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Hypoglycemic Test Of Catfish Oil Extracts (*Pangasius hypophthalmus*) In The Oral Glucose Tolerance Test And Histology Of The Pancreas Of Male Rats (*Rattus norvegicus*).

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ABSTRACT

Cat fish oil extracts (*Pangasius hypophthalmus*) contains omega-3 and albumin which controlling the blood glucose levels. Diabetes mellitus is a metabolic disease characterized by hyperglycemia syndrome and impaired metabolism of carbohydrates, fats, and proteins. The purposes of this study were to evaluate the effect of cat fish oil extracts in oral glucose tolerance test and pancreas histology of male rats and to determine the most effective dose of cat fish oil extract in decreasing the blood glucose level. This research used a completely randomized design with 6 groups of 4 male rats each. All treatments were injected with alloxan monohydrate at a dose of 150 mg/kg intraperitoneally, excluding normal control treatment. Normal control administered with Na-CMC 0,5% 1 mL/200 gr BB, negative control with Na- CMC 0,5% 1 mL/200 gr BB, and positive control with glibenclamide 0,45 mg/kg BB. The other groups were administered a dose of the extract as much a 18,2 mg/kg BB, 36,4mg/kg BB, and 72,8 mg/kg BB. Extraction was conducted using wet rendering method. Observed parameters were the oral glucose tolerance test (OGTT) and histology of the pancreas. The results showed that the cat fish oil extracts possessed an effect on OGTT and histology of the pancreas. Cat fish oil extracts dose of 72,8 mg/kg BB was the most effective dose in lowering blood glucose OGTT and in repairing island of Langerhans which were necrotic and the amount of blood glucose was closed to normal.

Keyword: cat fish oil extracts, hypoglycemic

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INTRODUCTION

Catfish (*Pangasius hypophthalmus*) is a freshwater fish which are the most sought and consumed by the Indonesian people. Catfish has the potential to be extracted into fish oil which contains omega-3 fatty acid that is comprising linolenic acid, eikosa-pentanoat (EPA), dandokosa-heksaenoat (DHA) ^[1]. Omega-3 fatty acids that contained in fish oil can control the content of the blood glucose and increase the insulin production in the beta cells of Langerhans ^[2]. (Minarul, 2014). Fish contains albumin which acts as the antioxidants to prevent the damage of normal cells and help to regenerate the damaged of pancreatic tissue ^[3].

Diabetes mellitus is a metabolic disease that characterized by chronic hyperglycemia syndrome and impaired metabolism of carbohydrates, fats, and proteins that caused by insufficiency of insulin function ^[4]. Treatment is usually done by people with diabetes mellitus is using injection of insulin or antidiabetic oral administration of medicinal chemistry. Treatment of diabetes mellitus in this way can cause the unwanted side effects and has a cost that is relatively expensive, because it is used in a long time period ^[5]. Based on statement above, it is necessary to find an antidiabetic drug that has low side effects, the cheap price, easy to obtain, which is derived from the extracts of catfish.

The observation of the decrease in blood glucose was done by oral glucose tolerance test parameters and histology of the pancreas. Oral glucose tolerance test was conducted to see a decrease in blood glucose levels and determine the effect of the test material to see the curve of glucose tolerance ^[6]. The observation of pancreas histology structure was observed to determine the damage and refinement that occurs in pancreatic beta cells that had been induced alloxan ^[2].

This purpose of this study is to evaluate the effect of oil extracts of catfish toward the oral glucose tolerance test and histology of the pancreas of male rats and determine the conditions of hyperglycemia catfish oil extract dose that is effective to reduce the levels of glucose in an oral glucose tolerance test of male rats were hyperglycemic conditions.

MATERIALS AND METHODS

Chemicals

The materials that is used in this research are the catfish, akuades, 1% bentonite, white male rats, husks, standard feed, alloxan, NaCl 0.9%, glibenclamide, Na-CMC0, 5%, glucose, glucose test strip, cotton, 70% alcohol, ether, formaldehyde 10%, high rise alcohol, xylene, paraffin, and the dye of haematoxylin-eosin.

