

Liver Histopathology of Rats Induced by High-Fat Feed After Giving Neem Leaf Ethanol Extract

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Abstract. Foods that contain high levels of fat can cause hyperlipidemia, which is one of the triggering factors for non-alcoholic fatty liver disease. Neem leaves contain flavonoids, alkaloids and tannins which have the ability to act as hepatoprotectors. This study aimed to determine the Liver Histopathology of Rats Induced by High-Fat Feed After Giving Neem Leaf Ethanol Extract. Twenty-four the male white rats (*Rattus norvegicus* L.) were divided into 6 treatment groups, namely: normal control (P0); negative control (P1: given high-fat diet); P2 treatment (P1+ 8 mg/200gBW simvastatin); and P1+ the dose of neem leaf ethanolic extract of 75; 100; and 125 mg/200gBW (P3; P4; and P5). Fixation process with 10% Neutral Formalin Buffer (NFB) solution. Liver histopathological preparations were made by paraffin method and Hematoxylin-Eosin staining, histopathological observations with a 400x magnification microscope. Liver histopathology was analyzed descriptively, homogeneous and normally distributed data of liver weight and hepatocyte diameter were analyzed statistically using ANOVA followed by Duncan's test with a significance level of 5% using SPSS 16.0 software. The results showed that the administration of ethanolic extract of neem leaves could improve the liver histology structure. From this study it was concluded that the ethanolic extract of neem leaves can be used as an alternative hepatoprotector.

Key words: Hepatoprotector; Herbal plant; Hyperlipidemia; Non-alcoholic fatty liver disease

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INTRODUCTION

The liver is an organ that has very important and complex functions, especially in fat metabolism, glycogen storage, body defense, remodeling of old red blood cells, detoxification of body wastes, hormones, drugs and other foreign compounds (Sherwood, 2011). The liver is an organ that is very susceptible to the influence of chemical compounds. The liver is often damaged by the entry of toxic substances. Junqueira et al. (2017) stated that liver damage can be caused by various agents including viruses, alcohol and drugs (such as isoniazid, aspirin, tetracycline). These agents can cause impaired liver function in the form of carcinoma or liver cirrhosis. Furthermore, Lu (2012) stated that about 80% of the blood supply to the liver comes from the digestive tract, so toxic materials that are absorbed by the intestine will be carried to the liver through the portal vein. Toxic substances can cause various types of toxic effects such as steatosis, necrosis, cholestasis, and cirrhosis.

Foods with high levels of fat, especially cholesterol, can cause hyperlipidemia. This statement is in accordance with Hariaji's research (2019) that total cholesterol levels in rat blood plasma increased in all groups given chicken egg

yolks for two weeks. The increase in cholesterol levels is caused by one chicken egg yolk containing 220-250 mg of cholesterol and saturated fat. Increased blood cholesterol levels are one of the triggering factors for non-alcoholic fatty liver disease. Rukmini (2007) states that used cooking oil contains free radicals with an indicator of peroxide (COO*). Chemicals that enter the liver will form free radicals, these free radicals bind to O₂ in the body to form peroxy (peroxy radicals), peroxy absorbs hydrogen atoms from unsaturated lipid molecules, resulting in further reactions. The reaction produces other peroxides namely peroxy nitrite, peroxy. Peroxy nitrite is lipophilic which causes lipid peroxides on the membrane and will attack the mitochondria, then release ribose and the endoplasmic reticulum, so that the energy supply needed to maintain the function and structure of the endoplasmic reticulum is inhibited and protein synthesis decreases drastically so that cells lose the power to secrete triglycerides, resulting in decreased synthesis. proteins. There is accumulation of triglycerides in the liver and liver cell damage, which can lead to necrosis of liver cells. Furthermore, Dowman et al. (2011) stated that fatty liver can be diagnosed early by

microscopic examination to determine changes in its histological structure. The spectrum of liver disorders in NAFLD (Nonalcoholic Fatty Liver Disease) includes steatosis (fatty liver), steatohepatitis (fatty and inflammation of the liver, Non-Alcoholic Steato Hepatitis / NASH), liver fibrosis and liver cirrhosis.

