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THE EFFECT COMBINING SNAKEHEAD FISH EXTRACT, MENIRAN, AND TEMULAWAK ON GLYCEMIC STATUS AND PANCREATIC HISTOPATHOLOGY IN HIGH-FAT DIET DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, frequently associated with obesity, dyslipidemia, hypertension, and cardiovascular complications, particularly in type 2 diabetes mellitus (DM). In Indonesia, traditional medicine is often favored for its safety and cost-effectiveness compared to synthetic drugs. This study involved 25 male Wistar rats, which were divided into five groups: a control group, an alloxan and high-fat diet group, a pioglitazone group, a plant extract group, and a combination group. Obesity was induced over 27 days through a high-fat diet, followed by the administration of alloxan to elevate blood glucose levels. Glucose measurements were taken at specified intervals, with HbA1c assessed on day 26, and pancreatic histopathology was analyzed post-study. The Kruskal-Wallis test revealed significant differences in weight gain ($p = 0.016$) and blood glucose levels ($p = 0.003$) among the groups. Although no significant difference was observed in blood glucose reduction ($p = 0.05$), the combination group exhibited the most substantial decrease. One-way ANOVA results demonstrated that the combination of the extract and pioglitazone significantly reduced HbA1c levels ($p < 0.001$), with the second group showing the highest levels and the fourth group displaying the most pronounced reduction. Histopathological analysis indicated damage to the islets of Langerhans in both the pioglitazone and extract groups. The combination of snakehead fish extract, meniran, temulawak, and pioglitazone effectively lowers blood glucose and HbA1c levels; however, it does not appear to ameliorate islet damage, likely due to oxidative stress resulting from the treatments.

KEYWORDS Diabetes mellitus , Snakehead Fish Extract, Meniran, Temulawak, Glycemic Status, Pancreatic Histopathology.

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INTRODUCTION

The pancreas is an accessory organ in the digestive system that is located in the Retroperitoneal and is flattened like a sponge, measuring about 12 to 15 cm long and 2.5 cm thick. The pancreas comprises three parts: a head surrounded by a duodenum, a central or torso, and a blunt and pointed tail on the left. The pancreas acts as exocrine and endocrine glands. The islands of the pancreatic Langerhans tend to be more abundant in the tail of the pancreas, while the head of the pancreas is dominated by exocrine tissue. About 99% of the pancreatic structure is exocrine tissue that secretes 1,200 to 1,500 mL/day of pancreatic fluid. In the endocrine part, the island of Langerhans pancreas produces insulin and glucagon (Saladin et al., 2010).

Diabetes mellitus (DM) is a group of common metabolic disorders in which hyperglycemia is the main feature. The variation of the type of diabetes mellitus is influenced by the complex interaction between genetic and environmental factors. Diabetes mellitus is a condition in which there is damage to the cells of the pancreatic Langerhans (in the form of necrosis and vacuolisation), thus indicating damage or breakdown in the beta (Jameson, 2010) cells of the Langerhans. In this state, lymphocytes can penetrate into the pancreatic Langerhans, thus indicating the presence of an autoimmune process against the Langerhans beta (β) cells. Based on the causes, factors that trigger hyperglycemia include decreased insulin secretion, inefficient use of glucose, and increased glucose production (Jameson, 2010) (Margaretha, 2016).

Diabetes mellitus is closely related to obesity, specifically type 2, and is a risk factor for dyslipidemia, hypertension, as well as heart and vascular disease, which later becomes the main complication and leading cause of death in individuals with type 2 diabetes mellitus. In addition, a lifestyle that lacks physical activity, leading to overweight and obesity, is one of the factors that can be changed in the occurrence of diabetes mellitus. Other risk factors such as an unbalanced diet, a history of impaired glucose tolerance, or disturbances in fasting blood glucose levels (Ardiani et al., 2021) (N. N. Sari, 2018).

In type 1 diabetes mellitus, pancreas's the beta (β) ceeas are damaged by autoimmune processes, resulting in the absence of insulin production. This condition leads to fasting hyperglycemia, in which the liver produces glucose without proper regulation. Although glucose from food circulates in the blood and causes *postprandial* hyperglycemia, the liver is unable to store that glucose. When blood glucose levels are very high, the kidneys are unable to reabsorb the glucose that has been filtered, causing glucose to appear in the urine, known as diabetes. The osmotic diuresis process is the process of excretion of excess glucose along with excess electrolytes, which results in increased frequency of urination (polyuria) and excessive thirst (polydipsia) (Lestari & Zulkarnain, 2021).

