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Effect Of Combination Of Snakehead Fish Extract (Channa Striata), Meniran (Phyllanthus Niruri L.), And Temulawak (Curcuma Xanthorrhiza) On Histopathological and Liver Function Of Rats Model Diabetes Mellitus with High Fat Diet

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ABSTRACT
Diabetes mellitus is one of the metabolic disorders characterized by a progressive decrease in insulin sensitivity, resulting in hyperglycemia which causes oxidative stress and damage to liver function. Currently, anti-fibrogenic treatment options are still limited, This study aims to determine the effect of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) on a high fat diet of diabetic melitus model rats. This type of research is an experimental laboratory in vivo with a randomized post test-only control group design. The population of this study is male rats (Rattus norvegicus) of the wistar strain with a sample size of 30 mice divided into 5 groups of rats. Data analysis used the SPSS version 26 program which was analyzed by the Kruskal-Wallis and Mann-Whitney statistical tests. The results of this study found that there was a difference but not significant between SGOT and SGPT between all groups (p>0.05). Therefore, from these results, it can be stated that there is an effect of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) in diabetic mellitus model rats with a high fat diet in improving liver function. It is hoped that further researchers can continue this research by using a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) in repairing damage to other organs other than the liver as a result of diabetes mellitus complications.

Keywords: Liver, Snakehead Fish, Imitation, Temulawak, Diabetes Mellitus High Fat Diet

Introduction

Diabetes mellitus (DM) is one of the metabolic disorders where high blood sugar levels or called hyperglycemia. DM occurs when glucose levels in the blood experience impaired insulin production, insulin action, or both (Dewi et al., 2021).

Diabetes mellitus (DM), hyperglycemia (high levels of glucose in the blood) occurs due to insulin deficiency, insulin resistance, or both, as previously stated. Where insulin is a hormone produced by pancreatic β cells to control blood glucose by regulating the storage and use of glucose. Not only that, diabetes mellitus can also be caused by insulin resistance, or a decrease in insulin's ability to stimulate glucose use, or a decrease in the response of target cells, such as

in muscles, tissues, and the liver (Hardianto, 2020).

Diabetes mellitus is one of the metabolic diseases with a fairly high prevalence rate. Especially in DM type 2 (T2D), which is characterized by a progressive decrease in insulin sensitivity, resulting in cell dysfunction β pancreas. The main characteristic of DM, hyperglycemia, causes oxidative stress and various organ damage, such as diabetic retinopathy disorders, diabetic nephropathy, cardiovascular diseases, and other endocrine disorders (Asokan et al., 2019).

In developing countries, type 2 DM has experienced a drastic increase in cases. According to data (WHO, 2022), around 422 million people in the world suffer from diabetes mellitus. It can be caused by a sedentary lifestyle and a fairly high obesity rate. In patients with type 2 DM, prolonged hyperglycemia can cause metabolic disorders, as well as complications if not treated appropriately (Nurrahma et al., 2021).

Type 2 diabetes mellitus (T2D) and hyperinsulinemia are closely related to developmental changes in fat in the liver ranging from simple steatosis to steatohepatitis, as well as advanced liver diseases, such as liver fibrosis and cirrhosis (Uyanıkgil et al., 2015).

Fatty liver, liver fibrosis, and cirrhosis, including non-alcoholic fatty liver disease (NAFLD) as a result of diabetes (Samadi-Noshahr et al., 2021). Non-alcoholic fatty liver disease (NAFLD) is a condition in which the liver contains too much fat, but develops without the influence of alcohol consumption, and its diagnosis can be established through laboratory examinations as well as microscopic and ultrastructural anatomy (Nurrahma et al., 2021).

Many studies have been conducted, but the mechanism underlying this liver disease is still not known for sure. Decreased antioxidant defense with oxidative stress has been found to be associated with liver fibrosis, regardless of the etiology and degree of development of the fibrosis (Uyanıkgil et al., 2015).

Oxidative stress occurs when the balance between the formation of reactive oxygen species or ROS and the antioxidant defense system is disrupted. Hyperglycemia will directly induce oxidative stress through protein glycation, glucose autooxidation, and increased production of mitochondrial superoxide anions. Where mitochondria belong to the reaction pathways that produce ROS (Samadi-Noshahr et al., 2021).