Treatment for lowering lipid levels, especially cholesterol, uses cholesterol-lowering or anti-cholesterol drugs which are widely circulated in the market, both synthetic drugs and traditional medicines, but the use of synthetic drugs can have side effects on users (Dwipayanti & Sutomo, 2019). The side effects of using synthetic drugs can be reduced by using natural medicines or traditional medicines from plants. One of the plants that can be used as an alternative to lower cholesterol levels is neem. Neem (*Azadirachta indica* A. Juss) belongs to the Meliaceae family and is widely found in tropical countries, one of which is Indonesia. Its use as a traditional medicine is because the neem plant contains bioactive compounds, especially in the leaves. Phytochemical screening of neem leaves showed that neem leaves contain bioactive compounds including alkaloids, anthraquinones, saponins, cardiac glycosides, quercetin 3-galactoside, phenols, flavonoids, tannins, and ascorbic acid (Awotedu et al., 2019; Pandey et al., 2014; Rao et al., 2019). Bisht & Sisodia (2010) in their research on the antihyperglycemic and antidiabetic potential of neem leaf extract in Streptozotocin-induced STZ-Diabetes Mellitus showed that repeated administration of neem ethanol extract in diabetic rats could prevent an increase in TC, TG, LDL and VLDL cholesterol levels compared to diabetic rats treated with not given neem leaf extract, while HDL cholesterol levels increased significantly.

Based on the facts, it is important to conduct research on the liver histopathology of Wistar strain white rats fed a high-fat feed after being treated with neem (*A. indica*) leaf ethanol extract. This study is expected to provide information about the potential of neem (*A. indica*) leaves and their benefits in the health sector in order to develop knowledge related to non-pharmacological treatment and as a reference for further research. So that in the future alternative treatments can be found to overcome liver histopathology caused by hyperlipidemia by using herbal plants.

METHODS

Making high-fat diet

A high-fat diet is made by mixing commercial feed and used cooking oil obtained by the deep fat frying technique, namely one liter packaged cooking oil which is used to fry 450 g of tofu for 10 minutes at a temperature of 150-165°C, then the oil is heated 9 times (Muhartono et al., 2018; Hanung et al., 2019). Furthermore, the rats were given a high-fat diet of 30 g/day and given 2.5 ml of duck egg yolk.

Preparation of the neem leaf ethanolic extract

The neem leaves were taken from the campus of the Faculty of Science and Mathematics, Diponegoro University. After that, the neem leaves were put in the oven for 10 days at a temperature of 40-50°C. The maceration method was used to extract neem leaves with 70% ethanol as done by Hasana et al. (2019)

Treatment of test animals (No.101/EC/H/FK-UNDIP/X/2020)

Twenty-four male Wistar rats aged two months and weighing about 200 g. Acclimatization for 1 week was determined by 1 rat in each rearing cage. Feeding and drinking on an ad libitum basis. The rats were weighed and checked for cholesterol levels before treatment. Completely Randomized Block Design was used in this study with an experimental study. The test animal group consisted of six treatments and four replications, namely: Control group was given a standard diet (P0); Given a high fat diet and duck egg yolk orally 2.5 ml/200gBW (P1); P1 + 8 mg/200gBW simvastatin in 1 ml aquades (P2); P1 + 75 mg/200gBW ethanolic extract of neem leaves in 1 ml of distilled water (P3); P1 + 100 mg/200gBB of ethanolic extract of neem leaves in 1 ml of distilled water (P4); and P1+ 125 mg/200gBB of ethanolic extract of neem leaves in 1 ml of distilled water (P5). Giving ethanol extract of neem leaves in the afternoon per day. Feeding a high-fat diet a mixture of commercial feed and used cooking oil in the morning per day, while giving duck egg yolks in the morning for 2 days. Giving treatment for 44 days orally.

