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Antidiabetic Activity Test of Ethanol Extract Combination of Moringa Oleifera L. Leaves and Snakehead Fish *(Channa Striata)*

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ABSTRACT

Diabetes mellitus DM is a serious global health issue caused by the body's inability to use insulin optimally, resulting in uncontrolled blood sugar levels. Moringa leaves Moringa oleifera L. and snakehead fish Channa striata are known to have antidiabetic potential; moringa leaves contain quercetin, while snakehead fish are rich in arginine and leucine, which help regulate blood sugar levels. This study aims to test the antidiabetic activity of the combination of Moringa oleifera L. leaf ethanol extract and Channa striata ethanol extract CMOCS in male white mice. A total of 25 Wistar rats were divided into five groups: negative control distilled water, positive control glibenclamide 0.45 mg/kg body weight, and three combination groups of CMOCS 100, 200, 300 mg/kg body weight. Diabetes was induced by alloxan. Blood glucose levels were measured on days 0, 7, and 14. The results showed that alloxan successfully induced hyperglycemia. After 14 days, all combination groups reduced blood glucose levels. CMOC III was the most effective, reducing glucose levels by 31.91%, approaching glibenclamide 37.78%. The Mann-Whitney test showed significant differences CMOCS I produced a p-value of 0.021, CMOCS II a p-value of 0.036, and CMOCS III a p-value of 0.047 p < 0.05 between the negative control and the treatment groups, confirming the antidiabetic activity of this extract combination.

Keywords: *Diabetes mellitus DM , Moringa leaves Moringan oleifera L., Snakefish Channa striata, blood glucose levels*

INTRODUCTION

The International Diabetes Federation IDF reports that in 2021, there were approximately 537 million people worldwide suffering from diabetes mellitus DM between the ages of 20 and 79. Indonesia ranks fifth with 19.5 million cases of DM Feby Febriany, 2023. Diabetes mellitus is the third leading cause of death in Indonesia, with an estimated mortality rate of 14.7% in urban areas and around 5.8% in rural areas. The high mortality rate due to diabetes certainly has a negative impact on the life expectancy of the current Indonesian population and future generations Fauzi & Isnawati, 2023.

Diabetes mellitus is a condition where the body's metabolism is disrupted due to the hormone insulin, which is useful for maintaining blood sugar balance, not being able to function optimally, causing uncontrolled blood sugar Zakiudin *et al*., 2023 The cause of diabetes is a combination of genetic and environmental factors Lestari *et al*., 2021. When blood sugar is more than 126 mg/dL during fasting and more than 200 mg/dL for 2 hours, diabetes is diagnosed Bakri *et al*., 2023

In traditional medicine, one plant that is often used to lower blood glucose levels is moringa leaves *Moringa oleifera* L. Utami *et al*., 2022. According to research by Surya SR 2020, moringa leaf extract at a dose of 14 mg per 20 grams of body weight in rats successfully lowered blood sugar levels. In addition, moringa leaves have various other benefits such as facilitating urination, being anti-allergic and anti-inflammatory, and being used in the treatment of hepatitis, urinary tract infections, bacterial infections, as well as being anti-hypersensitive

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and antianemic Prasetya, 2021. The quercetin content in moringa leaves has antioxidant effects and can reduce glucose absorption in the intestines by inhibiting fructose and glucose transport, thereby helping to lower glucose levels systemically Bobaya *et al*., 2023

In addition to plants, natural ingredients from animals are also widely used in traditional medicine. One animal believed to lower blood sugar levels is the snakehead fish *Channa striata* Hasibuan, 2020. At a dose of 250 mg/kgBW, snakehead fish protein can help lower blood glucose levels Salsabila *et al*., 2024. The amino acid content of snakehead fish protein, namely arginine and leucine, functions to control blood glucose levels in hyperglycemia Soniya & Fauziah, 2020.