Non-alcoholic fatty liver disease (NAFLD) can cause chronic liver damage, such as liver cirrhosis to cancer. Liver fibrosis is one of the chronic liver damage, where the condition occurs as a response to repair the damage. Chronic liver damage can be caused by many factors, such as the use of certain medications, hepatitis virus infections, parasitic infections, alcohol consumption, cholestasis, and non-alcoholic fatty liver disease (NAFLD), as mentioned earlier. This damage often results in the death of liver-forming cells or hepatocytes. After hepatocytes, the cells will be absorbed and dictated by macrophages or liver cell (HSC). HSCs will then be activated and secrete profibrotic cytokines. Fibrotic cytokines are substances that trigger the formation of scar tissue in the liver (Lu et al., 2023).

There are several effective therapies used for the treatment of liver fibrosis, so far no drug has been approved as an anti-fibrotic agent in humans. Anti-fibrogenic treatment options are currently limited, where anti-fibrotics will protect hepatocytes from oxidative stress, as well

as reduce inflammatory damage Based on previously conducted research, it is stated that different types of antioxidants can reduce complications in diabetes including fatty liver disease and liver fibrosis (Uyanıkgil et al., 2015).

Currently, herbal medicine is considered relevant today. One of them comes from plants and animals. In NAFLD patients, antioxidant therapy can improve the histopathological picture and liver function. However, not all antioxidants will provide satisfactory results, and their effects on metabolic disorders, especially diabetes mellitus, are not widely known. In this study, we will test capsules containing snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and temulawak (Curcuma xanthorrhiza) on the improvement of liver fibrosis in rats. Where, these extracts have an antioxidant effect that can neutralize ROS (reactive oxygen species) (Naufal et al., 2020).

Channa striata is one of the types of fish that is widely found in Indonesia. Channa striata or snakehead fish is usually used by the community as a postoperative wound healing process. The high nutrient content in Channa striata not only supports the healing process, but also has anti-inflammatory, anti-nociceptive, and anti-microbial potential. The albumin content in Channa striata extract can be used to speed up the recovery process of damaged tissues or cells of the body. (Fitriana Nur, 2021).

Several studies have been conducted to prove the benefits of Channa striata extract in various treatments. One of them is the research on the effects of Channa striata extract as an anti-inflammatory that has been researched in Banjarmasin (Izzaty et al., 2014) and the research on the benefits of cork extract for wound healing in patients with diabetes mellitus (Udayanti & Noviyani, 2022).

Other researchers have obtained the potential of Channa striata extract in the treatment of osteoarthritis (Ayu Dessy Satriani Putri, P. A., Udayanti, S., Ita Purnama Dewi, P., & Noviyani, 2022) and also the potential of Channa striata extract as an antioxidant (Rasimi et al., 2020). A study in Jember stated that the administration of snakehead fish albumin extract capsules (Channa striata) at a dose of 1500 mg/day divided into 3 doses for 1 month can significantly reduce IFN-γ levels in patients with pulmonary tuberculosis in the intensive phase (Allest Pratama, H., Efendi, E., Riyanti Faculty of Medicine, R., & Jember Jln Kalimantan, 2016).

Meniran herb (Phyllanthus niruri L.) is one of the plants that can provide hepatoprotective effects. Based on various studies that have been carried out, meniran has pharmacological activities, including anticancer, antispasmodic, antifungal, antioxidant, antimicrobial, antibacterial, antidiabetic, antihypertensive, anti-inflammatory, antiviral, antianaemic, and immunomodulator. Through a number of literature, it is shown that Phyllanthus niruri exerts an influence on hepatoprotectors through the mechanism of reducing lipid peroxidation and maintaining glutathione in reduced form. The water extract contained in it has the effect of significantly reducing the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, which is $p < 0.001$. In addition, the protein isolates in it also protect liver tissue against oxidative damage and help stimulate the repair mechanisms present in the liver. There are major compounds in the form of phyllantins, where the phyllentine will provide hepatoprotective activity and show antioxidant effects. Therefore, Phyllanthus niruri L. has the potential for hepatoprotector activity that provides improved

connectivity in patients with liver disease (Putu Rika Noviyanti, N., & Chandra Yowani, 2023).

A study shows that plants originating from the Euphorbiaceae tribe can be used as natural immunomodulators because they have flavonoid compounds, such as quercetin, quercithin, isoquercithin, astragalin, and rutin which function as immunomodulators (Perdana, 2022).

The usefulness of meniran herbal extract as an antibacterial has also been proven based on research in Kediri (Widiyawati, D., Biology, J., Science, F., 2017). Another study in Manado also succeeded in testing meniran extract as an antipyretic in fever-induced wistar rats (Jansen, I., Wuisan, J., & Awaloei, 2015).