In type 2 diabetes mellitus, insulin resistance in muscle and liver cells and pancreatic beta cell dysfunction are the central damaging pathophysiology of type 2 diabetes mellitus. Recent research suggests that pancreatic beta cell failure occurs earlier and is more severe than previously thought. Other organs that participate in type 2 diabetes mellitus are adipose tissue (experiencing increased lipolysis),

gastrointestinal (experiencing incretin deficiency), alpha cells of the pancreas (experiencing *hyperglycagonia*), kidneys (experiencing increased glucose absorption), and brain (experiencing insulin resistance) thus causing impaired glucose tolerance. Currently, three new pathogenesis pathways have been identified in the concept of "*ominous octet*," which plays a role in the occurrence of hyperglycemia in type 2 diabetes mellitus (Indonesia, 2021).

Patients with diabetes mellitus need a variety of treatments to reduce the risk of micro and macrovascular complications. Drugs from the *thiazolidindione* group, such as pioglitazone, can be used as one of the options in the treatment of diabetes mellitus. This drug works by increasing sensitivity to insulin so that it is able to overcome insulin resistance problems and its complications without causing hypoglycemia. However, the use of this group of drugs can cause side effects such as edema, weight gain, heart failure, and risk of fractures. (Lebovitz, 2019) (Malihah & Emelia, 2022)

In Indonesia, many people prefer the use of traditional medicine as a method of prevention and treatment of various diseases, including diabetes mellitus (DM), rather than using synthetic drugs. The reason is that traditional medicines are claimed to have fewer side effects, are affordable, and are easy to get. Traditional medicine can be in the form of ingredients or mixtures of plants, animals, minerals and galenic preparations that have been used for generations to overcome various diseases, including diabetes mellitus (Anam et al., n.d.).

One of the natural ingredients that can be used for the treatment of diabetes is a combination of snakehead fish *(Channa striata),* meniran *Phyllanthus niruri* L.*),* and temulawak (*Curcuma xanthorrhiza),* which are available in finished capsule preparations. *Channa* striata extract is known as a source of animal protein that is rich in essential nutrients, which plays an important role in increasing the body's stamina after childbirth, surgery, and the recovery process from certain diseases, and has anti-inflammatory, antioxidant and protective abilities against peptic ulcers (Yulizal et al., 2020) .α −glucosidase. This enzyme is responsible for converting carbohydrates into glucose. Thus making it effective in helping to control glucose levels in the blood (Soniya & Fauziah, 2020).

Temulawak *(Curcuma xanthorrhiza)* is a plant native to Indonesia that has the potential to be an antidiabetic. Secondary metabolites contained in curcuma are believed to reduce blood glucose levels. In addition, (Nurcahyani, 2022)*Curcuma xanthorrhiza* has properties such as anti-inflammatory, antibacterial, antioxidative, neuroprotective, nephroprotective, antitumor, and hepatoprotective activity (Grace Et Al., 2021).

Green Meniran *(Phyllanthus niruri* L.) is a plant that is known to have effects that can increase the body's immune system or immunostimulant. Its main contents include flavonoids, phyllants, *hypophylantines*, resin, and tannins. These ingredients are believed to be beneficial as diuretics, antioxidants, anti-inflammatory, antidiabetic, and antipyretic, and can increase appetite. In medical activities, this plant is used to treat various conditions, such as kidney stones, dyspepsia, hepatotoxicity, and has the potential as an antihyperglycemic agent.

However, studies on the antihyperglycemic and antioxidant potential of meniran (H. Sari et al., 2019). *(Phyllanthus niruri* L.*)* is still fairly rare (Kumar et al., 2019).

Based on the description above, extracts from snakehead fish *(Channa striata),* meniran *(Phyllanthus niruri* L.*),* and temulawak (*Curcuma xanthorrhiza)* show potential as antihyperglycemic agents in the treatment of diabetes mellitus. Based on these findings, researchers became interested in further studying the effects of animal and plant extracts formulated in combination capsule preparations as an alternative treatment for diabetes mellitus.