Preparations and Staining of Paraffin Sections

The liver of the test animals was prepared for histological observation using the paraffin method with 10% neutral buffered formalin (NBF) fixative

sectioned at $\pm 4 \mu\text{m}$. Then dehydrated with graded alcohol (80%, 90%, and 95%) for 2 hours per concentration. The tissue was then cleaned for 60 minutes with xylol I and II and continued with the paraffin infiltration process. then deparaffinization process with xylol I and II for 5 minutes per concentration, and continued with alcohol concentration (95%, 90%, and 80%) for 2 minutes per concentration and then washed with distilled water. Staining using Hematoxylin-Eosin. The slides were dipped in Hematoxylin for 2 minutes and rinsed with water. Then the slides were immersed in eosin for 5 minutes, then in alcohol (80%, 90%, and 95%) for 2 minutes and xylol I and II for 2 minutes each. Finally, attach the adhesive to the preparation and cover it with a cover slip.

Observation of Preparations

All slides were observed using an Olympus BX51 microscope equipped with a photomicrograph at 4x100 magnification. The histopathological observations of the liver were analyzed descriptively. Measurement of hepatocyte diameter by measuring the widest and narrowest part of the hepatocyte cell then dividing by 2. Measurement of liver weight with a digital scale with an accuracy of 0.001.

Data analysis

Liver histopathology was analyzed descriptively, the data on liver weight and diameter of hepatocytes which were homogeneous and normally distributed were statistically analyzed by ANOVA followed by Duncan's test at a significance level of 5% assisted by SPSS 16.0 software.

RESULTS AND DISCUSSION

The results of the analysis of the effect of exposure to ethanolic extract of neem leaves (*Azadirachta indica* A. Juss) on the liver

histopathology of white rats (*Rattus norvegicus* L.) fed a high-fat diet using the Analysis of Variance (ANOVA) test and continued with the Duncan Multiple Range Test with a level of confidence 95%, are presented in Table 1.

The results of the analysis of liver weight obtained in Table 1. show that the liver weight of the P1 treatment group was significantly different from the P2, P3 and P5 treatment groups, the P0 treatment group was not significantly different from P4, P0 and P4 was not significantly different from P1 and P2, P3 as well as P5. This is because the administration of a high-fat diet (high-fat feed and duck egg yolk orally 2.5 ml/200gBW) in white rats (*Rattus norvegicus* L.) caused the liver weight of the P1 treatment group to increase compared to the control group's liver weight (P0) but did not show a significant difference in results. Isdadiyanto (2009) states that the difference in liver weight can be caused by fat accumulation in the liver. Fatty liver, which is characterized by enlarged vacuoles and an increase in liver weight, is caused by abnormalities in lipid metabolism. Anggraini (2008) in his research stated that fat degeneration occurs in the liver, which will result in an increase in liver weight.

Simvastatin is a drug that has been proven to be able to lower cholesterol levels so that it can prevent weight gain, this is according to research by Rusdi et al. (2018) Simvastatin is a drug commonly consumed for the treatment of hyperlipidemia and is used as a positive control because this drug is very effective in lowering total and LDL cholesterol. Lajuck (2012) stated that simvastatin works by inhibiting the HMG-CoA reductase enzyme which is a precursor of cholesterol synthesis. Inhibition of the first step in the mevalonate pathway in cholesterol synthesis, increases the affinity of LDL receptors and the rate of LDL catabolism and the extraction of hepatic LDL precursors so that plasma LDL levels

Table 1. The mean of liver weight and hepatocyte diameter of white rats

Treatment	Variable	
	The mean liver weight (g) \pm SD	The mean hepatocyte diameter (μm) \pm SD
P0	9.41 ^{ab} \pm 1.1	7.01 ^a \pm 0.19
P1	11.28 ^b \pm 1.36	9.55 ^c \pm 0.08
P2	7.99 ^a \pm 1.35	8.78 ^b \pm 0.17
P3	8.01 ^a \pm 0.53	8.85 ^b \pm 0.30
P4	9.31 ^{ab} \pm 1.30	8.76 ^b \pm 0.22
P5	8.71 ^a \pm 2.09	8.36 ^b \pm 0.92

Note: Numbers that have the same superscript in the same column show no significant difference ($P > 0.05$). The control group was given a standard diet (P0); Given a high fat diet and duck egg yolk 2.5 ml/200gBW (P1); P1 + 8 mg/200gBW simvastatin in 1 ml aquades (P2); P1 + 75 mg/200gBW ethanolic extract of neem leaves in 1 ml of distilled water (P3); P1 + 100 mg/200gBB of ethanolic extract of neem leaves in 1 ml of distilled water (P4); and P1+ 125 mg/200gBB of ethanolic extract of neem leaves in 1 ml of distilled water (P5).