Another study in South Jakarta also concluded that 70% ethanol extract of meniran herb has the potential to have a pharmacological effect as a strong antioxidant (Marisi Tambunan et al., n.d.) The potential of meniran (Phyllanthus niruri L.) as an antiviral and anticancer for the cervix was also successfully proven in a study in Yogyakarta, where the corilagin compound in meniran has a high degree score so that it has the potential to be an antiviral and anticancer agent for the cervix (Arifin & Febriansah, 2022)

Curcuma xanthorrhiza is also known as Javanese turmeric or temulawak. Curcuma xanthorrhiza is a type of herbal plant native to Indonesia that is often used as a raw material for traditional medicines. Besides being cheap, these materials are also very easy to get. Temulawak is known to have a high content of curcumin and xanthorizzol. Curcumin contained in curcumin has antioxidant effects that can neutralize ROS, including hydroxyl radicals (H2O) and nitric oxide (NO). Meanwhile, the content of xanthorrhizol in temulawak is known to provide anticancer, antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive, antiplatelet, nefroprotective, and hepatoprotective effects. Based on research that has been conducted in China, the results show that administering curcumin extract to rats with acute liver injury due to alcohol can significantly reduce SGOT/SGPT levels in the high-dose group (Naufal et al., 2020).

Several other studies have also been conducted to prove Curcuma xanthorrhiza extract. One of them is a study in Surabaya, where the study was able to prove the antibacterial effect in administering Curcuma xanthorrhiza extract against P. acnes bacteria in vitro. The compounds xanthorrizol, alkaloids, flavonoids, tannins, curcuminoids, and terpenoids contained in it are suspected to be the antibacterial effects (Zahrah et al., 2018).

The utilization of Curcuma xanthorrhiza has also been successfully proven to have good natural antioxidant activity (Rosidi et al., 2016). The combined effect of ethanol extract of sambiloto herb and curcuma rhizome given orally for 28 days has an effect in increasing appetite in male wistar rats. This has been successfully proven based on research conducted in Surabaya (Azalia, Lia et al., 2015).

Channa striata, Phyllanthus niruri L., and Curcuma xanthorizza have been extracted to make it easier to prepare and consume. Commercially available, capsule preparations containing a combination of these three extracts are available. This is the background of the author's interest in researching the effects of Channa striata, Phyllanthus niruri L., and Curcuma xanthorizza on liver repair in diabetic melitus rats with liver fibroblast complications.

The purpose of this study was to determine the effect of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) on a high fat diet of diabetic melitus model rats. To determine the levels of SGOT and SGPT in Aloxan-induced diabetes mellitus model mice with the administration of Metmorphine and a combination capsule of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and temulawak (Curcuma xantorrhiza). To determine the histopathological picture of the liver in Aloxan-induced diabetes mellitus model mice with the administration of pioglitazone and a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and temulawak (Curcuma xantorrhiza).

The benefit of this study is to increase knowledge about the effect of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) on diabetes mellitus with liver disorders. If it is proven that the administration of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) can improve liver function in patients with diabetes mellitus, then this can help the community as an alternative drug and affordable in terms of cost and effectiveness of treatment.

Research Methods

1. Research Design

This study is an experimental type of laboratory in vivo using a randomized post test-only control group design with 31 male Wistar mice measuring 150 to 250 grams aged four to five months. This design was chosen because both the sample and the treatment were more controlled, measurable, and more reliable (Indriputri & Maulana, 2022).

In this study, the researcher wanted to determine the effect of capsules containing a combination of snakehead fish (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) extracts on male white rats of the Wistar strain (Rattus norvegicus sp) which were dissonized with fat from lard and chicken egg yolk for approximately 3 weeks. Furthermore, an assessment of SGOT and SGPT levels was carried out, as well as a picture of liver histopathology.

2. Research Place and Research Time

The research will be carried out in a standardized laboratory with complete equipment and experience in raising experimental animals, namely at the Ellio Medan Laboratory. The research will be carried out after receiving the approval of the Research Ethics Commission of the Faculty of Medicine, Prima Indonesia University, carried out from April 2024 – June 2024, including the stages of preparing materials and tools, treatment, examination and preparation of reports.

3. Research Population and Sample

a. Population

The population in this study is male rats (Rattus norvegicus) of the wistar strain.

b. Sample

The sample in this study used male rats (Rattus norvegicus) of aloxan-induced wistar strains and met the following criteria:

1) Inclusion Criteria
   a. Male rat
   b. Rat age 6-8 weeks
   c. Rat weight 150-250 grams
   d. The mice are in good health and there are no anatomical abnormalities
   e. Mice that have never been used in previous studies
2) Exclusion Criteria
   a. Rats that died during the study
   b. Actively immobile rats

The size of the sample in this study was calculated by Federer's formula and the following results were obtained:

$$(R-1)\ (T-1) \geq 15$$

Information:
r : Number of samples in each treatment group
t : Number of treatment groups

$$(R-1)(5-1) \geq 15$$
$$4(R-1) \geq 15$$
$$4R-4 \geq 15$$
$$r \geq 4.75$$

Based on the results of the calculation, a result of 4.75 was obtained, so it was rounded to 5 rats in each treatment group. In this study, we increased 1 rat each in each treatment group so that it became 6 male wistar rats in each treatment group to anticipate the presence of dead rats.