RESEARCH METHOD

This study is an experimental research with a randomized post-test-only control group design. The object of the study used was male rats of the Wistar strain (Rattus norvegicus) induced by aloxan to assess the glycemic status and histopathological picture of the pancreas. This research has received an ethical permit with the number 046/KEPK/UNPRI/III/2024.

The research data source consisted of 30 male rats of the Wistar strain aged 6-8 weeks with a body weight of 150-250 grams, obtained from the Ellio Medan Laboratory. The study population was male rats of the Wistar strain, while samples were taken based on strict inclusion and exclusion criteria. The inclusion criteria included healthy mice, had no anatomical abnormalities, and had not been used in previous studies. Exclusion criteria included mice that died during the study or were not actively moving. The sample size was calculated using Federer's formula, resulting in the need for a minimum of 5 rats per treatment group, which was then rounded to 6 rats per group to anticipate the death of rats.

Research techniques and tools include providing a high-fat diet to induce diabetes and using capsules with a combination of snakehead fish extract, temulawak, and meniran, as well as pioglitazone as a positive control. Blood glucose levels were measured using a glucometer, while HbA1c levels were measured using an i-CHROMA™ device. Pancreatic histopathology preparations were made using the paraffin technique, stained with Hematoxylin and Eosin (HE), and examined using the Olympus Cx-21 microscope.

Data analysis was carried out using the SPSS version 29 program. The data obtained were tested for normality using the Shapiro-Wilk test. For the analysis of the effect of the combination of extracts on the glycemic status of mice, a variant analysis (ANOVA) was used if the data were normally distributed ($P > 0.05$). In contrast, the Kruskal-Wallis H test was applied to the normally undistributed data ($P < 0.05$), in order to determine the mean difference in results between each treatment group.

RESULT AND DISCUSSION

Effect of High Fat Diet in Rats

The study showed that mice in the group before and after the treatment experienced weight gain as a result of feeding a high-fat diet. Each group experienced an average weight gain of 10 grams. The following table shows the complete data on the weight changes:

Table 1. Shapiro-Wilk Test of Rat Body Weight

Parameters	Treatment Groups	Mean ± SD	P value	Distribution Data
Initial Weight Loss	**K-1**	151.20 ± 1.30	0,421	Usual
	K-2	151.80 ± 1.92	0,223	Usual
	K-3	152.00 ± 0.70	0,325	Usual
	K-4	150.40 ± 0.54	0,314	Usual
	K-5	151.80 ± 1.23	0,006	Abnormal
Weight Loss After Treatment	**K-1**	153.80 ± 2.07	0,656	Usual
	K-2	164.80 ± 0.83	0,314	Usual
	K-3	162.60 ± 5.77	0,866	Usual
	K-4	164.60 ± 4.50	0,957	Usual
	K-5	164.40 ± 1.67	0,314	Usual

The results of the Shapiro-Wilk test, based on the data above, show that certain groups have a p < value of 0.05 while other groups have a p > value of 0.05. This indicates an abnormal distribution of weight data. Therefore, the analysis continued by applying the Kruskall-Wallis H non-parametric test to assess the significant weight difference between body weight before and after the high-fat diet treatment.

Table 2. Kruskal-Wallis Test of Rat Body Weight

Parameters	Group Treatment	Weight (Gram)			P value
		Median	Min	Max	
Initial Weight Loss	**K-1**	150	150	150	0,128
	K-2	152,5	152	153	
	K-3	152	151	153	
	K-4	151	150	152	
	K-5	150	150	151	
Weight Loss After Treatment	**K-1**	153	150	157	0,016
	K-2	165	164	166	
	K-3	162	155	171	
	K-4	165	158	170	
	K-5	164	162	166	

The results of the Kruskal-Wallis test showed that the value on body weight after treatment showed a p-value of 0.016 (< 0.05), which indicates a significant influence of high-fat diet on the difference in body weight of male rats of the Wistar strain (Rattus norvegicus). The weight of the rats in this study ranged from 150 grams to 171 grams.