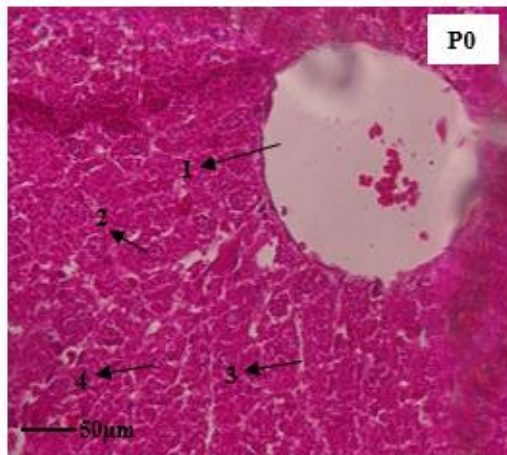


Figure 1. Histological structure of P0 with a magnification of 4x100. (H & E staining). 1= Central Vein, 2= Normal Hepatocytes, 3= Sinusoid, 4= Binucleic Cells.

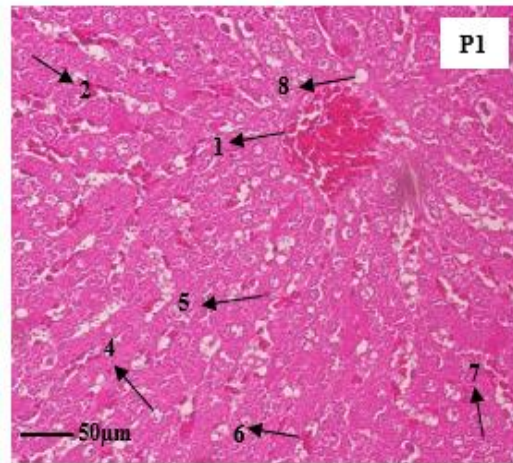


Figure 2. Histological structure of P1 with a magnification of 4x100. (H & E staining). 1= Central Vein, 2= Normal Hepatocytes, 4= Binucleic Cells, 5= Pyknotic Nucleus, 6= Inflammatory cell infiltration, 7= Necrotic, 8= Fatty degeneration.

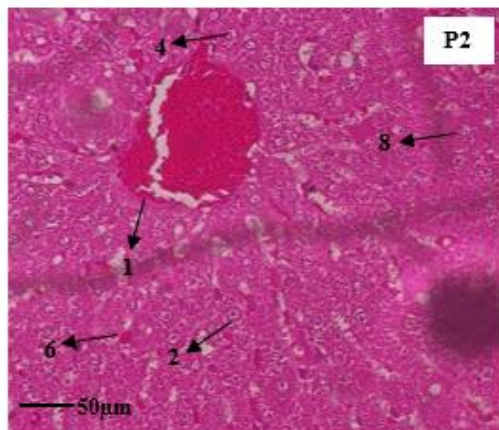


Figure 3. Histological structure of P2 with a magnification of 4x100. (H & E staining). 1= Central Vein, 2= Normal Hepatocytes, 4= Binucleic Cells, 6= Inflammatory cell infiltration, 8= Fatty degeneration.

