limits and did not show significant mean changes (p=0.120 and p=0.811, respectively). The pre-and post-intervention lipid profile (cholesterol and triglyceride levels) in all groups showed significant differences in means (p=0.023 and p=0.036, respectively). From the posthoc test, it was found that the lipid profile of the HFD-CCl_4 group was different from the other groups. In conclusion, a NAFLD modeling with a combination of HFD intervention (30% fat content) and CCl_4 administration for 12 weeks gave better results than NAFLD modeling only with HFD intervention.

Keywords: *Animal model, NAFLD, liver function, lipid profile.*

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the main element of liver disease and its prevalence keeps increasing worldwide. NAFLD has a broad spectrum that represents non-inflammatory fat-accumulating macrovesicles (simple steatosis) and lobular inflamed in the liver of non-alcoholic individuals [1]. The prevalence of NAFLD is 15-30% in western countries and around 5-18% in Asia [2]. The estimated incidences of NAFLD in western countries and Asia are 52.3 per 1000 person/year and 28 per 1000 person/year respectively. NAFLD prevalence increases with age, with the highest incidence in men aged 40 to 65 years. The risk factor for increased prevalence of NAFLD is the metabolic problems as type II diabetes mellitus, dyslipidemia, obesity, and arterial hypertension. Western food that is high in protein and fat is known as a contributing factor to both NAFLD and obesity. A high-fat diet (HFD) may cause obesity and fat accumulation in hepatocytes and adipocytes [3-4].

Animal models are often used to facilitate studies on NAFLD. This modeling can be carried out by dietary intervention, chemical administration, or genetic modification [5]. The use of dietary intervention could give a more similar metabolic profile as in human NAFLD. Dietary interventions in NAFLD modeling are often conducted with either a high-fat diet (HFD), a choline and methionine deficiency diet, or a fructose diet. However, the outcome of this intervention was influenced by gender, strain, and species of the experimental animal used [6]. Diet intervention tends to take a longer time to induce NAFLD, as opposed to the more rapid chemical intervention. Carbon tetrachloride (CCl_4), streptozotocin, and diethylnitrosamine are often used to chemically induce NAFLD. CCl_4 is a xenobiotic that can induce lipid toxicity through lipid peroxidation. In the endoplasmic reticulum of the liver, CCl_4 is broken down by cytochrome (CYP2E1) into trichloromethyl free radicals (CCl_3) that cause hepatotoxic manifestations in the form of inflammation, fat infiltration, centrilobular necrosis, fibrosis, and cirrhosis [7]. Therefore, combining HFD and chemical intervention to induce NAFLD is preferable. The use of CCl_4 will accelerate the influence of an HFD in experimental animals so that NAFLD is formed more rapidly. This modeling can provide an overview of metabolic syndrome and pathogenesis of NAFLD as in humans with a shorter development period [5].

This preliminary study to induce NAFLD in rats using a combination of HFD and CCl_4 administration was meant to investigate the effects and the success rate of the combined intervention in experimental animals. The liver has many roles and consequently, it requires many tests to assess its function. One of the most common tests to detect liver dysfunction and hepatocyte injury is the measurement of alanine transaminase (ALT) and aspartate transaminase (AST) [8]. The dysregulations of lipid metabolism in the liver include increasing free fatty acids (FFAs) intake to the liver, decreased FFA oxidation, increased fatty acid production, and low production of very-low-density lipoprotein (VLDL). These conditions will lead to intrahepatic fat accumulation, resulting in the development of NAFLD. Therefore, this research was conducted to explore the influence of combined HFD intervention and CCl_4 induction on liver function (ALT and AST levels) and lipid profile (cholesterol and triglycerides levels) as the metabolic parameters in the NAFLD rat model.

2. METHODS

2.1. Time and Place

The study was done in April-July 2021 at the Laboratory of Physiology, Faculty of Medicine, Gadjah Mada University. The experimental method was accepted by the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University (reference number: KE/FK/0408/EC/2021). The experimental protocols comply with the ethical principles of the Declaration of Helsinki and all addendums regarding the humane treatment of animal subjects.