Based on this background, this study was conducted to examine the antidiabetic activity of a combination of ethanol extracts of moringa leaves *Moringa oleifera* L. and snakehead fish *Channa striata* in male white rats. Previous studies focused on the active fractions of ethanol extracts of bay leaves *Syzygium polyanthum* and snakehead fish *Channa striata* as antihyperglycemic agents in rats.

MATERIAL AND METHODS

Equipment and Materials

The tools used in this study were analytical scales, digital scales Electronic Kitchen Scale, water baths, injection syringes, probe syringes, scissors, animal cages, blood glucose testing equipment; glucometers Sinoheart and glassware. The materials used in this study were ethanol extract of moringa leavesBatu Materia Medica Herbal Laboratory, snakehead fish extract Crystal Of The Sea Official Shop, alloxan Kimia Jaya, male Wistar strain white rats, alcohol swabs, tissues, cotton wool, rat feed pellets, 0.5% Na CMC Kimia Jaya, 5 mg glibenclamide PT. First Medipharma, and distilled water Kimia Jaya.

Methods

This study is an experimental study conducted at the Pharmacology Laboratory and Biology Laboratory of Al-Irsyad University Cilacap to determine the effect of the combination of Moringa oleifera L. leaf ethanol extract and Channa striata ethanol extract is CMOCS throughout the entire manuscript as antidiabetic agents in male white rats. The test animals were divided into 5 groups consisting of 5 test animals. Group 1 was only given distilled water negative control. Group 2 was given Glibeclamide suspension at a dose of 0.45 mg/kg BW as a positive control. Groups 3, 4, and 5 were given extracts with different doses of CMOCS I 100mg/kgBW+ 250 mg/kg BW, CMOCS II 200mg/kgBW+ 250 mg/kg BW and CMOCS III 300mg/kgBW+ 250 mg/kg BW

Preparation Sample

The ethanol leaf extract samples *Moringa oleifera L*. used in this study were obtained from the Batu Materia Medica Herbal Laboratory, and the snakehead fish extract powder *Channa striata* to be used was obtained from the “Crystal Of The Sea Official Shop” marketplace, which has obtained a CoA certificate. To prevent the degradation of active compounds, the ethanol extracts of moringa leaves and snakehead fish were stored in airtight containers protected from light in a cool, dark place.

Preparation of Test Animals

Male Wistar rats Rattus norvegicus were divided into 5 groups, each consisting of 5 rats that had been acclimatized for 7 days.

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Preparation of Glibenclamide Suspension

Glibenclamide is administered at a human dose of 5 mg. The dose of glibenclamide administered to rats is converted based on the body weight of 200 g rats, which is: 0.018 x 5 mg = 0.09 mg.

Therefore, the dose of glibenclamide for 1000/200 g rats is 0.45 mg. Therefore, the glibenclamide dose for mice is 1000/200 x 0.09 = 0.45 mg. A total of 0.45 mg of glibenclamide is dissolved in 1 ml of distilled water. The prepared glibenclamide solution is administered orally to the test animals Pongoh *et al*., 2020.

Preparation of Alloxan

The alloxan dose for rats is 120 mg/kgBW. Based on the dose conversion, the amount of alloxan administered to rats is first converted based on the body weight of the rats, which is 200 g = 200 g/1000 g x 120 mg = 24 mg/200 g. The alloxan monohydrate powder was weighed and then dissolved in 0.5% Na CMC that had been dissolved using hot distilled water at a temperature of 90 °C to a volume of 10 ml Susmawati *et al*., 2020.

Antidiabetic Activity Test

Before administering alloxan, fasting blood glucose levels were measured first. The mice were fasted for 8 to 12 hours and were not given any food other than drinking water. The administration was performed intraperitoneally and given once. After 72 hours of alloxan induction, blood glucose levels were then checked. Mice with blood glucose levels above 135 mg/dL were declared diabetic and were ready for research Lengkong et al., 2023. The test solution was administered orally at a dose of once daily for 14 days, and blood glucose levels were measured every 7 days, namely on days 0, 7, and 14. Blood was taken from the tip of the mouse's tail, which had been sterilized using 70% alcohol, by making a small cut, and the glucose level was measured using a glucometer.