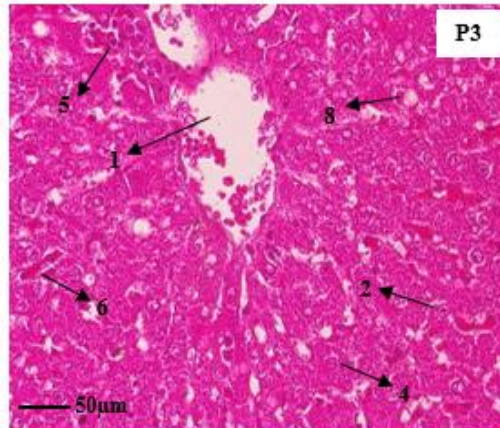


Figure 4. Histological structure of P3 with a magnification of 4x100. (H & E staining). 1 = Central Vein, 2 = Normal Hepatocytes, 3 = Sinusoid, 4 = Binucleic Cells, 5= Pyknotic Nucleus, 6= Inflammatory cell infiltration, 7= Necrotic, 8= Fatty degeneration.

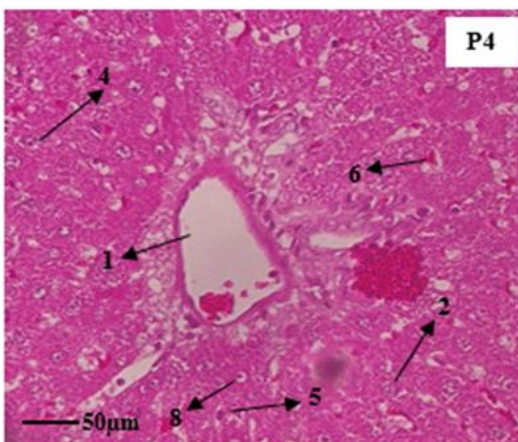


Figure 5. Histological structure of P5 with a magnification of 4x100. (H & E staining). 1= Central Vein, 2= Normal Hepatocytes, 3= Sinusoid, 4= Binucleic Cells, 5= Pyknotic Nucleus, 6= Inflammatory cell infiltration, 7= Necrotic, 8=

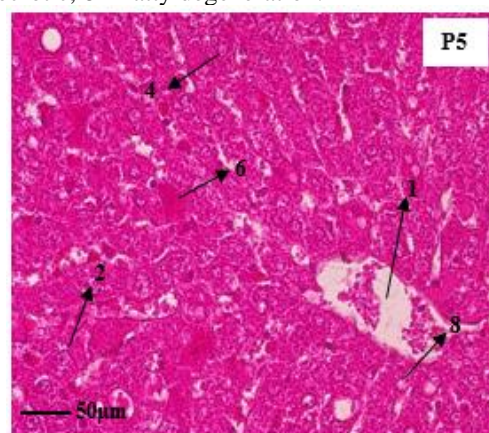


Figure 6. Histological structure of P3, P4 and P5 with a magnification of 4x100. (H & E staining). 1= Central Vein, 2= Normal Hepatocytes, 3= Sinusoid, 4= Binucleic Cells, 5= Pyknotic Nucleus, 6= Inflammatory cell infiltration, 7= Necrotic, 8=

provision of high-fat diet led to a fairly high weight gain. From Table 1. it can be seen that P1 was significantly different from P2, P3 and P5, this indicates that the ethanolic extract of neem leaves is able to function like simvastatin, namely reducing cholesterol levels so that the liver weight

is significantly different from the group given high fat diet. Pankaj et al. (2011) stated that nimbidine, the active ingredient that can be isolated by ethanol extraction, functions as a hypoglycemic agent so that it is thought to cause a decrease in energy availability for the metabolism of test animals. Colville and Bassert (2008) stated that a decrease in the availability of energy in the body will cause a decrease in liver weight. This proves that the ethanolic extract of neem leaves has the same potential as simvastatin in preventing an increase in cholesterol levels so that it has the potential to be used to treat hyperlipidemia.

The results of hepatocyte diameter measurements showed a significant difference ($P < 0.05$) between P0 and the treatment groups P1, P2, P3, P4 and P5. while the treatment groups P2, P3, P4 and P5 were not significantly different ($P > 0.05$). These significantly different results indicate that a high-fat diet affects the diameter of the hepatocytes in rats, which causes the diameter of the hepatocytes to become larger. These results are consistent with Basaranoglu & Ormeci (2014) which states that excess fat consumption and disturbances in fatty acid beta-oxidation in hepatocyte cells can cause accumulation of triglycerides in hepatocyte cells resulting in swelling. Furthermore, Dewi and Saraswati (2009) stated that swelling of hepatocyte cells occurs due to accumulation of fluid in the cytoplasm, this can be caused by disturbances in the regulation of cell fluid, so that on observation under a microscope, the cells appear enlarged with an image of vacuoles in the cytoplasm of the cells. This significantly different result also showed that the ethanolic extract of neem leaves had a significant effect, namely being able to reduce hepatocyte diameter due to high fat diet.