2.2. Experimental Animals

As many as 28 male Sprague Dawley rats (within age 8-10 weeks, body weight between 160-210 grams) were acquired from the Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University. Before the intervention, all rats were acclimatized for seven days to adapt to the new environment. Rats were placed in standard plastic cages (40x20x12 cm^3) with a 12-hour light and dark cycle, the temperature of 24-26 °C, and a humidity of 60-65%. Each cage contains 2 rats. The plastic cage is equipped with a lid made of wire, a floor covered in the husk, a food container, and drinking water for the rats. Experimental animals were treated accordingly to the applicable code of ethics.

Rats were fed with normal food (RatBio®) and drinking water as well during the acclimatization. Rats then split into three groups, namely (I) control group, rats were given the standard food; (II) HFD group, the rats were given daily HFD intervention only (self-made pellets with a composition of 30% fat, 11% protein, 9% fiber, and 14% water); and (III) HFD-CCl_4 group, rats were given daily HFD intervention and intraperitoneal CCl_4 induction of 0.5 mg/ml/kg body weight two times a week. The treatments were carried out for 12 weeks. Rats were weighed weekly to monitor weight gain.

2.3. Standard Diet

The standard diet used was RatBio® produced by PT. Citra Ina Feedmill (Jakarta, Indonesia). This standard diet consisted of 4% fat, 20% protein, 4% crude fiber, 12%

calcium, and 12% water. The diet was provided daily (30 g/rat), and it was increased to 40 g/rat after the bodyweight reached 300 g.

2.4. High-Fat Diet Intervention

The HFD was self-made referring to a study by Ahmed et al. [9] with modifications. The HFD was in the form of pellets, made from lard (800 g), a standard diet mixture (365 g), casein (250 g), vitamins and minerals (60 g), quail egg yolk (10 g), DL-methionine (3 g), sodium chloride (1 ml), and yeast powder (1 g). The results of the proximate test conducted at the Food and Nutrition Laboratory, Universitas Gadjah Mada showed that it had a fat content of 29.84%, 10.76% of protein, 8.67% of crude fiber, and 14.38% of water. The type of fat contained was 100% unsaturated fat with a concentration of 69.431 ng/µl. HFD was given daily for as much as 30 g/rat, and it was increased to 40 g/rat after the bodyweight reached 300 g.

2.5. CCl_4 Administration

CCl_4 (Merck, Germany) with a concentration of 1.59 g/ml was dissoluble in peanut oil with end concentration of 0.5 mg/ml/kg body weight [10]. CCl_4 was injected intraperitoneally to the rats from the HFD-CCl_4 group. Behavioral observations were carried out for at least 30 minutes after administration.

2.6. Weight Measurement

Bodyweight was taken using a digital scale. At the time of weighing, the rats were placed in a box. In each weight measurement, the bodyweight of every rat was recorded twice. Rats were weighed once a week (every Wednesday morning).

2.7. Blood Examination

Blood tests were carried out before and after intervention (week 0 and week 12). The parameters measured were liver function (ALT and AST levels) and lipid profile (cholesterol and triglyceride levels). Blood samples were obtained from the orbital sinus in a volume of 1 ml after rats were anesthetized with ketamine (Ikapharmindo Putramas, Indonesia) 0.1 ml/kg body weight. Blood samples were temporarily stored in lithium-heparin gel tubes. The blood samples were then taken to LPPT Universitas Gadjah Mada. The measurements of AST and ALT levels were carried out using the enzymatic-photometric method. The enzymatic-photometric Cholesterol Oxidase Diaminase Peroxidase Aminoantipyrin (CHOD-PAP) method was used to measure the cholesterol level while the enzymatic-photometric Glycerol-3-Phosphate Oxidase (GPO) method was used to measure the triglyceride level.