Effect of Aloxan Induction on Increase in KGD and Extract Administration on Decrease in KGD in Rats

The results showed that blood glucose levels were in the normal range before naloxone induction, after induction, and after administration. Each group showed hyperglycemia conditions with glucose levels of more than 300 mg/dL and a decrease in blood glucose levels after 14 days of administration of the extract can be seen in the table below:

Table 3. Shapiro Wilk Test Results for Each Treatment Group

Treatment Groups	Mean ± SD				Shapiro Wilk (Sig.) Normality Test			
	Before	After	D+4	D+14	Before	After	D+4	D+14
K-1	83 ± 3.39	130.4 ± 2.97	124.2 ± 6.30	117.2 ± 3.35	0.564	0.777	0.414	0.616
K-2	80.8 ± 5.63	469.2 ± 90.6	466.6 ± 132.12	399.4 ± 82.18	0.272	0.169	0.129	0.341
K-3	88.6 ± 5.08	478.6 ± 121.08	396.8 ± 132.04	317.8 ± 82.17	0.965	0.215	0.515	0.642
K-4	89.6 ± 8.08	402.0 ± 128.18	374.4 ± 85.05	212.8 ± 143.73	0.548	0.914	0.957	0.018
K-5	92.0 ± 5.83	428.8 ± 69.08	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 rats after induction with aloxan 100 mg/kgBB, with an increase in blood glucose levels of around 300 mg/dL in the treatment group that received aloxane. On the 14th day, treatment with a combination of snakehead fish *extract (Channa striata),* meniran *(Phyllanthus niruri* L.), curcuma *(Curcuma xanthorrhiza),* and pioglitazone lowered blood glucose levels in 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 (Aloxan + *high-fat diet* + *pioglitazone* on day 14) p < 0.05 showed abnormal data distribution. Therefore, the *Kruskall-Wallis test* was conducted to test for significant differences between the groups.

Table 4. Kruskal-Wallis Test After Administration of H+14 Extract

Treatment	*Test Statistic (D+14)*		
	Kruskall Wallis H	***Df***	***Asym. Sig***
K-1	16,315	4	0,003
K-2			

K-3	
K-4	
K-5	

Based on the Kruskal-Wallis test, a *p-value* of 0.003 (< 0.05) was obtained; this value showed that there was a significant difference in the average blood glucose level in male rats of the Wistar strain *(Rattus norvegicus)* on D+14. To further examine the differences between the treatment groups, a Mann Whitney post hoc test was performed, as seen in the following graph:

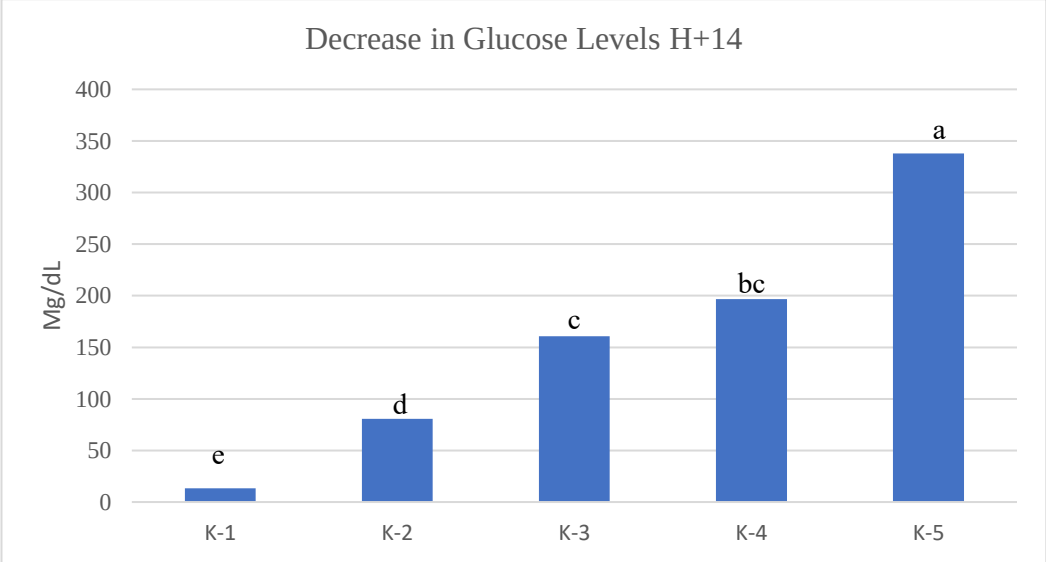

Figure 1. Graph of H+14 Glucose Level Decrease

The same letter notation showed that there was no difference in the reduction of glucose levels between treatments, based on *Mann Whitney's post-hoc test* at the singnifiability level of α = 0.05. The K-5 treatment (aloxan + *high fat diet* + pioglitazone + extract) showed the highest average decrease in glucose levels compared to other treatments. This shows that the K-5 treatment is the most effective in lowering blood glucose levels in the Wistar strain male rats *(Rattus norvegicus).* Thus, it can be concluded that the combination of snakehead fish extract, meniran, temulawak, and pioglitazone significantly lowers blood glucose levels.

Effectiveness of Snakehead Fish Extracts, Meniran, Temulawak, and Pioglitazone on HbA1c Levels in Wistar Rats

HbA1c measurements were performed to assess glycemic control in people with diabetes mellitus. HbA1c was measured after the mice underwent acclimatization, aloxane induction, and treatment with snakehead fish extracts, meniran, temulawak, and pioglitazone for 26 days. The examination of HbA1c levels in rat serum obtained the results in the following table:

Table 5. HbA1c Levels in Mouse Serum

Group	Result	Units
K1 (Normal)	3.7	%
K1 (Normal)	3.6	%
K1 (Normal)	4.0	%
K1 (Normal)	3.5	%
K1 (Normal)	3.8	%
K2 (Aloxan + CMC + *High Fat Diet*)	9.3	%
K2 (Aloxan + CMC + *High Fat Diet*)	8.8	%
K2 (Aloxan + CMC + *High Fat Diet*)	8.9	%
K2 (Aloxan + CMC + *High Fat Diet*)	9.0	%
K2 (Aloxan + CMC + *High Fat Diet*)	8.7	%
K3 (Aloxan + *High Fat Diet* + Pioglitazone)	3.9	%
K3 (Aloxan + *High Fat Diet* + Pioglitazone)	3.8	%
K3 (Aloxan + *High Fat Diet* + Pioglitazone)	4.1	%
K3 (Aloxan + *High Fat Diet* + Pioglitazone)	3.6	%
K3 (Aloxan + *High Fat Diet* + Pioglitazone)	4.2	%
K4 (Aloxan + *High Fat Diet* + Extract)	3.2	%
K4 (Aloxan + *High Fat Diet* + Extract)	3.3	%
K4 (Aloxan + *High Fat Diet* + Extract)	3.1	%
K4 (Aloxan + *High Fat Diet* + Extract)	3.5	%
K4 (Aloxan + *High Fat Diet* + Extract)	3.6	%
K5 (Aloxan + *High Fat Diet* + Pioglitazone + Extract)	4.3	%
K5 (Aloxan + *High Fat Diet* + Pioglitazone + Extract)	4.0	%
K5 (Aloxan + *High Fat Diet* + Pioglitazone + Extract)	3.9	%
K5 (Aloxan + *High Fat Diet* + Pioglitazone + Extract)	4.1	%
K5 (Aloxan + *High Fat Diet* + Pioglitazone + Extract)	3.5	%

The HbA1c level was first analyzed using the Shapiro-Wilk test to check whether the data was distributed normally, and the results of the normally distributed data were obtained ($p > 0.05$). Then the data was analyzed by the ANOVA one-way test. The results of this analysis are shown in the following table:

Table 1. Normality Test and One Way ANOVA

Group Treatment	Mean ± SD	Normality Shapiro Wilk	One Way Annova	
			F Calculate	*P-value*
K-1	3.72 ± 0.19	0,928		
K-2	8.94 ± 2.32	0,685		
K-3	3.92 ± 0.23	0,899	445,193	< 0.001
K-4	3.34 ± 0.20	0,754		
K-5	3.96 ± 0.29	0,777		

The results of *the ANOVA one-way* test showed a *p-value* of < 0.001, which indicates that the combination of snakehead fish extract, meniran, and temulawak significantly affected the decrease in HbA1c levels in the serum of male rats of the wistar strain *(Rattus norvegicus)* after 26 days of treatment. To identify further

differences between treatment groups, a *post hoc test of Tukey LSD* was performed, as seen in the graph below:

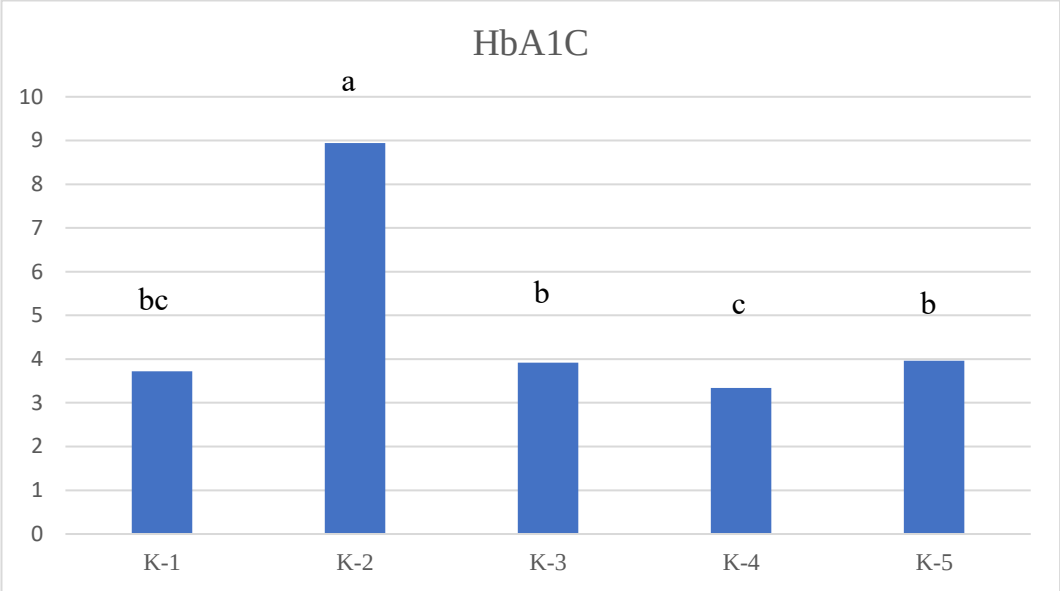

Figure 2. Tukey LSD Post Hoc Chart

The same letter notation showed no significant difference in the decrease in HbA1c levels between treatments based on *the Tukey LSD post hoc test* at a significance level of α = 0.05. The K-2 treatment (aloxan + *high fat diet)* showed the highest average HbA1c levels compared to other treatments, while the K-4 treatment (aloxan + *high fat diet* + extract) had the lowest average HbA1c levels. This showed that K-4 was the best treatment group in lowering HbA1c levels in male rats of the Wistar *strain (Rattus norvegicus)* compared to other treatments. Therefore, it can be concluded that the combination of snakehead fish extract, meniran, and temulawak has a significant effect on reducing HbA1c levels in rats.

Overview of Pancreatic Histopathology in Diabetes Mellitus Model Rats with *a High Fat Diet* After Treatment

The results of the study showing the effect of giving a combination of snakehead fish *(Channa striata),* meniran *(Phyllanthus niruri L.),* and temulawak *(Curcuma xanthorrhiza)* extracts for 26 days can be seen in Figure 3.