Results and Discussion

Research Results

1. Difference in Sample Weight Before and After High-Fat Diet Feeding

The weight measurement of the sample prior to feeding a high-fat diet was carried out after the mice were adapted for 1 week which was kept in a light/dark cycle for 12 hours on a standard chow diet. After feeding a high-fat diet, the body weight was re-measured. The difference in sample weight before and after feeding a high-fat diet in white rats (Rattus Norvegicus) for 28 days can be seen in table 1. The following.

Table 1. Weight Difference Before and After High-Fat Diet Feeding

Group	*Mean±Standard Deviation (gr)*		*P value*
	Before	**After**	
K1 (normal)	144.6±0.55	147.4±2.07	0,063
K2 (aloksan + *high fat diet*)	154.6±1.14	164.8±0.84	0,041*
K3 (aloksan + *high fat diet* + pioglitazone at a dose of 0.27 mg/200 grBB)	152.2±0.45	162.6±5.77	0,043

K4 (aloksan + *high fat diet* + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB)	153.6±0.55	164.6±4.51	0,043*
K5 (aloksan + *high fat diet* + pioglitazone 0.27 mg/200 grBB + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200grBB)	150.4±0.55	164.4±1.67	0,043*

*Significant

Based on the results of the research in Table 1. above, the body weight of white rats (Rattus novergicus) before being fed a high-fat diet for 28 days at K1, K2, K3, K4, and K5 was 144.6±0.55 g, 154.6±1.14 g, 152.2±0.45 g, 153.6±0.55 g, and 150.4±0.55 g. After being treated with high-fat diet feed for 28 days, the average body weight of the samples in all groups increased to 147.4±2.07 grams, 164.8±0.84 grams, 162.6±5.77 grams, 164.6±4.51 grams, and 164.4±1.67 grams.

The results of the Wilcoxon test showed that there was a significant difference in average body weight before and after being fed a high-fat diet for 28 days in K2 (aloksan + high fat diet), K3 (aloksan + high fat diet + pioglitazone with a dose of 0.27 mg/200 grBB), K4 (aloksan + high fat diet + capsule combination of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB), and K5 (aloksan + high fat diet + pioglitazone 0.27 mg/200 grBB + capsule combination of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200 grBB) where the average weight change in the rat group was 10 gr. While K1 (negative or normal control) was not found to have a significant change in body weight ($p>0.05$) because all of the rats in this group were not given a high-fat diet.

2. Differences in Liver Weight in White Rats

After being treated by each group, liver samples were taken as histopathological preparations that were observed under a microscope to see the differences between the control group and the treatment group. Liver samples were fixated with 10% formalin neutral buffer (BNF) for 24 hours, planted on a block of paraffin cut with a thickness of 5 µm placed on an object glass. then deparaphinized, dehydrated and stained with GiemSA and hematoxylin-eosin (HE), then liver weight was measured.

The results of liver weight measurement after staining with giemsa and hematoxylin-eosin (HE) obtained the difference in the average liver weight, which can be seen in Table 2. next.

Table 2. Liver Weight Difference

Group	*Mean±Standard deviation*	*P value*
K1 (normal)	6.5±0.76	0,957
K2 (aloksan + *high fat diet*)	6.1±0.03	

K3 (aloksan + *high fat diet* + pioglitazone at a dose of 0.27 mg/200 grBB)	5.6±1.78
K4 (aloksan + *high fat diet* + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB)	6.4±2.25
K5 (aloksan + *high fat diet* + pioglitazone 0.27 mg/200 grBB + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200grBB)	6.2±1.19

Based on the results of the research in Table 2. Above it can be seen that the average weight of liver samples in K2 (aloksan + high fat diet), K3 (aloksan + high fat diet + pioglitazone with a dose of 0.27 mg/200 grBB), K4 (aloksan + high fat diet + combination capsules of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB), and K5 (aloksan + high fat diet + pioglitazone 0.27 mg/200 grBB + combination capsules of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200 gr) of 6.1±0.03 gr, 5.6±1.78 gr, 6.4±2.25 gr, and 6.2±1.19 gr, while the weight of the K1 liver (normal) was 6.5±0.76 gr.