RESULT AND DISCUSSION

Result

Figure 1 shows the percentage increase in blood glucose levels after administration of alloxan in all treatment groups.

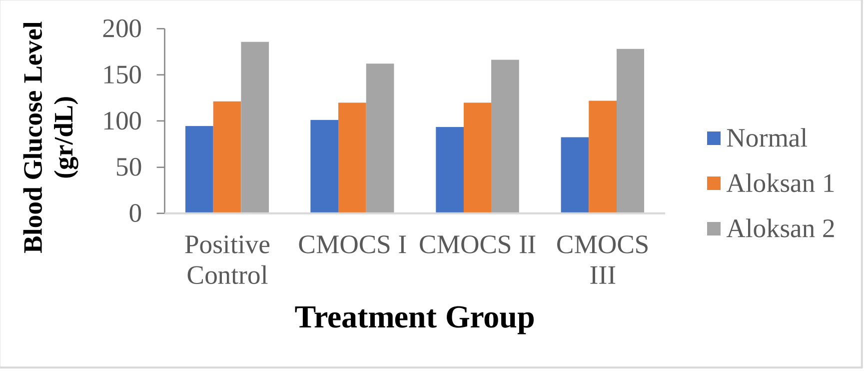

Figure 1. Result Before and After Aloxan Treatment

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Figure 2 shows the body weight obtained in mice that were treated and those that were not treated.

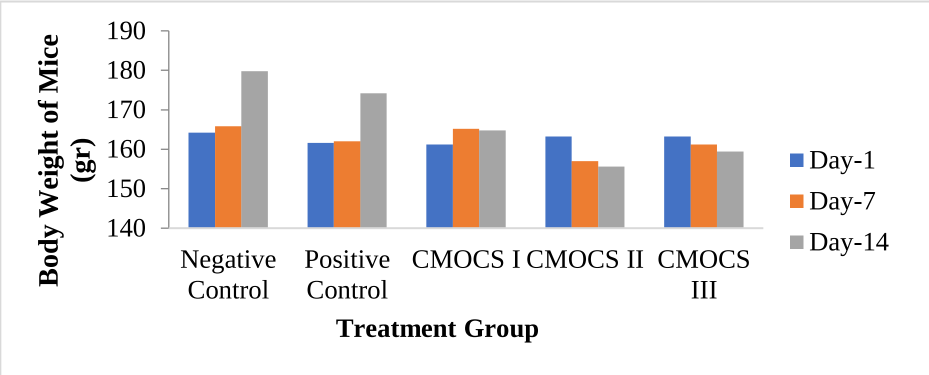

Figure 2. Mouse Body Weight

Table 1 shows the average decrease in blood sugar levels in test animals.

Table 1. Average Decrease in Blood Glucose Level

	Average Blood Sugar Level mg/dL			
	Day-0 mg/dL	Day-1 After Alloxan Induction mg/dL	Day-7 mg/dL	Day-14 mg/dL
Negative Control	98,4	-	96,6	94,8
Positive Control	94,4	185,8	166,6	115,6
CMOCS I	101,2	162	149,6	121,6
CMOCS II	93,4	166,2	142,6	117,6
CMOCS III	82,4	178	150,2	121,2

Figure 3 shows the percentage decrease in blood glucose levels in rats in both the positive control and treatment groups.