2.8. Data Analysis

Statistical Package for the Social Sciences (SPSS) version 26 (IBM®, Chicago, USA) was used for statistical analysis. T-test was performed to analyze pre- and post-intervention data. The normality of the data was tested by the Shapiro-Wilk test. If the data had normal distribution and the variance was the same, the data were tested using ANOVA. Otherwise, the Kruskal-Wallis test was used. Bonferroni posthoc tests were used when the data showed differences significantly ($p < 0.05$). Data were shown in mean ± standard deviation (SD).

3. RESULTS

Table 1 showed before intervention (week 0) and after intervention (after week 12) characteristics of the experimental animals. All groups showed an increase in body weight (p=0.000). However, the bodyweight of post-intervention and average weight gain between the control, HFD, and HFD-CCl_4 groups did not differ (166.88 + 33.87 g, 188.44 + 30.39 g, and 173.30 + 30.75 g, respectively). The post-intervention results showed that AST, total cholesterol, and triglycerides levels showed significant differences in each group. The AST levels of the HFD-CCl_4 group was lower than the HFD

Table 1. Bodyweight, liver enzyme levels, and lipid profile in rats given normal food (control), High-Fat Diet (HFD), and High-Fat Diet combined with CCl_4 induction.

	Pre-intervention			*p* value#	Post-intervention			*p* value#
	Control	HFD	HFD+CCl_4		Control	HFD	HFD+CCl_4	
Body weight (g)	194.1±8.6	185.6±17.9	192.9±16.4	0.462	361±34.5^{d}	374.1±41.4^{d}	366.2±31.8^{d}	0.753
AST (U/L)	158.6±31.9	144 ±32.7	124.8±19.1	0.055	66±6.4^{d}	73.3±8.8^{d}	61.9±7.5$^{b, d}$	0.013*
ALT (U/L)	78.8±15.7	67.9±8.3	70.8±14.3	0.230	115.9±27.8^{d}	108.4±7.5^{d}	116±32.9^{d}	0.772
Cholesterol (mg/dL)	100.4± 11.9	106.1±16.3	98.5±12.5	0.476	72.6±7.7^{d}	91.1±17^{a}	95.9±11^{c}	0.002*
Triglycerides (mg/dL)	108.5± 42.5	77.2±18.3	70.6±10.5^{c}	0.018*	132±22	195.7±105^{d}	224.8±138.7$^{c, d}$	0.031*

Data were presented as mean ± SD; #, *p*-value from one-way ANOVA; AST, aspartate aminotransferase; ALT, alanine aminotransferase; *, significantly different (*p*-value < 0.05); a, significantly different between HFD and control; b, significantly different between HFD+CCl_4 and HFD; c, significantly different between HFD+CCL4 and control; d, significantly different between pre- and post-intervention levels, analyzed with paired t-test.

