Blood glucose level of white rats (*Rattus norvegicus*) after giving catfish biscuit (*Pangasius hypophthalmus*)

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**Abstract.** Glucose is an important carbohydrate which is more absorbed into the bloodstream as glucose and other sugar is changed into glucose in the liver. Blood glucose level is closely related to diabetes mellitus. Diabetes mellitus is a disease that arises in a person which is indicated by the presence of blood glucose levels exceeding normal (hyperglycemia) due to the deficiency of the insulin hormone in the body. The design of this research was using RAL with 24 male rats that divided into 6 treatments which were normal with the giving of Na-CMC 0.5% 1mL/200 gram BB, negative with the giving of Na-CMC 0.5% 1mL/200 gram BB, positive with the giving of glibenclamide of 0.45 mg/kg BB and 3 other treatments by giving catfish biscuit (Formula A, Formula B, and formula C) with 4 replications. All treatments were injected with monohydrate alloxan at a dose of 150 mg/kg BW, except for the normal treatment. The results showed that the formulation of catfish biscuit can lower the blood glucose level of white rats. The composition of biscuit with formula C has the highest decreasing percentage which was 80.20% to the blood glucose level of white rats.

### 1 Introduction

Glucose is an important carbohydrate which is more absorbed into the bloodstream as glucose and other sugar is changed into glucose in the liver. Glucose is also the main fuel in the body tissues and has the function to generate energy. Blood glucose level is closely related to diabetes mellitus [1]. Diabetes mellitus is a disease that arises in a person which is indicated by the presence of blood glucose levels exceeding the normal level (hyperglycemia) due to the deficiency of the insulin hormone in the body. If the disease is uncontrolled or the patient is unaware of the disease, there will be various fatal chronic complications [2]. Increasing the prevalence of diabetes mellitus in Indonesia can cause negative impact which is the decreasing of human resources (HR) due to this chronic disease and also in the case of medication, it is not cheap because the patient must consume drugs in a long time.

One symptom that is often found in diabetes mellitus patients is polyphagia that is a condition of having an appetite which increases but the patient loses his/her weight [3]. The existence of this appetite affects people with diabetes mellitus to consume food besides the staple food, in this case, it is by consuming biscuit. The biscuit that is circulating in the public is not given by vegetable or animal foods and nutritional content that is beneficial to health. Moreover, dietary factor becomes the main key to diabetes mellitus prevention and treatment. One alternative that can be used is by consuming functional foods that are proven to help to keep the blood glucose level at the normal level [4].

Based on that explanation, it is necessary to find out the antidiabetic biscuits that have relatively low side effects, cheap prices, easy to obtain, and derived from animal extracts, and one of them derived from catfish extract. Catfish contain various components that are very beneficial for the health of the body which are minerals, vitamins, and omega 3 fatty acids that are very beneficial to human health. The catfish have high-fat content and are a very good source of unsaturated fatty acids, including omega 3 fatty acids that have a positive function for human health. This research was previously published in RJPBCS with the use of catfish extract in the form of oil.

This study aims to determine the effect of catfish biscuit on blood glucose level of white rats and determine the formula of catfish biscuit that is effective toward blood glucose level.

### 2 Materials and Methods

#### 2.1 Materials and equipments

Materials used in this study were catfish, aquadest, alloxan, Na-CMC 0.5%, glibenclamide, wheat flour, eggs, margarine and animal test that was used as the object of this research which was male white rat as many as 24 Wistar strains with 150-200 grams weight and 5-6 months old.

The tools used in this research were cage, drink bottle, scale, large tray, stirrer, printer, oven, and **Blood Glucose Test Meter**.

#### 2.2 Methods

**2.2.1 Catfish Filtrate Making**

The method used was steam for 90 minutes with ± 10 mL aquadest addition.
2.2.2 Catfish Biscuit Making
The biscuit was roasted in an oven at 160 °C for 30 minutes.