The results of the Kruskal-Wallis test can be stated that there is a difference in liver weight of white mice but not significantly between all groups (p=0.957; p>0.05). Therefore, the treatment that has been carried out by each group does not affect the liver weight of the sample.

3. Differences in Blood Glucose Levels of Samples Before, After, D+4, and D+14 Aloxan Induction

Before being induced by aloxan, all samples were first measured blood glucose levels. Aloxan was induced to all white rats (Rattus novergicus) intraperitoneally with an allocan concentration of 0.04% dissolved in sterile normal saline at a dose of 100 mg/KgBB for the positive control group, and the treatment group was carried out on day 36, while the white rat group on the negative or normal control group was not given aloxane induction. After being given aloxan, the rats were left alone for 72 hours, then on the 38th day the blood glucose levels were checked again, D+4 and D+14.

The difference in blood glucose levels in white rats (Rattus novergicus) before, after, D+4 and D+14 of aloxane-induced can be seen in Table 3. The following.

Table 3. Difference in Blood Glucose Levels Before, After, D+4, and D+14 Aloxan Induction

Group	*Mean±Standard deviation (mg/dL)*			
	Before	**After**	**D+4**	**D+14**
K1 (normal)	83.6±6.23	125.8±10.92	124.2±6.30	117.8±3.56
K2 (aloksan + *high fat diet*)	80.8±5.63	469.2±90.62	466.6±103.12	336.6±70.54
K3 (aloksan + *high fat diet* + pioglitazone at a	88.6±5.08	478.6±121.08	396.8±132.04	150.4±32.31

dose of 0.27 mg/200 grBB)				
K4 (aloksan + *high fat diet* + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB)	89.6±8.08	402.0±128.18	374.4±85.06	261.8±102.10
K5 (aloksan + *high fat diet* + pioglitazone 0.27 mg/200 grBB + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200grBB)	92.0±5.83	367.0±92.86	290.8±78.78	135.4±40.50
P value	0,061	0,000*	0,005*	0,001*

*Significant

Prior to induction of Aloxan with a concentration of 0.04% dissolved in sterile normal saline at a dose of 100 mg/KgBB, the results of the one-way ANOVA statistical test could state that there was a difference in blood glucose levels of white rats (Rattus novergicus) but not significantly between all groups (p=0.061; p>0.05). From the results of this test, it means that the blood glucose levels in white rats (Rattus novergicus) are under the same or normal conditions.

After induction with Aloxan concentration of 0.04% dissolved in sterile normal saline at a dose of 100 mg/KgBB, the blood glucose levels of the sample increased. The results of the Kruskal-Wallis statistical test can be stated that there is a significant difference in blood glucose levels of white rats (Rattus novergicus) after (p=0.000; p<0.05), D+4 (p=0.005; p<0.05), and H+14 (p=0.001; p<0.05) of aloxane induction. Blood glucose levels in the group of rats given aloxan were higher than the levels of the normal group of rats. Thus, there is an effect of Aloxan induction in increasing blood glucose levels.

4. Differences between SGOT and SGPT of White Rats After Treatment

SGOT and SGPT measurements were carried out after white rats (Rattus novergicus) of the diabetes mellitus high fat diet model were treated using blood serum through the photometric method by mixing serum with SGOT/SGPT reagents which were carried out at room temperature. The differences between SGOT and SGPT after being given the treatment of each group can be seen in Table 4 below.

Table 4. Difference Between SGOT and SGPT

Group	*Mean±Standard deviation*			
	SGOT	***P value***	**SGPT**	***P value***
K1 (normal)	175.3±30.09		69.7±13.43	
K2 (aloksan + *high fat diet*)	198.3±63.36		74.3±11.59	
K3 (aloksan + *high fat diet* + pioglitazone at a dose of 0.27 mg/200 grBB)	224.7±7.02		89.3±8.51	
K4 (aloksan + *high fat diet* + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB)	188.7±56.86	0,321	70.3±9.07	0,102
K5 (aloksan + *high fat diet* + pioglitazone 0.27 mg/200 grBB + combination capsule of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200grBB)	148.3±26.58		62.7±10.12	

Based on the results of the study, the average SGOT was obtained from the blood serum of white rats (Rattus novergicus) model of diabetes mellitus high fat diet after being treated with K2 (aloksan + high fat diet), K3 (aloksan + high fat diet + pioglitazone with a dose of 0.27 mg/200 grBB), K4 (aloksan + high fat diet + combination capsules of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB), and K5 (aloksan + high fat diet + pioglitazone 0.27 mg/200 grBB + capsule combination of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200 grBB) of 198.3±63.36; 224.7±7.02; 188.7±56.86 and 148.3±26.58, while SGOT K1 (normal) was 175.3±30.09. From the results of the one-way ANOVA statistical test, it can be stated that there is a difference in SGOT of white rats (Rattus novergicus) model of diabetes mellitus high fat diet after treatment but not significantly between all groups ($p=0.321$; $p>0.05$).