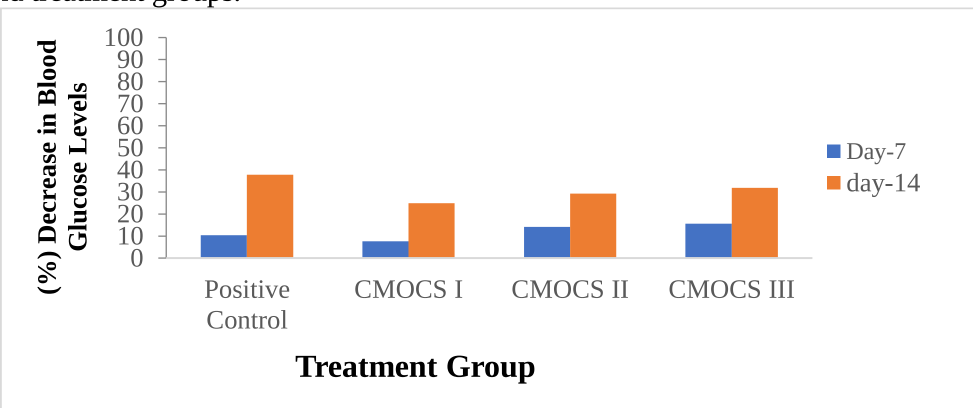

Figure 3. Persetage of Decrease in Blood Glucose Levels

Description :
Negative control: Given distilled water
Positive control: Given glibenclamide treatment 0.45 mg
CMOCS I: Given treatment EEDK 100 mg/kgBW + EIG 250 mg/kgBW
CMOCS II: Given treatment EEDK 200 mg/kgBW + EIG 250 mg/kgBW
CMOCS III: Given treatment EEDK 300 mg/kgBW + EIG 250 mg/kgBW

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Discussion

The test animals used in this study were male Wistar rats *Rattus norvegicus*. Rats were chosen as test animals because they are larger than mice and easier to maintain in large numbers, and because rats have a digestive system, absorption process, and drug metabolism system that are relatively similar to those of humans. Male rats were chosen because their hormones are more stable than those of female rats, whereas female rats experience estrus, pregnancy, and lactation, which affect the psychological condition of the test animals. Male white rats do not produce estrogen, although male rats have very small amountsIsrul *et al*., 2020. Wistar rats are more suitable for studies on body metabolism because they have a fast metabolism Putri & Zebua, 2024.

Alloxan was chosen as a diabetes inducer because it damages pancreatic β cells by forming superoxide radicals that lead to a redox cycle. These radicals then transform into hydrogen peroxide. When this increased activity of superoxide free radicals occurs, the concentration of calcium in the cytosol increases. This causes rapid damage to pancreatic β cells. The result of this damage is a decrease in insulin production, which in turn reduces the process of glycogenesis and glucose transport in cells. In addition, glycogenolysis becomes uncontrolled, causing blood glucose levels in experimental animals to increase Azizah *et al.*, 2022.

Based on Figure 1, there was an increase in blood glucose levels after aloxan administration in all treatment groups. The average initial blood glucose level was within the normal range of approximately 82.4-101.2 mg/dL. There was a significant increase after the first administration of alloxan alloxan 1, with the average reaching a range of 119.8-121.8 mg/dL, and the increase continued after the second administration of alloxan alloxan 2 to exceed 162 mg/dL. In the positive control group, blood glucose levels increased gradually, with an average of approximately 94.4 mg/dL to 121 mg/dL after alloxan 1, and rose again to 185.8 mg/dL after alloxan 2. A similar pattern was observed in CMOCS I, from an average of approximately 101.2 mg/dL to 120 mg/dL, then increasing to 162 mg/dL. CMOCS II increased from an average of approximately 93.4 mg/dL to 119.8 mg/dL, then rising to 166.2 mg/dL. CMOCS III showed the most significant increase, from an average of approximately 82.4 mg/dL to 121.8 mg/dL, and finally reaching approximately 178 mg/dL after alloxan 2. The graph shows that the administration of alloxan successfully induced hyperglycemia, with a consistent pattern of increase in all groups. This indicates that the diabetic animal model has been successfully established and can be used for further testing of therapeutic efficacy. Referring to previous studies showing that administration of alloxan at a dose of 120mg/kgBW can increase fasting glucose levels in rats to more than 125 mg/dL, even reaching more than 135mg/dL, despite the less stable hyperglycemic effect and the possibility of returning to normal after 9-10 days due to the possible regeneration of pancreatic β cells Wulandari *et al*., 2024