The diameter of the hepatocytes in the P2 treatment group was smaller than the hepatocyte diameter in the P1 treatment group, but not the same as the hepatocyte diameter in the P0 treatment group. This shows that giving simvastatin can help repair swollen liver cells due to excessive fat consumption, Khairunnisa et al. (2016) stated that through inhibition of HMG-CoA reductase, simvastatin prevents endogenous

cholesterol production. Reduced cholesterol concentrations in hepatocytes promote up-regulation of LDL receptor expression, which promotes uptake of LDL and LDL precursors from the systemic circulation. This statement shows that the action of simvastatin in lowering cholesterol is not only limited to reducing cholesterol biosynthesis, but also through the clearance of LDL from plasma. Simvastatin also results in inhibition of apoB-100 synthesis in the liver and reduced synthesis and secretion of triglyceride-rich lipoproteins.

The diameter of the rat hepatocytes in the P3, P4 and P5 treatments gave significantly different results in the control treatment group (P0) and the P1 treatment group. The results showed that the mean hepatocyte diameter in the P3, P4, and P5 treatment groups was smaller than the hepatocyte diameter in the P1 treatment group fed a high-fat diet, but the diameter in the P3, P4, and P5 treatment groups was smaller. not close to the hepatocyte diameter of the P0 treatment group. (control). This is presumably due to the flavonoid content in the ethanolic extract of neem leaves which can repair liver damage caused by fatty liver. This is in accordance with the statement of Tandi et al. (2016) and Choy et al. (2019) that flavonoids as antioxidants can reduce oxidative stress, this can trigger a decrease in ROS production. Decreased ROS production will inhibit oxidative stress so that it does not trigger inflammation and the secretion of the proinflammatory cytokine TNF-. Inflammation causes an increase in hepatocyte diameter.

The results of microscopic observations to determine the histopathological picture of the liver in rats in the P0 treatment group can be seen in Figure 1. This shows that there is no damage to the histological structure of the liver. Histological observation of the liver showed normal hepatocyte cells and sinusoidal structures that were still tight and regular in size and location, although there were still some dilated sinusoidal structures near the central vein. Binucleic cells were found some but not in large numbers. These results indicate that the histological structure of the rat liver in the P0 treatment group was normal. This is supported by the opinion of Muliawan et al. (2012) stated that normal histology can be seen from several liver cells close to the central vein with a normal appearance and clear sinusoidal images and regularly located. Slight widening of the sinusoids due to sinusoidal congestion was found to be possible due to exposure to fat obtained from commercial feed consumed by mice in the control

group (P0).

The histopathology of the liver of rats in the P1 treatment group (Figure 2) showed so many abnormalities in the histological structure as shown in Figure 2. The sinusoids looked wide and irregular, cells Normal hepatocytes are found only in small numbers and pale in color. Some binucleic cells were found not in large numbers. Pyknotic nuclei were also found, inflammatory cell infiltration, fat degeneration and even necrosis of some cells were found. The sinusoids appear dilated as a result of sinusoid congestion caused by fat that causes the sinusoids to widen and become irregularly located. Judging from the hepatocyte cells of the P1 treatment group, many of them experienced fat degeneration which was characterized by the presence of swollen vacuoles and in severe cases pushing the nucleus to the edge, this was due to the accumulation of excess fat due to the administration of a high-fat diet.