2.2.3 Test Animal Maintenance and Treatment
The mice were first in acclimatization for 1 week. Acclimatization was intended to allow the test animal to adapt to the conditions of the test place. Rats were fed, drank, and put in the same cage intending to not affect the results and the rats were able to adapt to the conditions of test place (laboratory). The cage was equipped with a drinking bottle and was covered with chaff evenly and cleaned at least 2 times a week. The cage was placed in a ventilated room to regulate the air circulation and radiation system so that light and dark conditions respectively for over 12 hours.

2.2.4 0.5% Na-CMC Solution Making
0.5g Na-CMC was weighed and fed into an Erlenmeyer containing ± 10 mL aquadest that had been heated. It was set aside for 15 minutes until it was obtained a transparent mass, then it was mixed until homogeneous. After that, Na-CMC solution was transferred to a 100 mL measuring flask and be sufficient volume with the aquadest to the tera mark (Darmawi, 2015). The provision of a 0.5% Na-CMC solution was done orally with a given dosage of 0.5 mg/kg BB of rat (Annex 1).

2.2.5 Glibenclamide Solution Making
The 5 mg weighed glibenclamide was then finely crushed, and dissolved in 1% Na-CMC solution in a 25 mL flask. The given dose to rats was 0.45 mg/kg BB of rats (Appendix 1) (Siwaka, 2013).

2.2.6 Alloxan Solution Making and Induction
Alloxan solution is the monohydrate alloxan powder which was weighed as much as 0.5 g then dissolved with aquadest up to a volume of 10 mL. The dissolved alloxan was injected immediately before any color change occurs. The given dose was 150 mg/kg BB of the rat by intraperitoneal injection.

2.2.7 Treatment Giving and Blood Glucose Level Measurement
The tested animal that had been fasted for 12 hours and measured its fasting blood glucose level then was given the food treatment (catfish biscuit) for about 5 gram per serving orally and dissolved in cold aquadest (Nissa & Madjid, 2016) for 15 days with drink provision by using ad libitum way (unlimited) and balanced with standard feeding. The measurement was made 6 times on the first, third, sixth, ninth, twelfth, and the last measurement day was on the fifteenth day of the treatment (Fahri et al., 2005). The ways of blood glucose level measurement were as follows:
1. Rats’ tail was cleaned and injured
2. Blood was taken using a glucometer strip
3. Blood glucose level result was awaited for 10 seconds

2.2.8 Percentage Calculation of Blood Glucose Level Changes
The percentage calculation of the blood glucose level decreasing was measured by the following formula:
\[
\% \text{ Decreasing} = \frac{\text{First BGL} - \text{Final BGL}}{\text{First BGL}} \times 100\%
\]
(Astuti, 2012).

2.3 Data Analysis
The collected data was quantitative, including the level of the blood glucose level of the rats. The data were analyzed by using the Kolmogorov-Smirnov test to know the normality of the data distribution and Levene's Test to find out the homogeneity of the data variance. The obtained data were distributed into normal and homogeneous then it was followed by ANOVA test and real difference test (Duncan). The data which was not distributed into normal but homogeneous then transformed by Log, Ln, and SQRT and non-parametric test using the Kruskal-Wallis test was done and continued by Real Difference test (Duncan).

3 Results and Discussion
3.1 Blood Glucose Level of White Rats
Based on the results of statistical analysis, from the use of ANOVA test for the normal and homogeneous distribution and the use of Kruskall-Wallis test for the non-normal distributed but homogeneous and also continued by the use of the Real Difference test using Duncan test, the obtained result is that catfish biscuit giving have a significant effect on the blood glucose level of white rats. The level of the blood glucose level of catfish biscuit treatment is still within the normal range. The blood glucose normal level of white rat is recorded 50-135 mg/dL. The average level of blood glucose (mg/dL) can be seen in Table 1.
2.2.3 Test Animal Maintenance and Treatment