Experimental

Cat Fish Oil Extraction

The extraction method that used in this research was the wet rendering method which the catfish that has weighs about 750 grams, then it cuts into small pieces in order to facilitate the extraction process. Catfish is added to stainless steel panic and added 500 mL of akuades. After that, the catfish is boiled until boiling, then let it for 30 minutes while stir it slowly. Between the crude oil and fish stew result are filtered until it separated. Oil and water layers are separated by a separating funnel ^[7].

Table 1. The Weighing Materials Extraction of Oil Catfish

Components	Weighing
Materials Extraction	
The total weight of catfish	2kg (700-750 grams)s
Akuades	6 liters
Bentonite needed	1,6 grams

The separated layer of oil that has been put into the beaker was heated at a temperature of 55 ° C-60 ° C for 5 minutes. The purification process was done with the addition of 1% bentonite. Oil reheated to a temperature of 80 ° C for 30 minutes. Oil centrifuged at 10,000 rpm for 10 minutes at a temperature of 10 ° C and the oil was separated from the other deposition ^[8]. The weighing catfish oil extraction that is used can be seen in the following table 1

The Maintenance and Treatment of Animals Test

The animal that is used in this research test are male rats (*Rattus norvegicus*), which are aged 2-3 months and have weight around 150 to 200 grams. The test animals obtained from Yogyakarta in order to obtain a homogeneous subject. First of all, the rats are acclimatized for 1 week, before being treated according to the research design. The rats are fed, watered, and have the same enclosure in order to not affect the results and the rats were able to adapt with the test conditions (laboratory).

Producing and Inducing the Alloxan Monohydrate Solution

The test animals were induced a dose of 150 mg/kg monohydrate alloxan that were injected intraperitoneally. The test animals have induced alloxan, then they adapted for 3 days. If there is an increase in blood glucose levels of the rats that became ± 200 mg/dL, then the test animals need to get a treatment for 14 days ^[9].

Producing the Suspension of Na-CMC 0.5%

The researcher fused 0,125 grams Na-CMC with 5 mL of hot akuades, then it is allowed to stand for approximately 15 minutes and then it became homogenized. After that, added cold akuades into 20 mL of Na-CMC solution ^[10].

Producing the Suspension of Glibenclamide

The dose of glibenclamide that is given to each rats are 0,45mg/kg rats weight ^[11]. Glibenclamide is weighed as much as 5 mg and it dissolved with the Na-CMC as much as 25 mL.

Preparation of Glucose Solution

The dose of glucose that used in each rat was 2g/kg rats weight^[12]. Glucose is weighed as much as 4 grams and it dissolved with akuades as much as 10 mL.

Research design

This research is an experimental research. The research design of the research is completely randomized design (CRD) with 6 treatments and 4 replicates for each treatment. Grouping patterns of the treatment are:

1. Normal control : The treatment was not given alloxan, but it is only given 1 mL/ 200g Na-CMC 0.5%
2. Negative control: The treatment was given alloxan and 1 mL/200gr Na-CMC 0.5%
1 mL/200gr
3. Positive control : The treatment was given alloxan and 0.45 mg/kg glibenclamide
4. Group A : The treatment was given alloxan and 18.2 mg/kg catfish oil extract
5. Group B : The treatment was given alloxan and 36.4 mg/kg catfish oil extract
6. Group C : The treatment was given alloxan and 72.8 mg/kg catfish oil extract

The dose catfish extract oil that is used for the test animal based on the dose of Hidayaturrahmah research (2015) ^[7] that has been converted into a rat.

The Measurement of Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was done by giving the glucose to the test animals and did the test on day 14^[13]. The rats were fasted for \pm 12 hours before the test^[14]. The measurement of blood glucose levels were done before the rats got the treatment. The rats were treated based on the research design for each group of rats. The rats were given a dose of 2 g/kg oral glucose after 30 minutes being treated. Blood glucose levels of rats are measured in some minutes: they are 0, 30, 60, 90, and 120 minutes^[15].