Observation of the histopathological structure of the liver of rats in the P2 treatment group (Figure 3.) showed almost entirely normal hepatocyte cells and many binucleic cells were found. Sinusoids look more regular, but there are still abnormalities in the presence of fatty degeneration and inflammatory cell infiltration, but the number is not much. Histological structure of the liver in this treatment group showed that simvastatin was able to improve the histological structure of the liver of rats fed a high-fat diet which could be seen with the least number of necrotic cells and pyknotic nuclei found. This is in accordance with the research results of Ruslin et al. (2019), the results of which showed that the histology of the liver that was given simvastatin after exposure to a high-fat diet improved, which could be seen from the increase in the number of normal hepatocytes by 50%, the reduction in fat degeneration by 5%, and the reduction in necrotic cells by up to 20%. This is due to the reduced number of agents that cause cell damage, caused by intake of cholesterol, fatty acids and triglycerides. The administration of simvastatin in the positive group caused a decrease in the damage to fatty degeneration in the liver because statins had the greatest LDL cholesterol-lowering effect, so statins were used as the main drug to treat hyperlipidemia. This is also supported by the research results of Isdadiyanto et al. (2020) which showed that the total cholesterol, LDL cholesterol and triglycerides of rats fed a high-fat diet and simvastatin were lower than rats fed a high-fat diet alone, the amount of HDL cholesterol in rats given simvastatin was higher than rats fed only a high-

fat diet.

Histopathological microscopic observation of rat liver on treatment P3, P4 and P5 (Figures 4,5 and 6) showed a histological picture of the liver with an increase in normal hepatocytes and binucleate cells, compared to treatment P1, sinusoids also appeared to be more regular. On treatment P4 and P5 sinusoids appear normal and more regular. This increase in the number of binucleate cells is a sign of liver cell repair that occurs due to the addition of neem leaf ethanol extract. Jayabalan et al. (2010) explained that the regeneration process can occur in hepatocytes, liver parenchyma cells that are able to replicate. Hepatocytes work like stem cells (stem cells) meaning hepatocytes can multiply. After the hepatocytes proliferate, other cells will also follow suit and break down into a variety of different cells. These new cells then form new structures, resembling new liver lobules. This ability can only be done hepatocytes if the damage that occurs to the liver is minor damage. The number of cells that experienced fat degeneration also decreased, even in the P5 treatment the amount of fat degeneration that occurred was very small. There was still a small amount of inflammatory cell infiltration in the P3, P4 and P5 treatment groups, but P5 showed a very small number of inflammatory cells.

Microscopic observation of liver histopathology in rats given the ethanolic extract of neem leaves showed that the best liver histopathological structure could be improved in the P5 treatment, namely by administering the ethanolic extract of neem leaves. The dose of 125 mg/200gBB of neem leaf ethanol extract showed a very large increase in the number of normal cells and binucleic cells and almost all of them showed normal cells (Figure 6). This is in accordance with Hayong et al. (2019) which stated that administration of neem leaf ethanol extract at a dose below 400 mg/200gBW was effective as hepatoprotective against fatty liver caused by high-dose aspirin, which was indicated by an increase in the number of normal cells and a decrease in the number of necrotic cells.

The decrease in the amount of fatty degeneration and inflammatory cell infiltration in the liver can also be a parameter of the ability to add ethanolic extract of neem leaves, where the most effective repairing effect was shown by giving 125 mg/200gBW of ethanolic extract of neem leaves in 1 ml of distilled water, which was indicated by almost no fatty degeneration. and reduced inflammatory infiltration. According to

the research of Hayong et al. (2019) Administration of neem leaf ethanol extract at doses of 280 mg/200 gBW and 360 mg/200gBW showed the most effective results in reducing fatty degeneration and congestion. This is presumably because the ethanolic extract of neem leaves can reduce the accumulation of triglycerides that allow fatty hepatocyte cells to occur.

