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ANTIDIABETIC ACTIVITY OF THE COMBINATION OF SEPAT RAWA FISH FLOUR (*Trichopodus thricopterus*) AND ETHANOL EXTRACT OF RAMANIA LEAVES (*Bouea Macrophylla Griffith*)

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ABSTRAK

Diabetes melitus merupakan penyakit metabolik dengan gangguan sekresi insulin. Ikan Sepat Rawa terbukti mengandung asam alfa-amino yang dapat meningkatkan kerja insulin dengan meningkatkan energi dalam siklus metabolisme sel. Daun ramania mengandung senyawa pengobatan antidiabetes seperti saponin dan flavonoid. Penelitian ini bertujuan untuk mengetahui dosis terbaik untuk meningkatkan aktivitas antidiabetes dari kombinasi tepung ikan sepat rawa dan ekstrak etanol daun ramania. Kegiatan penelitian ini menggunakan pre and posttest with control group design. Sebanyak 25 ekor mencit yang digunakan dibagi menjadi 5 kelompok, yaitu kelompok I: kontrol negatif (CMC Na 0,5%), kelompok II: kontrol positif (glibenklamid 3 mg/kgBB), kelompok III: tepung ikan sepat Rawa dosis 300 mg/kgBB. KgBB, kelompok IV: ekstrak daun ramania dengan dosis 300 mg/kgBB, kelompok V: kombinasi tepung ikan 300 mg/KgBB dan ekstrak etanol daun ramania 300mg/kg BB. Mencit yang diinduksi aloksan mengalami hiperglikemia. Pengamatan penurunan kadar gula darah masing-masing kelompok uji dilakukan pada hari ke 7 setelah induksi aloksan dengan cara mengambil darah ekor mencit secukupnya dan diukur menggunakan glukotes. Hasil analisis ANOVA (p=0,05) diperoleh bahwa ekstrak Ramania kelompok tunggal dosis 300 mg/KgBB menunjukkan aktivitas terbaik dengan persen penurunan kadar gula darah sebesar 28,438, dosis kombinasi sebesar 25,107% dan dosis Sepat Rawa sebesar 21,675%. Ketiganya tidak berbeda nyata dengan kontrol positif.

ABSTRACT

Diabetes mellitus is a metabolic disease with impaired insulin secretion. Sepat Rawa fish has been proven to contain alpha-amino acids, which can increase insulin action by increasing energy in the cell metabolic cycle. Ramania leaves contain compounds for antidiabetic treatment, such as saponins and flavonoids. This study aims to find the best dose to increase the antidiabetic activity of the combination of sepat rawa fish meal and ethanol extract of ramania leaves. This research activity uses pre- and post-tests with a control group design. The 25 mice used were divided into 5 groups, namely group I: negative control (CMC Na 0.5%), group II: positive control (glibenclamide 3 mg/kgBW), group III: sepat Rawa fish meal dose of 300 mg/kg. KgBW, group IV: ramania leaf extract at a dose of 300 mg/kg BW, group V: a combination of fish meal 300 mg/KgBW and ethanol extract of ramania leaf 300mg/kg BW. Mice were induced by alloxan to experience hyperglycemia. Observations on the decrease in blood sugar levels of each test group were carried out on the 7th day after alloxan induction by taking sufficient blood from the tails of mice and measuring using glucose. Data was analyzed for variance (ANOVA) (p=0.05). The result is that a single group of Ramania extract with a dose of 300 mg/KgBB shows the best activity with a percent decrease in blood sugar levels by 28.438, a combination dose of 25.107 %, and a Sepat Rawa dose of 21,675%. The Third of them was not significantly different with positive control.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease with hyperglycemic characteristics. Diabetes mellitus (DM) is classified into two major diseases: type 1 DM and type 2 DM (Chaidir, 2017). Type 1 DM is an autoimmune disease in which the immune system of the body attacks insulin-producing pancreatic β cells, resulting in low insulin production. This condition leads to insulin-dependent DM. On the other hand, type 2 DM was caused by inadequate insulin action in the action site (Hossain *et al.*, 2016). The number of people with DM in the world has significantly increased, and it was found that as many as 425 million people in 2017 will increase to 592 million people in 2035 (IDF, 2017). Most types of DM suffer, and the prevalence continues to increase in type 2 DM with the number of cases the most, that is, with 90% of all cases of DM in the world (WHO, 2013).