This study also found the average SGPT from the blood serum of white rats (Rattus novergicus) model of diabetes mellitus high fat diet after being treated with K2 (aloksan + high fat diet), K3 (aloksan + high fat diet + pioglitazone with a dose of 0.27 mg/200 grBB), K4 (aloksan + high fat diet + combination capsules of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB), and K5 (aloksan + high fat diet + pioglitazone 0.27 mg/200 grBB + combination capsules of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200 grBB) of 74.3±11.59; 89.3±8.51; 70.3±9.07; and 62.7±10.12, while SGOT K1 (normal) was 69.7±13.43. From the results of the one-way ANOVA statistical test, it can be stated that there is a difference in SGPT of white rats (Rattus novergicus) in the diabetes mellitus high fat diet model after treatment but not significantly between all groups ($p=0.102$; $p>0.05$).

The K5 diabetes mellitus high fat diet model mice had the lowest SGOT and SGPT levels, while the K4 diabetes mellitus high fat diet model mice saw higher SGOT and SGPT levels than the K5 diabetes mellitus high fat diet model mice but these levels were still lower when compared to the diabetes mellitus high fat diet model mice at K2 and K3. Therefore, the results of this study can be stated that there is an effect of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and temulawak (Curcuma xantorrhiza) in diabetic mellitus model rats with a high fat diet in improving liver function.

5. Shapiro Wilk Test Results for Each Treatment Group

Table 5. Results of Shapiro Wilk Test for Each Treatment Group

Group	*Mean±SD*				Shapiro Wilk (Sig.) Normality Test			
	Before	After	D+4	D+14	Before	After	D+4	D+14
K1	83 ± 3.39	130.4 ± 2.97	124.2 ± 6, 30	117.2 ± 3.35	0.564	0.777	0.414	0.616
K2	80.8 ± 5.63	469.2 ± 90.6	466.6 ± 132.12	399.4 ± 82.18	0.272	0.169	0.129	0.341
K3	88.6 ± 5.08	478.6 ± 121.08	396.8 ± 132.04	317.8 ± 82.17	0.965	0.215	0.515	0.642
K4	89.6 ± 8.08	402.0 ± 128.18	374.4 ± 85.05	212.8 ± 143.73	0.548	0.914	0.957	0.018
K5	92.0 ± 5.83	322.4 ± 113.4	322.4 ± 113.4	107.2 ± 11.12	0.351	0.669	0.674	0.002

The above data showed that there was an increase in blood glucose levels in mice after induction with alloxan of 100 mg/kgBB, with an increase in blood glucose levels of about 300 mg/dl in the treatment group, which received alloxan. On the 14th day, treatment with a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus nururi L.), curcuma (Curcuma xanthorrhiza), and pioglitazone reduced the blood glucose level of male rats of the Wistar strain (Rattus norvegicus) to below 100 mg/dl, as shown in the data above. The analysis of glucose level reduction in five treatment groups was carried out using the Shapiro-Wilk test, with most of the results of the p > value of 0.05, indicating normal distributed data, while K-5 9aloksan + high fat diet + pioglitazone on the 14th day) p value < 0.05 showed abnormal data distribution.

6. Overview of Liver Histopathology in Diabetes Mellitus Model Rats with a High Fat Diet After Giving Combination Capsules of Snakehead Fish Extract, Meniran, and Temulawak

The results of the study showed that the effect of giving a combination capsule of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xanthorrhiza) for 26 days can be seen in Figure 1.