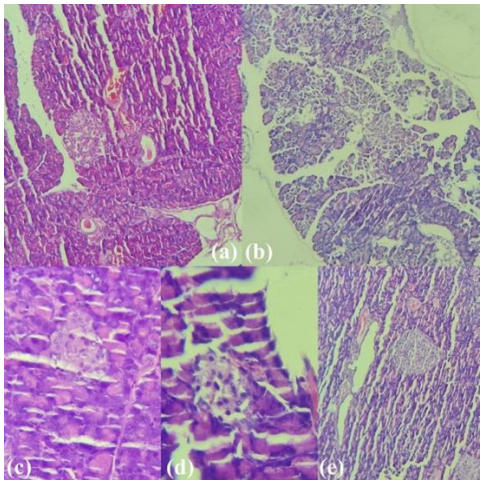

Figure 3. Overview of Histopathology of the Rat Pancreas

The histological picture of the pancreas of each treatment group shows differences, but there is one thing in common, namely the absence of atrial cell desquamation, as shown in Figure 3. The picture of pancreatic histology did not change in the normal group (a). Group 2 (b) (aloksan + CMC + *high fat diet*) also showed no abnormalities. Group 3 (c) (aloksan + *high fat diet* + pioglitazone) showed Langerhans islet atrophy, cell nucleus picnicosis, as well as fading cytoplasm on the pancreatic Langerhans islet. Group 4 (d) (aloksan + *high fat diet* + extract) showed similar results to group 3 (c), namely Langerhans islet atrophy, cell nucleus picnicosis, and fading cytoplasm on the pancreatic Langerhans islet. Group 5 (e) (aloxan + *high fat diet* + pioglitazone + extract) showed no abnormalities.

Discussion

The treatment group was given *a high-fat diet* for about 26 days, with additional feed in the form of lard 3 grams/200 gramsBB and duck egg yolk 2 grams/200 gramsBB, while group 1 (negative control) did not get any treatment. The treatment results showed that *a high-fat diet* increased the weight of rats by 10 grams. The analysis of the *Kruskal-Wallis test* showed that there was a significant difference in the weight gain of male rats of the Wistar strain with a value of p = 0.016 (< 0.05) between the treatment groups. Anonymous (2014), states that dyslipidemia is often associated with obesity and metabolic syndrome, including diabetes mellitus. Research using Wistar rats fed an (Indriputri & Maulana, 2022) ,*50-day high-fat diet* containing 0.86 g of lard and 0.43 g of chicken egg yolk showed an increase in blood glucose levels and lipid profiles.

Intraperitoneal induction of aloxan at a dose of 100 mg/kgBB led to an increase in blood glucose levels in groups 2, 3, 4 and 5 up to 300 mg/dL. After the increase, male rats of the Wistar strain were given a combination of snakehead fish extract, meniran, and temulawak, as well as pioglitazone for approximately 26 days. On the 14th day after the combination of the extract and pioglitazone, blood glucose levels dropped to 100 mg/dL. The *Kruskal-Wallis* test on day 14 showed a significant difference in blood glucose levels of male rats of the Wistar strain with a value of p = 0.003 (< 0.05) between treatment groups. The *Mann-Whitney test*

showed that there was no significant difference in the reduction of blood glucose levels between the treatment groups (p = 0.05). However, figure 13 shows that group 5 experienced the largest decrease in blood glucose levels compared to other groups. This showed that the combination of pioglitazone at a dose of 0.27 mg/200 gramsBB and the combination of extract at a dose of 9.9 mg/200 gramsBB was the most effective in lowering blood glucose levels.

The combination of snakehead fish extract, meniran, temulawak and pioglitazone for approximately 26 days had an effect on HbA1c levels in male rats of the Wistar strain. Based on the *ANOVA one-way test*, the combination of extract and pioglitazone significantly reduced HbA1c levels with a p < value of 0.001. *The Tukey LSD post hoc* test showed that there was no significant difference in the reduction in HbA1c levels between the treatment groups (p = 0.005). However, group 2 had the highest average HbA1c levels compared to other groups. Meanwhile, group 4 (aloksan + *high-fat diet* + extract) with a dose of 9.9 mg/200gramBB showed the most optimal decrease in HbA1c levels. In this study, drug interactions were also found. The interactions that occurred were in the form of synergistic/additive interactions and antagonists that could potentially cause *adverse drug reactions* (Al Mukminah & Indradi, 2021). In group 5, HbA1c levels decreased by 3.96%, while group 4 decreased by 3.34%. From these results, it can be concluded that there is an interaction between the combination of the extract and pioglitazone that affects the decrease in HbA1c levels in group 5.