Treatment is carried out by people with diabetes mellitus, like injection or the use of oral antidiabetic. Treatment in this way has side effects, including gastrointestinal disorders, water retention, and hyponatremia. In addition, another challenge is that it requires high costs due to its long-term use, so people with diabetes mellitus use regulating diet health and balance. They also use traditional methods to treat and control blood glucose levels (Putri & Isfandiari, 2013; Hidayaturrahmah et al., 2017; Chatterjee *et al.*, 2018). Besides being easy to obtain, the price is relatively cheap, and there are fewer side effects compared to synthetic drugs.

Treatment of diabetes mellitus has used a lot of plants, while the use of combination with animals is still very minimal. Therefore, animal products with a combination of plants should further enliven the trend of functional food in the markets of this country because the nutritional content in animals has the potential to be developed as an alternative treatment using natural ingredients (Prastari et al., 2017). Sepat rawa (*Trichopodus thricopterus*) are animals that are commonly found in Kalimantan and have a scope of life in Raway areas (Athar & Vitas, 2014). Another study showed that there was an increase in protein content of 8.7% in the quality of the sample after the addition of sepat rawa fish. Furthermore, the nutritional content and essential amino acid profile of sepat rawa fish meal were higher than that of fresh fish samples (Putra *et al.*, 2017). The dose of 50 mg/kgBW of Sepat Rawa Flour has the best tonic effect compared to the dose of 100 mg/kgBW and 150 mg/kgBW (Astuti *et al.*, 2023). Sepat rawa Flour with a dose of 300 mg/kgBW is known to have an anti-diabetic activity that has the potential to reduce blood glucose levels (Astuti & Fitriyanti, 2020). In test toxicity with a dose of 5000 mg/ kgbw, test animals do not show symptoms of acute toxicity as observed in previous doses. The value of LD_{50} obtained more than 5000 mg/ kgBW (Fitriyanti *et al.*, 2021). The test of chemical analysis showed that the protein content was 44.84%, the fiber content was 1.88%, the fat content was 4.12%, the ash content was 14.63%, and the water content was 5.98% (Endah *et al.,* 2022).

Picture 1. Sepat Rawa Fish

One of the typical Kalimantan plants that can be used as a DM treatment is ramania leaf (*Bouea macrophylla* Griffith). The chemical content contained in ramania leaves is flavonoids with levels of 167.06 g/mg (Rahman *et al*, 2017). Flavonoids can reduce blood glucose levels by inhibiting phosphodiesterase, thereby increasing cAMP in pancreatic beta

cells (Puspita et al., 2013). Increased cAMP will stimulate the release of protein kinase A (PKA), which stimulates insulin secretion to increase (Harapan et al., 2010).

Picture 2. Ramania's Leaves

There has been no research related to the specific potential of the combination of Rawa fish and ramania leaves which are commonly found in the area of South Kalimantan. So, this study was conducted to determine the synergistic effect in the form of increasing antidiabetic activity that occurred in test animals after being given a combination of sepat rawa fish and ramania leaves, which can be developed as an alternative medicine for diabetes.

METHODS

Materials

The Fish were collected from Amuntai, South Kalimantan. Ramania leaves were collected from Martapura City, South Kalimantan. The experimental animal in this research is male Balb/c mice about ages 6-8 weeks, with a body weight of about 20-35 grams. Other materials include Na-CMC, glibenclamide, alloxan (sigma), and 70% ethanol.

Tools

Tools are oven (Thermo Scientific®), rotary evaporator (IKA®), analytical balance (Ohaus®), dan waterbath (Memmart®).

Animal determination

Animal samples identified as *Trichopodus trichopterus*, determined at Ichtiologi Laboratory, Zoology Research Center for Biology, Cibinong, Bogor, Indonesia, with number B-5267/IPH.1/Ks.02.03/XII/2019.

Plant determination

Plant samples identified as *B. macrophylla* were determined at FMIPA Laboratory, Lambung Mangkurat University, Banjarbaru, South Borneo, Indonesia, with number 126a/LB.LABDASAR/ VI/2022.

Preparation Of Making Flour from Fish Sepat Rawa

Samples were carried out by preparing fresh samples of Sepat Rawa fish obtained in the Amuntai city of South Kalimantan. Then, in the process of making the fish meal, the entrails, eyes, scales, and fins are removed, and the body parts are taken. Next, the sample was refined using a blender and then steamed at a temperature of ± 90°C for 20 minutes. After that, it was continued by the roasting process at a temperature of ±70°C for 80 minutes. To obtain a sample of fish in the form of powder (Astuti & Fitriyanti, 2020).