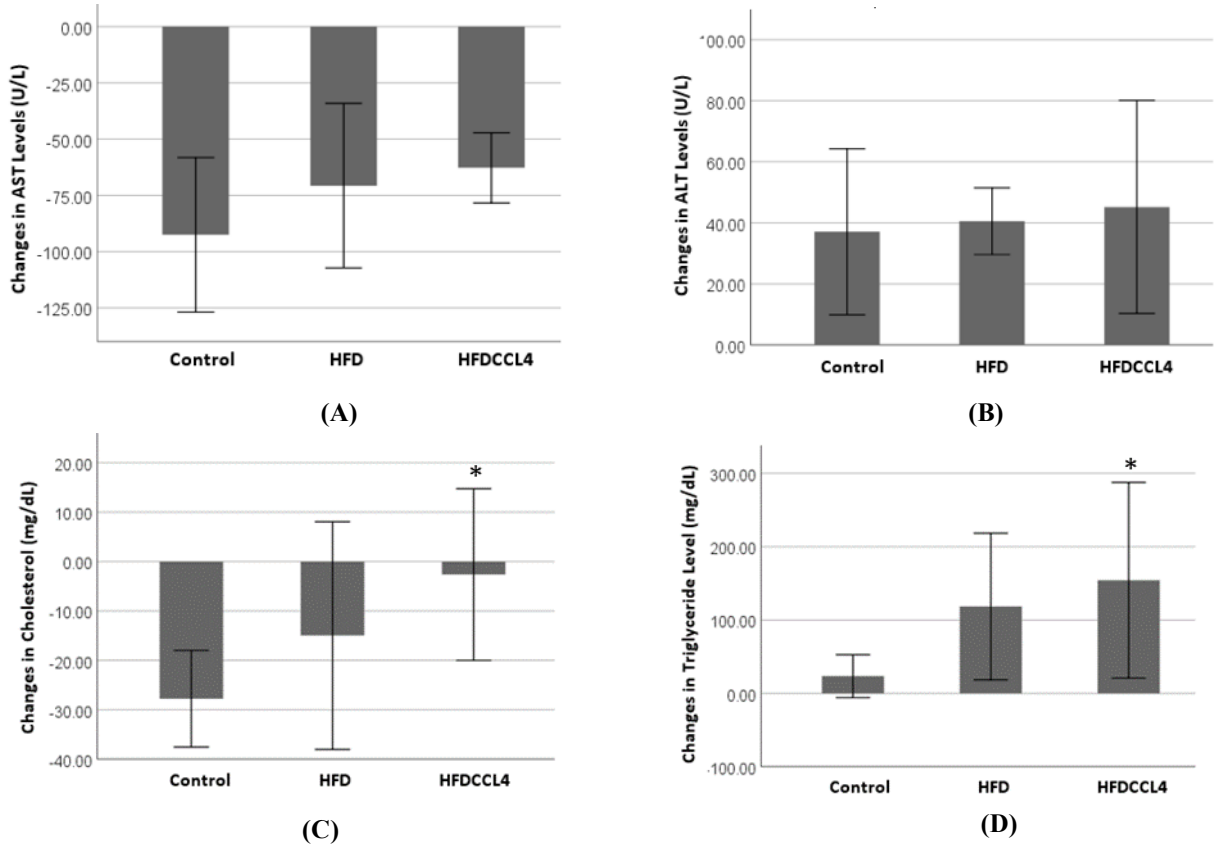

Figure 1. Average changes in liver enzyme levels and lipid profiles in the Control, HFD, and HFD-CCl_4 groups between pre- and post-intervention. (A) Changes in AST levels (I: -92.53±34.37; II: -70.73±36.53; and III: -62.82±15.61; p=0.120). (B) Changes in ALT levels (I: 37.08±27.17; II: 40.52±10.90; and III: 45.21±34.87; p=0.811). (C) Changes in total cholesterol levels (I: -27.76±9.77; II: -14.98+23.04; and III: -2.63±17.37; p=0.023; post hoc=0.020). (D) Changes in triglyceride levels (I: 23.53±29.20; II: 118.46±100.02; and III: 154.22±133.37; p=0.036. *Significantly different than control group (cholesterol levels, p=0.020; triglycerides levels, p=0.036)

group significantly. The post-intervention AST levels of all groups were lower than their pre-intervention levels significantly. In contrast, the post-intervention ALT levels of all groups were higher than their pre-intervention levels significantly. The total cholesterol levels in HFD and HFD-CCl_4 groups were higher than the control group. As for the triglyceride levels, the HFD-CCl_4 group was higher than the control group significantly.

The differences between before and after intervention values of liver enzyme levels and lipid profiles in all groups are shown in Figure 1. The pre-and post-intervention AST and ALT levels in all groups were still within the normal limits and did not show significant changes in means (p=0.120 and p=0.811, respectively) as shown in Figure 1A and 1B. The pre-and post-intervention lipid profile (cholesterol and triglyceride levels) in all groups showed significant differences in means with p values of 0.023 and 0.036 respectively (Figure 1C and 1D). The posthoc test revealed that the lipid profile of the HFD-CCl_4 group was different from other groups.