Histology Pancreas

Stages of production of a pancreas used paraffin method. The preparations made from the tissue preparation tools and materials/liquids as well as sample preparation, such as, anesthesia, surgery, and the isolation of tissue^[16]. Organ washed by using physiological NaCl and fixed it with 10% formaldehyde solution for 24 hours^[17]. The organ is cut around 5 mm (trimming). Then, the organ is also dehydrated by using alcohol 70% for 1 hour, alcohol 80% for 1 hour, 90% for 1 hour, and alcohol 100% (I and II) respectively for 2 hours^[18].

The purification process is done with *clearing agent* in the form xylene that start from xylene I for 2 hours and xylene II for 2 hours. Then, the xylene replaced with paraffin at the infiltration process that soak the tissue into liquid paraffin 3 times in the oven with a temperature of 60°C for 1 hour in each times^[17]. The process of embedding the tissue into liquid paraffin is done with three *paraffin bath* to create a block of paraffin in order to cut the tissue easily with a microtome. The tissue is put into a mold that is containing a hot paraffin by using tweezers and it allowed to stand for 24 hours^[3].

In sectioning process that used a microtome with a thickness of 5 μ m is done after the block of tissue in the paraffin is formed^[19]. Paraffin sheet which there is an expected tissue, then, it is moved carefully by using a small brush into above of the water surface that has been given the gelatin in the water bath at a 40°C temperatures. The sheets of tissue is taken by using the slide carefully with a scooping motion (*affixing method*). The slide that contained tissue slices are placed on a hotplate at 40°C temperatures in order to remove the paraffin^[3].

The tissue sheets entered the staining stage. The process of deparaffinization used xylene (I, II, and III) and each xylene took time for 2 minutes^[16]. The tissue preparation was dipped into decreased alcohol levels which were alcohol 100 %, 90%, 80% for about 2 minutes for each alcohol. The tissue preparation was soaked in the haematoxylin dye for 20 minutes. After 20 minutes, the tissue preparation was washed by using akuades. And then, it will be dipped into eosin dye for 20 minutes^[20].

The tissue preparation was dipped into graded alcohol levels which are 70%, 80%, 90%, and 100% and each tissue preparation was dipped for 5 times for each alcohol level. Then, the tissue preparation was dipped into xylene (I, II, and III) for 5 minutes for each xylene^[16]. After that, the tissue preparation was given entellan and closed by using mounting. The preparation was given label which consisted of the tissue information based on the treatment and repetition which conducted during the research and then it was observed by using microscope^[3]. The observation of histology picture of Langerhans islet was conducted qualitatively in the process of necrosis occurrence and conducted quantitatively by measuring the number of Langerhans island^[21, 22].

RESULT AND DISCUSSION

The cat fish which extracted into the cat fish oil were 3 fishes with 2 kg weight and for about 6-7 month of its age. The body parts of the cat fish which were extracted including the all its parts (head, flesh, and bone) to obtain the maximum number of the cat fish oil extracts. Based on the organoleptic test, the coarse cat fish oil extracts had turbid yellow color, there was white sediment and it smelled fishy. The pure cat fish oil extract had clear yellow color, smelled little bit fishy, and felt tasteless. The extraction result of fish cat oil can be seen in the table 2.

Table 2. The extraction result of cat fish oil

Component	Extraction Result of Weighing
Fish Dregs	1,2 Kg
Course oil	160 mL
Pure cat fish oil	75 gram

The Measurement of Blood Glucose Level by Using Oral Glucose Tolerance Test Method

The measurement of the blood glucose level showed the average score and the deviation standard from the group score. The increasing of blood glucose level occurred in the 30th minute after it had been given the glucose solution before. Based on the average score of blood glucose level (mg/dL) (Table 3), the blood glucose level which given the negative treatment in the 120th minute was manifestly different toward the blood glucose level which given normal, positive treatment and the catfish oil extract dose was 72,8 mg/kg BB. The giving of the cat fish oil extracts with 72,8 mg/kg BB dose can decrease the rats blood glucose level in the minute of T120 (120th minute)

Table 3. The average score ± The deviation Standard of Blood Glucose Level (mg/dL) in each time

TG	T (minute)				
	0	30	60	90	120
N	102,50±7,51	146,50±5,07	111,50±14,08*	99,25±5,25*	98,75±10,78*
-	164,50±56,51	386,50±75,66	361,75±87,24	259,25±129,06	249,50±126,37
+	168,75±79,45	246,25±146,20	186,25±128,43	113,75±45,99	82,00±17,46*
A	152,75±27,63	226,00±110,98	187,25±94,72*	152,00±67,55	142,50±65,79
B	174,00±53,05	228,25±124,18	174,75±66,61*	133,00±16,27	106,50±9,33
C	163,25±73,16	152,75±34,33	134,50±12,79*	113,00±7,66	89,25±10,72*

* Meaningfully different in the test standard $p < 0,05$, compare to the negative control group.