Preparation Extract of Ramania Leaves

Ramania leaves are obtained from the Martapura area, South Kalimantan. Fresh ramania leaves that have been picked and washed with water until clean and dry in the sun and covered with a black cloth. After drying, grind the ramania leaves until it becomes a powder. The powder was extracted using the maceration method using 70% ethanol as a solvent (Kumalasari et al., 2019).

Antidiabetic Activity Test (Decrease Blood Glucose in Test Animals)

His research was carried out after obtaining ethical approval number 42/Ka.Kom.et/70/KE/V/2023 from the ethics

committee of The Faculty of Medicine, Islamic University of Indonesia, Yogyakarta, Indonesia.

Before starting, the rats fasted for 18 hours (ad libideum), and then their blood glucose levels were measured using a glucometer and used as initial glucose levels. Diabetes induction was performed using alloxan intraperitoneally at a dose of 150mg/kg BW. After 48 hours, the rats showing blood glucose levels >200 mg/dL were divided into 5 groups : (a) The negative control group was given 0.5% NaCMC suspension (b) The positive control group was given glibenclamide at a dose of 0.5 mg/kg BW (c) Group 1 was given sepat rawa fish meal at a dose of 300 mg/kg BW (d) Group 2, was given ethanol extract of ramania leaves at a dose of 300 mg/kg BW (e) Group 3, was given a combination of sepat rawa fish meal and ethanol extract of ramania leaves with each dose of 300 mg/kg BW. Measurement of blood glucose levels was carried out on the 7th from the first day.

Data Analysis

Data on decreasing blood glucose levels from each group were carried out in triplicate. The decrease in blood glucose levels was analyzed using ANOVA (Analysis of Variance) using the SPSS program for a window with a 95% confidence level with (α= = 0.05)

RESULT AND DISCUSSION

Sepat Rawa fish was identified at the Research Center for Biology at the Indonesian Institute of Sciences. The result showed that the official name of the sample is *Trichopodus tricopterus*. Determination of animals is carried out to know the identity of an animal (Astuti & Fitriyanti, 2020)

The methods of making bog fish meal (T. trichopterus) in this study used steaming and continued with the roasting process. As for the leaves of ramania extracted by the maceration method. The yield of ramania extract was 13,46%, and Sepat Rawa Fish (*T. trichopterus*) was 15.67%. The high or low yield obtained in flour Rawa finches (*T. trichopterus*) can be affected by the manufacture of flour and the high water content of fish meat, so it can affect the yield value (Fatmawati & Mardiana, 2014).

In the results of the antidiabetic activity test, it was found that the average blood sugar level was blood and the average percentage decrease in blood sugar levels in mice. The results of the observations are shown in the following Table 1.

Table 1. Effect of Ethanol Extract, Flour Fish, and Combination In Alloxan Induced Diabetic Rats.

Groups	0^{th} Day (mg/dL)	2^{th} Day (mg/dL)	9^{th} Day (mg/dL)
Negative control	148±30,4	496,6±34,4	471,6±49,7
Positive control	140,5±29,3	347,6±42,2	258,4±60,8
Flour fish 300 mg/KgBW	167,4±64	370,8±51,9	297,8±48,7
Ramania 300 mg/KgBW	188,8±50,9	334,4±70,4	245,8±59,7
Combination	115,8±19,9	437,4±24,7	325,6±35,7

Based on Table 1, it can be seen that on day 0, before alloxan induction was given, the data obtained were blood sugar levels in mice below 200 mg/dL. This indicates that blood sugar levels are still in normal condition. Then, on the 2nd day after alloxan administration, all treatment groups were re-measured their blood sugar levels, and the results obtained were that the blood sugar levels of mice rose/above 200 mg/dL, which ranged from 334.4 mg /dL to 496.6 mg/dL. This is by the literature that alloxan can increase blood sugar levels in a relatively short time. Alloxan is one of the substances that are preferred because the price is lower than Streptozotocin (Susilawati et al., 2018). Alloxan is also selectively toxic to insulin-producing pancreatic beta cells, where the cytotoxic action of alloxan is mediated by free radicals (Samsul et al., 2020).