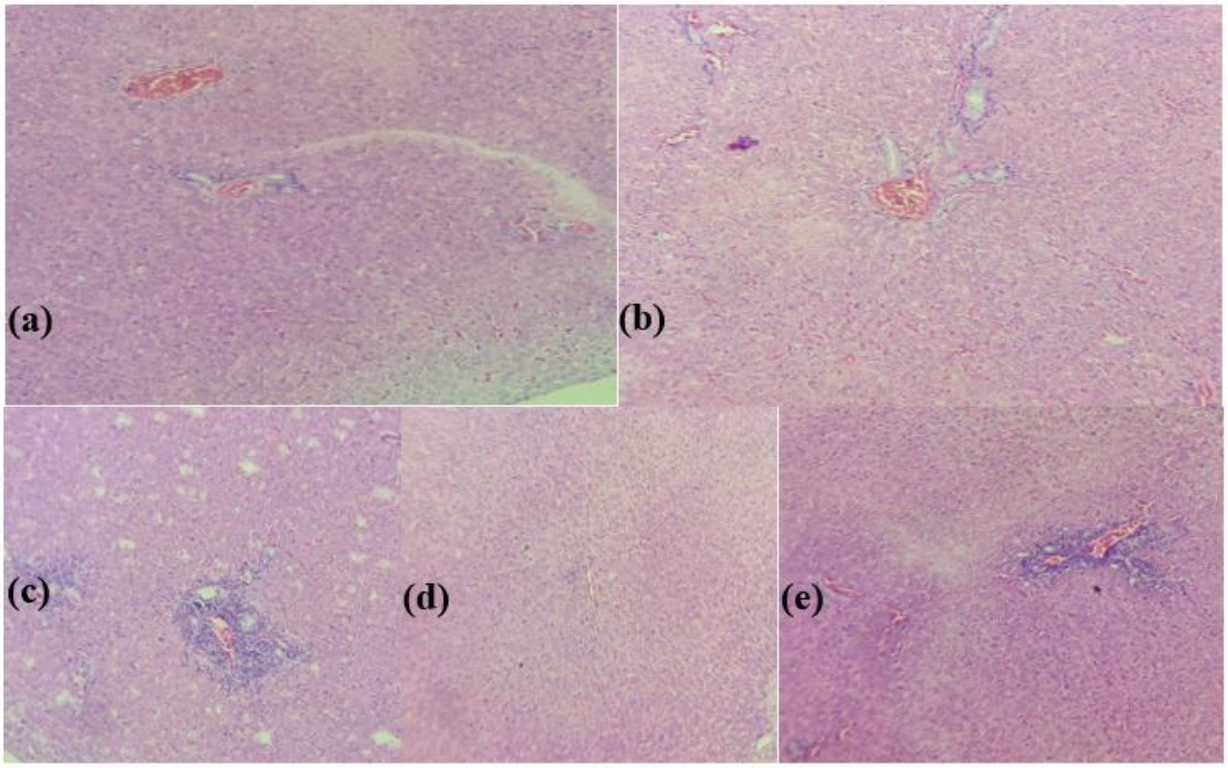

Figure 1. Overview of Histoptology of the Rat Pancreas

The histopathological picture of the liver in each group was different. However, there is one thing in common, namely no hepatocyte fatty as shown in Figure 4.1. The picture of liver histological did not change in the normal group (a). In group 2 (b), namely aloksan + high fat diet, no abnormalities were found. Group 3 (c), namely aloxan + high fat diet + pioglitazone 0.27 mg/200 grBB) was found to have hepatocyte hypertrophy and lymphocyte infiltration. Group 4 (d), namely aloksan + high fat diet + combination capsules of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mh/200 grBB showed similar results to groups 1 (a) and 2 (b). Meanwhile, group 5 (e), namely aloksan + high fat diet + pioglitazone 0.27 mg/200 grBB + capsule combination of snakehead fish extract, meniran, and temulawak with a dose of 9.9 mg/200 grBB, was found to have the same results as group 3 (c).

Discussion

Diabetes mellitus, especially type 2 DM (T2D), is one of the metabolic diseases due to a progressive decrease in insulin sensitivity, which causes oxidative stress and various organ damage, including liver organs (Asokan et al., 2019). This is because T2D is closely related to developmental changes in fat in the liver ranging from simple steatosis to steatohepatitis, as well as advanced liver diseases, such as liver fibrosis and cirrhosis (Uyanıkgil et al., 2015).

There are several drugs to overcome high blood sugar levels, including Pioglitazone. This drug is a type of thiazolidinedione drug class, namely a gamma peroxisome proliferatoractivated receptor (PPAR) agonist that has an insulin sensitizing effect. However, Pioglitazone has several side effects including weight gain, osteopenia, increased risk of fractures, fluid retention,

congestive heart failure, and bladder cancer. Weight gain that does not stop during treatment can cause significant problems (Anjani, 2023).

This can be seen from the results of this study which shows that the group of diabetic melitus mice who have been given Pioglitazone experienced a higher weight change compared to the normal group of mice. Rats that had been induced with Aloxan had a body weight of 152.2±0.45 grams before being given Pioglitazone and after treatment it was 162.6±5.77 grams, while rats that had been induced with Aloxan were found to have a body weight before being given Pioglitazone and capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and temulawak (Curcuma xantorrhiza) were 150.4±0.55 grams and after being 164.4±1.67 grams.