In this study, glibenclamide was used as a positive control because glibenclamide works by lowering blood glucose levels by stimulating active pancreatic β cells to release insulin Utama *et al*., 2025. The effective oral dose of glibenclamide for humans is 5 mg, which is converted to a dose for rats of 0.45 mg dissolved in 10 ml of distilled water Pongoh *et al*., 2020

Based on Figure 2, it can be seen that the negative control group experienced a fairly stable increase in body weight over 14 days, namely on day 1 with an average body weight of 164.2 g, day 7 165.8 g, and day 14 179.8 g, which means that there was no weight loss due to treatment. Meanwhile, the positive group with glibenclamide also showed an increase in body weight, but higher than the negative control group, with an average body weight of 161.6 g on day 1, 162 g on day 7, and 174.2 g on day 14. This could be due to improved glucose

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metabolism due to the drug's mechanism of action. In the treatment group, there were variations in weight. Combination I showed a slight increase, with an average weight of 161.2 g on day 1, 165.2 g on day 2, and 164.8 g on day 14. Meanwhile, in CMOCS II, on day 1 with an average body weight of 163.2 g, day 7 157 g, and day 14 155.6 g, there was a tendency for a decrease in body weight on days 7 and 14. In CMOCS III, with an average body weight on day 1 of 163.2 g, day 7 of 161.2 g, and day 14 of 159.4 g, there was a tendency to show a decrease in body weight, especially on days 7 and 14. This may occur not only due to the metabolic effects of administering the CMOCS, but may also be influenced by other factors such as test animal stress and fighting between test animals in response to the environment. Referring to a previous study that is in line with this, namely the study by Nadia, 2023 which explains that there were also variations in body weight in the treatment group, which were thought to be influenced by metabolic effects and fighting between rats.

The average blood glucose level measurements shown in Table 1 indicate that blood glucose levels in test rats receiving glibenclamide and CMOCS decreased from day 7 to day 14. On day 0, blood glucose levels were lower than on days 7 and 14, because on day 0, the mice had not yet received any treatment and blood glucose levels were within the normal range. The average blood glucose levels on day 0 were 98.4 mg/dL for the negative control, 94.4 mg/dL for the positive control, 101.2 mg/dL for CMOCS I, 93.4 mg/dL for CMOCS II, and 82.4 mg/dL for CMOCS III. On day 0, the lowest blood glucose level was found in the CMOCS III group, and the highest average blood glucose level was found in the combination I group. On day 7, the average blood glucose level after treatment in the negative control group was 96.6 mg/dL, in the positive control group was 166.6 mg/dL, in CMOCS I was 149.6 mg/dL, in CMOCS II was 142.6 mg/dL, and in CMOCS III was 150.2 mg/dL. The lowest blood glucose level after treatment was found in CMOCS II and the highest was in the positive control group given glibenclamide with a dose of 0.45 mg/kgBW. The average blood glucose levels on day 14 were 94.8 mg/dL for the negative control, 155.6 mg/dL for the positive control, 121.6 mg/dL for CMOCS I, 117.6 mg/dL for CMOCS II, and 121.2 mg/dL for CMOCS III. On day 14, the lowest blood glucose level was found in the positive control group given glibenclamide at a dose of 0.45 mg/kgBW and the highest in CMOCS I. The increasingly lower decrease in blood glucose levels indicates that glucose management is becoming more effective, and the lower the blood glucose levels, the better the glucose control in the test animals.