Research shows that therapy with snakehead fish extract at a dose of 300 mg/kgBB and metformin at a dose of 45 mg/kgBB is better than single therapy with EIG or metformin. In addition, it was found that meniran leaf extract at a dose of 200 mg/kgBB lowered blood glucose levels from 200 mg/dL to 90 mg/dL. The study also supported these findings with a dose of 18 mg/kgBB of temulawak extract could lower blood glucose levels from 206 mg/dL to 123 mg/dL, while a dose of 20 mg/kgBB lowered glucose levels to 101 mg/dL from 214 mg/dL. (Nurcahyani, 2022) (Yulizal et al., 2021) (H. Sari et al., 2019)

The combination of snakehead fish extract, meniran, temulawak, and pioglitazone for 26 days also provided a typical histopathological picture of the pancreas, especially Langerhans Island. In groups 3 and 4, it was shown that there was atrophy of Langerhans island, pyrosis of cell nucleus, and fading cytoplasm on Langerhans island pancreas and no desquamation was found. The other treatment groups did not show any abnormalities.

The study showed that histopathology in the pancreas of rats with aloxan-induced hyperglycemia showed that Langerhans Island was atrophied. In addition, it is also seen that the cell nucleus with psychosis or condensation of the cell nucleus is darker due to more intense staining. Langerhans Island the pancreas undergoes desquamation and cytoplasm that appears to fade. stated that various factors can cause pincosis, atrophy of the cell nucleus, and changes in the histopathology of the Langerhans islet pancreas of mice. Oxidative stress, which occurs as a side effect of pioglitazone, triggers the degeneration of endocrine cells, leading to picnicnics and atrophy. In addition, the presence of bioactive compounds such as flavonoids, which act as antioxidants can have different effects. The histopathological condition

of the pancreas may worsen if the dose or concentration of the extract is not appropriate or if there is a negative interaction with pioglitazone Solikhah (2024) (Walean et al., 2020).

(Maharani et al., 2023) revealed that aloxan does not always cause morphological changes in pancreatic tissue, such as pincosis or atrophy, although it can trigger necrosis. Aloxans tend to cause necrosis without causing more subtle changes, such as atrophy or pincosis. It is also suggested that histopathological results are affected by the dose of aloxan and the method of administration. Lower doses or certain methods of administration, such as intraperitoneal, may not be strong enough to alter significant histopathological changes, so the resulting picture differs from those of higher doses or other methods (Wulandari et al., 2024)v.

Several natural compounds work together to lower blood glucose levels HbA1c levels and improve the histopathological picture of the pancreas observed in this study. The amino acids in snakehead fish proteins, such as arginine and leucine, control blood glucose levels in hyperglycemia. The antihyperglycemic and anti-inflammatory properties of (Soniya & Fauziah, 2020) *C.xanthorrhiza* and xanthorrhizole extracts make it an effective antidiabetic agent in the treatment of diabetes mellitus. In addition, meniran contains alkaloids that function as antipyretic, antidiarrheal, and antidiabetic. Filantin and hypophyllant compounds that function as hepatoprotectors, as well as flavonoids, quercetin and nirurin that act as anti-carcinogens, also function as diuretics (Maulida & Indradi, 2019), (Risnawati et al., 2021).

CONCLUSION

The study findings indicate that the combination of snakehead fish extract (Channa striata), meniran (Phyllanthus niruri L.), curcuma (Curcuma xanthorrhiza), and pioglitazone is an effective method for reducing blood glucose levels in male Wistar rats. Furthermore, administration of a combination of snakehead fish extract, meniran, and curcuma for a period of 26 days demonstrated a notable reduction in HbA1c levels, indicative of enhanced glycemic control. However, histopathological analysis demonstrated that neither pioglitazone nor the combined treatment resulted in an improvement in the condition of the islets of Langerhans. This lack of improvement may be attributed to various contributing factors, including oxidative stress resulting from pioglitazone's side effects, potential inaccuracies in extract dosage or concentration, and the impact of alloxan induction, including its specific dosage and administration methods. These findings highlight the combination's potential efficacy in managing blood glucose and HbA1c levels, but underscore the need for further investigation into optimal dosing and mitigation of associated oxidative stress to support pancreatic health.

Further research is needed to understand the effects of combining snakehead fish extract, meniran, and temulawak with pioglitazone, on glycemic status and pancreatic histopathology in male rats. Furthermore, comprehensive studies from a biomolecular perspective are essential to enhance insights into the mechanisms and potential benefits of these extracts in the treatment of diabetes mellitus. Such research could illuminate their therapeutic roles and guide more effective applications in the management of diabetes.

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