In group 1, Na-CMC was given as a negative control. Administration of Na-CMC was carried out every day for 7 days. On the 9th day, blood sugar levels were re-measured in mice, and the results showed a decrease in blood sugar levels from 496,6 mmHg to 471,6 mg/dL. The literature states that there is a

decrease in blood sugar levels from day 7 to day 14 after alloxan induction, where alloxan is reversible in causing damage to the pancreas.

As a comparison in the form of positive control, Glibenclamide was given for 7 consecutive days. come along. Glibenclamide is an oral anti-diabetic drug belonging to the sulfonylurea group that increases insulin secretion by pancreatic beta cells so that levels of blood sugar drop (Rudijanto et. al., 2015). On the 9th day, the blood sugar levels (BSL) were checked, and the results were a decrease in BSL to 258.4±60.8 from the average BSL after alloxan induction in the form of 347.6±42.2 with percent a decrease of 29.144%. This shows that Glibenclamide given to this group has the best antidiabetic activity. The treatment of the test sample group in the form of 300 mg/kg BW dose of Sepat Rawa fish meal saw a decrease in BSL from 370.8±51.9 to 297.8±48.7 with a percentage decrease of 21.675%. In the Sepat Rawa fish meal group at a dose of 300 mg/kg BW, the mice developed diabetes on day 2 with an average BSL of 370.8±51.9 mg/dL. Blood sugar levels on the 9th day after being given treatment decreased to 297±48.7 mg/dL, with a percent decrease of 21.675 %. Sepat Rawa's ability to lower blood sugar levels in test animal white male mice was affected by the presence of amino acids contained in fish meal sepat rawa (*T. trichopterus*) (Astuti & Fitriyanti, 2021). Amino acids such as leucine, arginine, lysine, alanine, phenylalanine, isoleucine, and methionine (Adawyah *et al.*, 2020). This is also supported by several studies. One of the other is from Karnila's research (2012), which reports that acid amino acids such as leucine, arginine, lysine, alanine, phenylalanine, isoleucine, and methionine can stimulate insulin secretion from pancreatic beta cells that it can reduce the level of glucose in the blood.

In the dose group of 300 mg/kg, BW Ramania extracts, there was a decrease of 28.438%. Ramania leaves contain compounds that are as efficacious as antidiabetics, like flavonoids and saponins. Mechanism flavonoids can increase insulin secretion so that it can lower blood sugar levels (Gumelar *et al*, 2017). Flavonoids have antioxidant properties that can protect the damage of pancreatic cells by free radicals. The mechanism of action of saponins is that they are efficacious as antidiabetics because they act as inhibitors of the enzyme glucosidase. The α-glucosidase enzyme is an enzyme that plays a role in converting carbohydrates to glucose. Thus, when the enzyme-glucosidase is inhibited, so the level of -glucosidase glucose (sugar) in the blood will decrease, causing a hypoglycemic effect and decreased blood sugar and can be used as an adjunctive treatment for metabolic disorders (Fiana & Oktaria, 2016); (Yuwen *et al*, 2017)

The percentage of decrease in the combination of extract and the fish meal was 25.107%, which means it was smaller than the positive control and the single extract group of ramania. Calculation of the percentage decrease in blood glucose levels of mice from each treatment group. The results are presented in Table 2.

Table 2. Percentage of Blood Glucose Levels in Alloxan-Induced Mice of Ethanol Extract, Flour Fish, and Combination.

Groups	Percentage of Blood Glucose Level (%)
Negative control	6,160
Positive control	29, 144
Flour fish 300 mg/KgBB	21,675
Ramania 300 mg/KgBB	28, 438
Combination	25,107

The percentage of blood sugar levels was analyzed using SPSS 23. The first test was in the form of normality and homogeneity tests where sig values were 0.072 and 0.240 (> 0.05) so that it could be concluded that the data were normal and homogeneous. The test was continued with a parametric test in the form of ANOVA to see whether there were differences between groups. The results were obtained in the form of a sig. value of 0.663. Then, the post hoc test was to

see which groups differed significantly. The results obtained were significant differences between the negative control group and the positive control group. While the flour dose of 300 mg/kg BW, the extract dose of 300 mg/kg BW, and the combination were not significantly different from the positive control. This indicates that all dose groups have antidiabetic activity.

CONCLUSION

The result is that a single group of Ramania extract with a dose of 300 mg/KgBB showed the best activity, with a 28.438% decrease in blood sugar levels, a combination dose of 25.107 %, and a Sepat Rawa dose of 21,675%. The Third group was not significantly different with a positive control.

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