4. DISCUSSION

This study showed the effects of HFD intervention and CCl_4 administration on liver function and lipid profile in a fatty liver rat model. Each group showed an increase in bodyweight between pre-intervention and post-intervention significantly. However, the weight gain between groups did not differ (p>0.05). This might have been caused by the rats' low appetite for the HFD food pellets in HFD and HFD-CCl_4 groups, which was indicated by more leftovers of food pellets in the cage compared to the control group.

AST and ALT levels are indicators of hepatocellular injury. AST is expressed in many tissues except in bones while ALT is predominantly found in the liver, making ALT a more specific indicator for liver damage. Studies have shown that a high ALT level correlates with a higher risk of NAFLD. However, other studies also showed that 50% of patients with liver histology showing the presence of NAFLD and fibrosis had normal AST and ALT levels. Therefore, transaminase levels are weak predictive factors for NAFLD and are not related well with the metabolic and histological parameters of patients

with NAFLD [11-13]. After intervention, all groups showed a decrease in AST levels significantly ($p<0.05$). In contrast, the ALT levels of all groups increased even though it was not significant. The AST and ALT levels post-intervention were still within normal limits (male Sprague Dawley rat's normal range for AST is 42.9-67.4 U/L and normal ALT range is 92.3-122.5 U/L). These results are similar to Karacor *et al.* who also showed lower AST levels in the HFD group than normal [14]. Makoto *et al.* reported that HFD intervention with a composition of 64% fat, 14% protein, and 21% carbohydrate increased AST and ALT significantly after HFD intervention for 34-36 weeks. An increase in ALT is usually associated with a higher body mass index (BMI), but our finding showed that there was no difference in the mean weight gain in all groups after treatment [15-16].

In the liver, CCl_4 is converted to trichloromethyl radical (CCl_3) which can cause inflammation, fibrosis, and liver damage. Chronic low-dose CCl_4 administration can be used as a liver injury method. Variations in CCl_4 administration methods make it difficult to compare results. In NALFD modeling with dietary intervention, sometimes obesity or insulin resistance is not achieved. When it is combined with CCl_4, this chemical agent may potentiate the effects of HFD on NALFD development and its progression to fibrosis [5]. Kubota *et al*. showed that rats that were exclusively given HFD and CCl_4 had increased transaminase levels and hepatic steatosis was observed in the histological examination after 12 weeks of treatment [17]. However, the AST and ALT levels in the HFD-CCl_4 group in this study did not differ from the HFD and control groups. It is suspected that this different finding was caused by different intervention procedures, the CCl_4 solvent used, and the low appetite of the rats in the HFD-CCl_4 group.

A high-fat diet can increase total cholesterol and triglyceride levels. High fat consumption will cause intrahepatic triglyceride accumulation. Triglyceride accumulation arises from an imbalance in the acquisition and disposal of triglycerides formed by FFA and glycerol esterification in hepatocytes. Fatty acids can be obtained from the lipolysis in adipose tissue, food sources/hypercaloric diet, and the de novo process of triglyceride lipogenesis. Increased FFAs lead to higher hepatic triglyceride production and hepatic VLDL secretion, resulting in more circulating triglycerides [18]. Accumulation of triglycerides may also result from the nonspecific response of hepatocytes to various injuries [19].

The type of fat contained in the food pellet used in this study was 100% unsaturated fat with a concentration of 69.431 ng/µl. This study showed that the total cholesterol level of the control group was different from the other groups. Meanwhile, only the triglyceride level of the HFD-CCl_4 group differed from the other groups. These findings suggested that the combination of HFD intervention and CCl_4 administration has stronger effects on total cholesterol and triglyceride level. These results are similar to a study by Omagari *et al*. that compared an HFD intervention with 45% fat and a standard low-fat diet (LFD) with 10% fat [20]. The level of unsaturated fatty acids in the used HFD food pellets may lower the total cholesterol. Donald *et al.* reported the hepatoprotective properties of unsaturated fatty acids through their ability in controlling saturated fatty acid production and oxidation. Olive oil, peanut oil, and canola are examples that contain unsaturated fatty acids. Furthermore, unsaturated fatty acids decrease the fat level in the liver and improve the blood lipid profile [17, 21].