Direction:

- TG = Treatment group
- N = Normal control treatment without alloxan induction and it was given Na-CMC 0, 5%
- = Negative control treatment with alloxan induction and it was given Na-CMC 0, 5%
- + = Positive control treatment with alloxan induction and it was given glibenclamide 0,45 mg/kg BB
- A = Catfish oil extract with alloxan induction and it was given cat fish oil extracts with 18, 2 mg/kg BB dose
- B = Catfish oil extract with alloxan induction and it was given cat fish oil extracts with 36, 4 mg/kg BB dose
- C = Catfish oil extract with alloxan induction and it was given cat fish oil extracts with 72, 8 mg/kg BB dose
- T = Time
- T0 = Blood glucose level after giving glucose 2 g/kg BB
- T30 = Blood glucose level, 30 minutes after giving glucose 2 g/kg BB
- T60 = Blood glucose level, an hour after giving glucose 2 g/kg BB
- T90 = Blood glucose level, a half and an hour after giving glucose 2 g/kg BB
- T120 = Blood glucose level, 2 hours after giving glucose 2g/kg BB

The cat fish oil extracts can decrease the blood glucose level because it contains omega-3. Zinc is needed to form pro insulin which will be changed into insulin. EPA and DHA will stimulate the zinc into cell membrane and increase the process of insulin synthesis [23]. EPA is a precursor in the forming of eicosanoid hormone. The cell membrane is affected by eicosanoid which affecting toward the movement of calcium that enters inside and outside from the cell [24]. The closing process of *K channel* in the cell membrane caused the obstruction in the ion K^+ emission from the inside of cell which caused the occurring of cell membrane depolarization stage, and it will be followed by the *Ca Channel* opening stage. This condition enabled the ion Ca^{2+} to enter into the cell and insulin secretion would happen [25].

The blood glucose level in all treatment groups in the 60th minute had decreased. The increasing of the blood glucose level was occurred from the 30th minute until the 90th minute and the blood glucose level became normal in the 120th minute [26]. The decreasing of the blood glucose level in the period of 2 hours after the treatment was an indicator which indicated the success in controlling the glucose level in the blood [27]. The decreasing of blood glucose level in the positive group and 72, 8 mg/kg BB catfish oil extract dose showed that the decreasing of that blood glucose level was closer to the normal group than to another treatment group in the minute of T120 (120th minute).

The Measurement of Langerhans Island Number

The number measurement of Langerhans island in the 100 times zoom enlargement showed the average score and deviation standard from the group score. Based on the data of Langerhans island number (table 4), the number of Langerhans island in the negative treatment was manifestly different toward the number of Langerhans island in the normal treatment and catfish oil extract giving treatment with 72, 8 mg/kg weight dose.

Table 4. The Average Score ± Deviation Standard of the Langerhans Island Number Measurement