The biscuit was roasted in an oven at 160 °C for 30 minutes. The result was set aside for 15 minutes until it was obtained a chaff evenly and cleaned at least 2 times a week. The conditions of test place (laboratory). The cage was distributed into normal but homogenous then it was followed by ANOVA test and continued by Real Difference test (Duncan). Based on the results of statistical analysis, from the use treatment (Fahri, 2005). The ways of blood glucose level measurement were as follows: the rat by intraperitoneal injection. The percentage calculation of the blood glucose level of the rats. The data were known the normality of the data distribution and tested by Kolmogorov-Smirnov test. The data which was not distributed and the use of non-normal distributed but homogenous and also analyzed by using the Kruskall-Wallis test for the normal and homogenous conditions respectively for over 12 hours.

2.3 Data Analysis

The percentage calculation of the blood glucose level was obtained that the blood glucose level is 121 mg/dL, in the formula. C is 93 mg/dL. The changes in the blood glucose level of white rats in all treatments after the fifteenth day with biscuit giving are presented in graphical form (Figure 1). The average graph of blood glucose level (mg/dL) in all treatments can be seen in Figure 1.

### Table 1. The Average Value of Blood Glucose Level (mg/dL).

| P | Days | N | 0 | 1 | 3 | 6 | 9 | 12 | 15 |
|---|------|---|---|---|---|---|---|-----|-----|
| 16±±3.66 | 107±12.71 | 118±28.16 | 113±11.58 | 106±22.95 | 117±41.73 | 122±3.40 |
| 397±55.19 | 286±110.21 | 307±55.72 | 274±120.94 | 415±147.21 | 405±147.25 | 335±122.56 |
| 463±174.41 | 407±92.13 | 364±62.32 | 291±11.41 | 190±33.51 | 137±7.94 | 92±8.04 |
| A 472±96.27 | 251±105.13 | 291±98.98 | 270±120.3 | 244±117.44 | 174±56.09 | 121±23.69 |
| B 470±92.50 | 373±75.84 | 243±15.56 | 136±15.53 | 167±20.25 | 166±21.65 | 101±5.36 |
| C 466±160.45 | 266±129.9 | 249±105.73 | 163±33.38 | 128±21.40 | 127±34.95 | 93±4.21 |

* The figures that are followed by the same letter in the same column show no real difference (P> 0.05), while the figures with different letters in the same column indicate the values are the real difference (P< 0.05).

### Table 2. The Decreasing Percentage of Blood Glucose Level

| Treatment | Decreasing Percentage (%) |
|-----------|---------------------------|
| Normal | 26.80 |
| Negative | 15.62 |
| Positive | 80.23 |
| Formula A | 74.46 |
| Formula B | 78.47 |
| Formula C | 80.20 |

The changes in blood glucose level from all treatments (Appendix 4 & Appendix 5). It can be also used to find out the decreasing percentage in all treatments that had been tested. The highest percentage of all treatments in formula C is similar to the positive control treatment. The calculation of the decreasing percentage can be seen in Table 2 below:

3.2 Changes Percentage of Blood Glucose Level from all Treatments

The calculation of blood glucose level decreasing percentage aims to know the ability of the treatment in decreasing the blood glucose level which had been tested for 15 days (Appendix 4 & Appendix 5). It can be also used to find out the decreasing percentage in all treatments that had been tested. The highest percentage of all treatments in formula C is similar to the positive control treatment. The calculation of the decreasing percentage can be seen in Table 2 below:

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The treatment of catfish biscuit giving at various levels of the formula at the end of the whole observation shows the percentage of the effect that is comparable to the increase in the formula. Normal, negative, and positive control at the end of this treatment was 23.80%, 15.61%, and 80.22%. The catfish biscuit giving treatment that has the highest decreasing percentage was in formula C which was 80.20%, while for formula A and formula B were 74.46% and 78.47%.

### 4 Conclusion

It can be concluded that the catfish biscuit can affect the blood glucose level and can decrease the blood glucose level with the decreasing percentage as many as 80.20%.

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