Reduced inflammatory cell infiltration indicates reduced toxic substances and free radicals that enter liver cells. This is in accordance with Baratawijaya (2002) inflammation or inflammatory reaction is an important mechanism needed by the body to defend itself from various dangers that disturb the balance and improve the structure and function of tissue disorders caused by these hazards. So that if the inflammatory cells found are reduced, it can be seen that toxic compounds that have the potential to damage cells can be suppressed. This is presumably because neem leaf extract contains antioxidant compounds. This assumption is supported by the results of Hartono & Prabowo's research (2019) which states that various antioxidant phytochemical compounds in neem leaf extract function as hepatoprotectors because they are able to reduce the formation of free radicals by direct scavenging, namely reducing the formation of ROS, which have a toxic effect on phospholipid membranes and cause inflammation. broad spectrum of cell damage, thereby reducing oxidative stress. Sinusoids in treatment P4 and P5 were clearly and regularly seen due to a decrease in fat degeneration that occurred due to the effect of repairing the histological structure of the liver given a dose of 100 mg/200gBW and ethanol extract of neem leaves 125 mg/200gBW in 1 ml of distilled water. This is in line with research by Rarangsari (2015) which showed that sinusoids that experienced widening due to the presence of toxic substances were seen again clearly and regularly when given soursop leaf extract which was thought to be due to antioxidants from the flavonoids contained. Furthermore, Bisht & Sisodia (2010) reported that the flavonoids contained in neem leaves have been reported to reduce LDL oxidation, reduce triglyceride levels and cholesterol levels in the blood.

Alkaloid content in the ethanolic extract of neem leaves can prevent excess absorption of cholesterol in the liver. Wahyudi's research (2009) shows that alkaloids work as antioxidants by donating hydrogen ions as in flavonoids. This hydrogen donor mechanism was further described by Brunetti et al. (2013) which states that the

antioxidant activity of flavonoid content, especially quercetin, has a structure that allows radical scavenging activity of flavonoids is the presence of 3,4-dihydroxyl such as dihydroxyl (catechol structure) in ring B, which acts as an electron donor and becomes a radical target. The 3-OH structure of the C ring is also favorable for the antioxidant activity of flavonoids. Conjugation of the double bond at C2-C3 with a 4-keto group, plays a role in delocalizing electrons from ring B thereby increasing the radical scavenging capacity. The presence of 3-OH and 5-OH groups in combination with the 4-carbonyl function and the C2-C3 double bond increases the radical activity. In the absence of an o-dihydroxy structure in ring B, hydroxyl substituents in ring A can be compensated and increase the antiradical activity of flavonoids. This ability is thought to be able to bind free radicals from used cooking oil which triggers the failure of triglyceride production.

Tannins can inhibit fat absorption in the intestine by binding to mucosal proteins and intestinal epithelial cells. Arief et al. (2012) stated that tannins in the body will bind to body proteins and will coat the intestinal wall, so that fat absorption is inhibited. This causes the formation of cholesterol in the liver is inhibited and the absorption of cholesterol in the intestine is inhibited, thereby causing a decrease in total cholesterol and triglyceride levels in the blood. Tannins are also binding and increase the expenditure of bile acids which will then be wasted with feces. Binding of bile acids by tannins causes bile acids to exit the enterohepatic cycle. The decrease in the amount of bile acids causes the liver to use cholesterol in the blood as a material to form new bile acids. Increased faecal bile acids or lost cholesterol can cause a decrease in plasma cholesterol, and increase the biosynthesis of cholesterol turnover in experimental animals. The results of this study indicate the potential of neem leaf ethanol extract in repairing the histological structure of the liver damaged by a high-fat diet. The results were clearly shown by the treatment groups P4 and P5. This is evidenced by the histological structure which is close to the histology structure of the liver in the P0 treatment (control).

Neem leaf is an herbal plant, a more in-depth study is needed so that its utilization is more optimal. Furthermore, it is important to conduct research to determine the levels of each phytochemical in neem leaves to obtain compounds that play an active role as hepatoprotectors. In this study the ethanolic

extract of neem leaves was confirmed as a hepatoprotector. Thus, this study informs about the potential sources of local herbal plants, namely neem leaves for the treatment of liver pathology.

CONCLUSION

The results showed that the administration of ethanolic extract of neem leaves could improve the liver histology structure. From this study it was concluded that the ethanolic extract of neem leaves can be used as an alternative hepatoprotector. Suggestions can be used as an alternative liver drug.

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