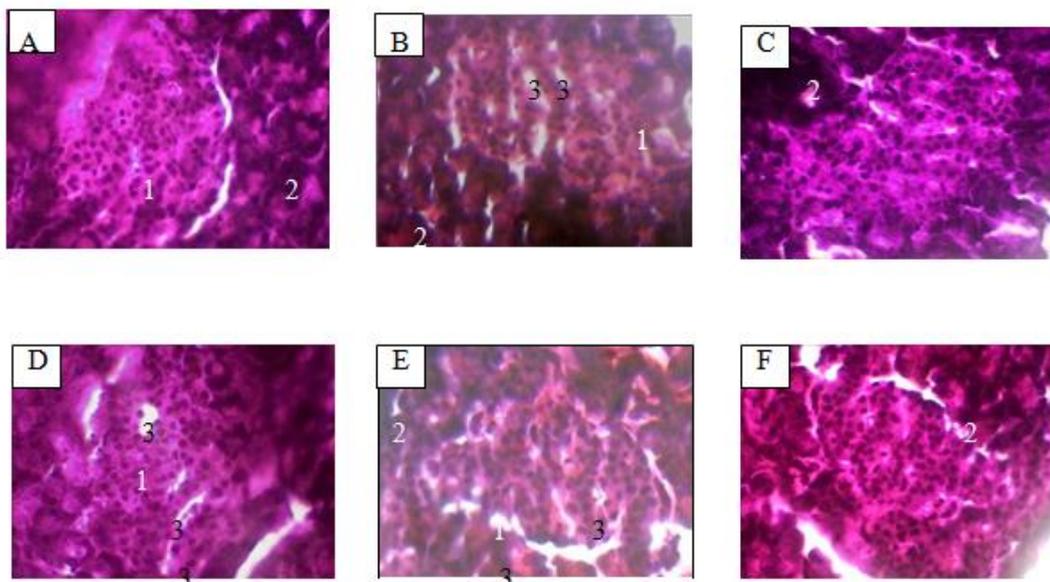
Group	Average ± DS
Normal control	3,5 ± 0,58*
Negative control	1,25 ± 0,96
Positive control	2,5 ± 0,58
Catfish oil extract A	1,5 ± 1,29
Catfish oil extract B	1,75 ± 1,26
Catfish oil extract C	3 ± 1,41*

* Meaningfully different in the test standard $p < 0,05$, compare to the negative control group.

Histology Observation of the Rats Pancreas

Based on the result of histology observation of the rats pancreas, the rats pancreas which was given normal treatment without induced with alloxan and just given Na-CMC 0,5% (picture 1.A) showed that there was an orderly structure from cells which spread out in the Langerhans island and there was no empty space in the central of Langerhans island (necrosis). The rats pancreas which was given negative treatment and induced with alloxan and also given Na-CMC 0,5% (Picture 1.B), there was an inflammation occurred which showed by the numerous number of the empty spaces in the central of the Langerhans island. Next, the rats pancreas that was given the positive treatment which induced with alloxan and given glibenclamide 0,45 mg/kg weight (Picture 1.C), the treatment for the rats A, B, and C which induced with alloxan and each of them was given 18,2 mg/kg catfish oil extract dose (Picture 1.D), 36,4 mg/kg catfish oil extract dose (Picture 1.E), and 72,8 mg/kg catfish oil extract dose (Picture 1.F) showed that there was an improvement or refinement of tissue that can be seen from the decreasing the empty spaces in the Langerhans island. So, it came near to the condition which the pancreas tissue became normal.

The giving of 72,8 mg/kg weight dose of cat fish oil extracts can affect the number and refine the cells in the Langerhans island. The cat fish oil extracts contain albumin which able to regenerate the damaged cells due to the occurrence of necrosis. The necrosis caused some changes which involve cell cytoplasm and it was marked by the appearing of empty spaces in the central part of the Langerhans island [28]. Albumin has a lot of sulfhydryl group (-SH) which can be functioned as free radical bond. Protein that is rich of -SH group will be able to bind dangerous metals as well as compounds which tend to be free radical [29].



Picture 1. The Pictures of the Rats Pancreas Histology without/induced with Alloxan after the treatment

- | | |
|--|---|
| A. Normal control (5200 X enlargement) | F. Catfish oil extract C (5200 X enlargement) |
| B. Negative control (400 X enlargement) | 1. Necrosis |
| C. Positive control (5200X enlargement) | 2. Asinus |
| D. Catfish oil extract A (5200X enlargement) | 3. Langerhans island |
| E. Catfish oil extract B (400 X enlargement) | |

CONCLUSION

Based on the research result, it showed that the cat fish oil extracts affected toward oral glucose tolerance test and histology pictures of the hyperglycemia condition of male rats. The 72,8 mg/kg weight dose of the cat fish oil extract was the most effective dose in OGTT and the histology of pancreas among the 18,2 mg/kg weight dose and 36,4 mg/kg weight.

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