The percentage decrease in blood glucose levels shown in Figure 3 was obtained after 7 days of treatment in the positive control group at 10.33%, CMOCS I at 7.65%, CMOCS II at 14.19%, and CMOCS III at 15.61%. On day 7, the lowest percentage decrease in blood glucose levels was found in CMOCS I at 7.65%, and the highest decrease in blood glucose levels was found in CMOCS III at 15.61%. . The percentage of blood glucose level reduction after 14 days of treatment in the positive control group was 37.78%, CMOCS I 24.93%, CMOCS II 29.24%, and CMOCS III 31.91%. The lowest percentage of blood glucose level reduction was found in CMOCS I at 24.93%. The highest reduction was found in the positive control group at 37.78% and CMOCS III at 31.91%. The results of the study on blood glucose levels showed that the combination of extracts given to hyperglycemic rats at higher doses resulted in a greater decrease in blood glucose levels, and the higher the extract dose, the more antidiabetic compounds it contained. The decrease in blood glucose in groups I, II, and III was also reinforced by the selection of extracts used, where this study used ethanol extracts that had better activity Owens *et* al., 2020. This study also used snakehead fish extract powder, which has been proven to have good antidiabetic activity due to its amino acid content that acts as an antidiabetic agent Soniya & Fauziah, 2020.

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The results show that the CMOCS III was able to reduce blood glucose levels better than other doses, which was almost as good as the positive control glibeclamide as a comparison. This is in line with the statement givenWibowo *et al*., 2024, which states that the administration of moringa leaf extract at a dose of 300 mg/kgBW showed an average of 280.145 nmol/L, effectively lowering blood glucose levels compared to doses of 100 mg/kgBW and 200 mg/kgBW. A dose of 100 mg/kg of moringa leaf extract provided lower activity compared to doses of 200 mg/kgBW and 300 mg/kgBW. According to a study Razoki *et al*., 2024, it was reported that snakehead fish extract at a dose of 250 mg/kgBW effectively lowered blood glucose levels in rats.

Based on the Mann-Whitney test conducted, there was a significant difference between the negative control group and all treatment groups on day 7 with a significance value of $p < 0.05$, indicating the success of the CMOCS as an antidiabetic agent in rats. On day 14, CMOCS I produced a p-value of 0.021, CMOCS II a p-value of 0.036, and CMOCS III a p-value of 0.047, indicating a significant difference between the negative control group and the treatment groups, proving the antidiabetic activity of the treatments administered. A comparison between the positive control group and the treatment group yielded a significance value of $p > 0.05$, indicating no significant difference between the positive control given glibenclamide and the treatment group given the extract combination. This means that the extract combination has an activity in lowering glucose levels similar to that of glibenclamide. Furthermore, the comparison between treatment CMOCS I, CMOCS II, and CMOCS III showed no significant difference with a significance value of $p > 0.05$, which means that there was no significant difference between the treatment combinations.

The results of the statistical analysis support the data on the decrease in blood glucose levels, indicating that the CMOCS has antidiabetic activity. The flavonoid content in moringa leaves has the potential to lower blood glucose levels due to its antioxidant properties Rohmawati *et al*., 2024. There are 15 types of amino acids in snakehead fish *Channa striata* protein, including 9 essential amino acids: histidine, threonine, arginine, methionine, valine, phenylalanine, leucine, isoleucine, and lysine. Two types of amino acids are those that can stimulate insulin and those that cannot stimulate insulin. The amino acid content of snakehead fish protein, namely arginine and leucine, functions to control blood glucose levels in hyperglycemia Soniya & Fauziah, 2020. CMOCS III is the combination with the best activity, with a percentage decrease in Figure 3 of 31.91%, which is almost equivalent to the positive control using glibenclamide with a percentage of 37.78%.

CONCLUSION

The CMOCS has antidiabetic activity, proven to be able to lower blood glucose levels in male rats induced by alloxan. The CMOCS III lowered blood glucose by 31.91%, close to glibenclamide at 37.78%. Statistical tests showed a significant difference CMOCS I produced a p-value of 0.021, CMOCS II a p-value of 0.036, and CMOCS III a p-value of 0.047 $p < 0.05$ between the negative control group and the treatment group, indicating that the extract combination has antidiabetic activity.

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