To anticipate the side effects caused by Pioglitazone, this study used capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) in improving the liver in diabetic melitus rats with liver fibroblast complications. The indicators used to indicate liver improvement in this study are through SGOT and SGPT levels.

SGOT (Serum Glutamic Oxaloacetic Transaminase) is an enzyme that is usually found in the liver. In addition, this enzyme is also found in the heart, muscles, kidneys, and brain, while SGPT (Serum Glutamic Pyruvit Transaminase) is the most abundant enzyme found in the liver. Abnormal SGOT and SGPT levels can indicate that prolonged treatment and therapy received by type 2 diabetes mellitus patients have caused damage to the liver (Supriyadi, S., & Dewi, 2022). Damage to the liver will cause these liver enzymes to be released into the bloodstream so that the level in the blood increases and indicates a disorder of liver function (Rasyid & Lio, 2020). This can be seen from the results of research conducted by Rachmawati stating that high blood sugar levels from time to time cause an increase in SGOT levels (Supriyadi, S., & Dewi, 2022).

Based on the results of this study, it can be stated that the group of rats that have been induced by Aloxan after the administration of a combination capsule of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and temulawak (Curcuma xantorrhiza) can improve liver function as shown by the average SGOT level of 188.7±56.86 and SGPT of 70.3±9.07. This number was lower than that of diabetic mice given Pioglitazone and the control group, but the levels were still higher than normal mice.

The existence of the ability to improve liver function from the combination capsule of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) in this study can be caused by the antioxidant ability contained in these ingredients. Antioxidants are substances necessary to neutralize free radicals and prevent damage to normal cells, proteins, and fats (Suhendi et al., 2020). This is caused by hyperglycemia in the condition of diabetes mellitus which has oxidative stress due to increased production of free radicals, especially reactive oxygen species (ROS). Hyperglycemia will directly induce oxidative stress through protein glycation, glucose autooxidation, and increased production of mitochondrial superoxide anions. Mitochondria include the reaction pathways that produce ROS (Samadi-Noshahr et al., 2021). The large number of free radicals in this complication of DM can affect liver function (Lailatul, N., Lyrawati, D., & Handaru, 2015).

The results of a previous study conducted by (Suhendi et al., 2020) stated that high doses

of snakehead fish extract (Channa striata) supplements have the potential to be antioxidants in rats experiencing oxidative stress. Snakehead fish extract contains albumin which acts as an antioxidant through a mechanism as a radical binder and ROS (Reactive Oxygen Species) capture, so the body will need antioxidants that will protect against free radical attacks by eliminating the negative impact of these compounds. Other researchers support the potential of Channa striata extract as an antioxidant (Rasimi et al., 2020).

Furthermore, another study from (Rusmana et al., 2017) confirmed that meniran extract (Phyllanthus niruri) and quercetin have great potential as natural sources of antioxidants. This is different from the research of (DARMAWAN, n.d.) which can be concluded that the combination of meniran extract and gotu gotu is administered orally at a dose of 50:50 mg/KgBB; 250: 250 mg/KgBB; and 1250:1250 mg/KgBB had no effect on SGOT, SGPT, BUN and creatinine of Wistar strain mice on sub-chronic administration for 2 consecutive days.

Based on research that has been conducted in China in (Naufal et al., 2020) shows that the administration of curcumin extract to rats with acute liver injury due to alcohol can significantly reduce SGOT/SGPT levels in the high-dose group. Curcumin contained in curcumin has antioxidant effects that can neutralize ROS, including hydroxyl radicals (H2O) and nitric oxide (NO). Meanwhile, the xanthorrhizol content in temulawak is known to provide anticancer, antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive, antiplatelet, nephroprotective, and hepatoprotective effects (Widyastuti et al., 2021).

The decrease in SGOT and SGPT levels in mice with diabetes mellitus who had a high fat diet after the administration of capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) showed that the test liver material could be used as an alternative in overcoming the problem of liver function damage caused by DM complications.

The administration of pioglitazone for 26 days provided a typical histopathological picture of the liver, namely the presence of hepatocyte hypertrophy and lymphocyte infiltration in groups 3 and 5. Meanwhile, in the other treatment groups, there were no abnormalities.

Conclusion

Based on the results of research that has been conducted on the effects of Channa striata, Phyllanthus niruri L., and Curcuma xanthorizza on liver improvement in diabetic melitus rats with liver fibroblast complications, several conclusions can be drawn that capsules containing a combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) have an effect in reducing SGOT and SGPT levels in mice model diabetes mellitus with a high fat diet. Diabetic mice that had been induced by aloxan and a high-fat diet with a combination capsule of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), and curcuma (Curcuma xantorrhiza) had a histopathological picture of the liver that was close to normal rats.

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