There are several limitations to this study. The fat content of the HFD pellets that we could prepare was only 30%, higher fat content is preferable. We could only estimate the pellet leftovers as the rats crushed the pellets and the leftovers were mixed with the husk and rats' manure. Histological features of the liver also need to be examined to confirm NAFLD in the target organ because NAFLD and fibrosis can be found in ALT levels below half of the upper normal limit [11].

In conclusion, a NAFLD modeling with a combination of HFD intervention (30% fat content) and CCl_4 administration for 12 weeks gave better results than NAFLD modeling only with HFD intervention. The modeling with HFD intervention and CCl_4 administration for 12 weeks affected blood cholesterol and triglyceride levels but it did not cause an increase in ALT and AST levels.

AUTHORS' CONTRIBUTIONS

All authors have contributed to the writing and in the experiment process. Material preparation, design study, data collection, and analysis are involved. The authors had read and agreed on the final manuscript.

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REFERENCES

[1] N. Chalasani, Z. Younossi, J.E. Lavine, Charlton, K. Cusi, M. Rinella, S.A. Harrison, E.M. Brunt, A.J. Sanyal, The diagnosis and management of

nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases, Hepatology, vol. 67(1), 2018, pp. 328-357. DOI: 10.1002/hep.29367.

[2] R. Adiwinata, A. Kristanto, F. Christianty, T. Richard, D. Edbert, Tatalaksana terkini perlemakan hati non alkoholik, Jurnal Penyakit Dalam Indonesia, vol. 2(1), 2015, pp. 53. DOI: 10.7454/jpdi.v2i1.65.

[3] Z. Younossi, Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: implications for liver transplantation, Gastroenterology and Hepatology, vol. 15(2), 2017, pp. 166-170. DOI: 10.1002/lt.25003.

[4] V. Ratziu, V. de Ledinghen, F. Oberti, P. Mathurin, C. Wartelle-Bladou, C. Renou, P. Sogni, M. Maynard, D. Larrey, L. Serfaty, *et al*., A randomized controlled trial of high-dose ursodeoxycholic acid for nonalcoholic steatohepatitis, Journal of Hepatology, vol. 54(5), 2011, pp. 1011-1019. DOI: 10.1016/j.jhep.2010.08.030.

[5] M.A. Van Herck, L. Vonghia, S.M. Francque, Animal models of nonalcoholic fatty liver disease—a starter's guide, Nutrients, vol. 9(10), 2017, pp. 1-13. DOI: 10.3390/nu9101072.

[6] J. Ka, C. Lau, X. Zhang, J. Yu, Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances, Journal of Pathology, vol. 241(1), 2017, pp. 36-44. DOI: 10.1002/path.4829.

[7] R.G.P. Panjaitan, E. Handharyani, Chairul, Masriani, Z. Zakiah, W. Manalu, Pengaruh pemberian karbon tetraklorida terhadap fungsi hati dan ginjal tikus, Makara Kesehatan, vol. 11(1), 2007, pp. 11-16.

[8] B.R. Thapa, A. Walia, Liver function tests and their interpretation, Indian Journal of Pediatrics, vol. 74, 2007, pp. 67-75. DOI: 10.1007/s12098-007-0118-7.

[9] M.M. Ahmed, E.S.A. Samir, A.M. El-Shehawi, M.E. Alkafafy, Anti-obesity effects of Taif and Egyptian pomegranates: molecular study, Bioscience, Biotechnol, and Biochemistry, vol. 79(4), 2015, pp. 598-609. DOI: 10.1080/09168451.2014.982505.

[10] G. Zhang, X. Wang, T.Y. Chung, W. Ye, L. Hodge, L. Zhang, K. Chng, Y.F. Xiao, Y.J. Wang, Carbon tetrachloride (CCl_4) accelerated development of non-alcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH) in MS-NASH mice fed western diet supplemented with fructose (WDF), BMC Gastroenterology, vol. 20(1), 2020, pp. 339. DOI: 10.1186/s12876-020-01467-w.

[11] S. Khosravi, S.M. Alavian, A. Zare, N.E. Daryani, S.M. Fereshtehnejad, N.E. Daryani, M.R. Keramati, S. Abdollahzade, S.T.T. Vakili, Non-alcoholic fatty liver disease and correlation of serum alanine aminotransferase level with histopathologic findings, Hepatitis Monthly, vol. 11(6), 2011, pp. 452-458.

[12] M. Noureddin, R. Loomba, Nonalcoholic fatty liver disease: indications for liver biopsy and noninvasive biomarkers, Clinical Liver Disease (Hoboken), vol. 1(4), 2012, pp. 104-107. DOI: 10.1002/cld.65.

[13] S. Jodi, A. Farshbafkhalili, R. Nikanfar, L. Javadi, Non-alcoholic fatty liver disease and its predictive factors among high-risk employees of Health Insurance Organization in Tabriz, Iran, Crescent Journal of Medical and Biological Sciences, vol. 5(1), 2018, pp. 34-39.

[14] K. Karaçor, M. Çam, N. Orhan, E. Coşgun, H. Demirin, High fatty diet effects on rat liver, European Journal of General Medicine, vol. 11(2), 2014, pp. 99-108. DOI: 10.15197/sabad.1.11. 47.

[15] M. Ito, J. Suzuki, S. Tsujioka, M. Sasaki, A. Gomori, T. Shirakura, H. Hirose, M. Ito, A. Ishihara, H. Iwaasa, *et al*., Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to a high-fat diet, Hepatology Research, vol. 37(1), 2007, pp. 50-57. DOI: 10.1111/j.1872-034X.2007.00008.x.

[16] K.A. Kwon, P. Chun, J.H. Park, Clinical significance of serum alanine amino transferase and lifestyle intervention in children with nonalcoholic fatty liver disease, Korean Journal of Pediatrics, vol. 59(9), 2016, pp. 362-367. DOI: 10.3345/kjp.2016.59.9.362.

[17] N. Kubota, S. Kado, M. Kano, N. Masuoka, Y. Nagata, T. Kobayashi, K. Miyazaki, F. Ishikawa, A high-fat diet and multiple administration of carbon tetrachloride induces liver injury and pathological features associated with non-alcoholic steatohepatitis in mice, Clinical and Experimental Pharmacology & Physiology, vol. 40(7), 2013, pp. 422-430. DOI: 10.1111/1440-1681.12102.

[18] G. Kanuri, I. Bergheim, In vitro and in vivo models of non-alcoholic fatty liver disease (NAFLD). International Journal of Molecular Sciences, vol. 14(6), 2013, pp. 11963-11980. DOI: 10.3390/ijms140611963.

[19] I. Semova, S.B. Biddinger, Triglycerides in nonalcoholic fatty liver disease: guilty until proven

innocent, Trends in Pharmacological Sciences, vol. 42(3), 2021, pp. 183-190. DOI: 10.1016/j.tips.2020.12.001.

[20] K. Omagari, S. Kato, K. Tsuneyama, C. Inohara, Y. Kuroda, H. Tsukuda, E. Fukazawa, K. Shiraishi, M. Mune, Effects of a long-term high-fat diet and switching from a high-fat to low-fat, standard diet on hepatic fat accumulation in Sprague-Dawley rats, Digestive Diseases and Sciences, vol. 53(12), 2008, pp. 3206-3212. DOI: 10.1007/s10620-008-0303-1.

[21] D.B. Jump, Fatty acid regulation of hepatic lipid metabolism, Current Opinion in Clinical Nutrition and Metabolic Care, vol. 14(2), 2011, pp. 115-120. DOI: 10.1097/MCO.0b013e328342991c.