Analysis of the Effect of Fish Bars Made of Bilih Fish (Mystacoleuseus padangensis Blkr) Flour to Reduce Oxidative Stress in a Diabetic Rat Model

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Summary This study aims to analyze the effect of Bilih fish bars (BFB) on oxidative stress based on the levels of MDA and SOD in diabetic rat models. This study used a Randomized Complete Design (RCD). Forty white male rats of the Sprague-Dawley strain were placed into the following five groups: normal and diabetic rats that were fed either the standard feed and metformin, BFB, BFB, and metformin or BFF with a zinc dose of 0.54 mg. A single dose of STZ (40 mg/kg) was used to induce diabetes in the rats. The intervention lasted for 30 d. The differences in MDA and SOD levels between groups were determined with one-way ANOVA followed by Duncan’s New Multiple Range Test, and the significance of the statistical level was set at p < 0.05. The intervention with BFB and metformin, BFB, and BFF resulted in a decrease in blood glucose levels. The levels of MDA in rats that received the intervention with Bilih fish were 8.236 ± 0.46 μmol/L for the BFB group and 8.266 ± 0.66 μmol/L for the BFF group, which were both lower compared to the control normal rats (8.279 ± 0.51 μmol/L). The levels of SOD in rats that received the intervention with BFB and BFF were higher compared to the diabetic rats with standard feed, but this increase was not significant (p > 0.05). BFB and BFF lowered blood sugar levels and decreased the oxidative stress levels based on MDA levels in a diabetic rat model.

Key Words blood glucose, oxidative stress, Bilih fish, MDA, SOD

Diabetes mellitus that is not controlled well for a long time can cause various complications/disorders, both acute and chronic. Various studies have been showing the critical role of zinc and the consequences of zinc deficiency on diabetes mellitus in various populations and age groups. Based on the Riskesdas 2018 data, the prevalence of diabetes mellitus in Indonesia in all age groups is 1.5% (1). Based on IDF data in 2017, Indonesia ranks sixth with the most diabetics after China, India, the United States of America, Brazil, and Mexico, and the number has increased from previous years of 10.3 million people with diabetes and is expected to increase to 16.7 million by 2045. However, Indonesia is still the fourth largest country with undiagnosed diabetes in 2017, which is 7.6 million (73.7%) in the age group of 20–79 y (2).

There are several reasons to assume that abnormal zinc metabolism plays a role in the pathogenesis of diabetes mellitus and some of its complications (3). Increased oxidative stress has been observed in diabetic patients, which indicates an increase in free radical production. Zn supplementation can increase SOD activity and reduce lipid peroxidation in diabetic rats (4).

Bilih fish is one of West Sumatra’s local food potentials, which has high nutrient content, especially zinc. The fresh Bilih fish contains zinc 4.76 mg/100 gr, while the zinc content of Bilih fish flour range from 12.83–22.92 mg/100 gr (5). The zinc content of this Bilih fish is higher than the zinc level of the other commercial fish such as Sardinella albella, Dussumeiria acuta, Latjanus fulvus, Hemiramphus far, Cephalipholis boenak which range from 0.28–1.80 mg/100 gr (6).

Fish flour is one of the fish products which until now has not been utilized optimally, especially for food, including Bilih fish. A snack bar is an effective form of between-meal food, has relatively excellent durability, and has full nutritional value. Fish bars (snack bars made from fish) are an innovative product of snack bars that have ranges of taste, from sweet to salty (savory) using fish as raw materials. Bilih fish bars can be used as an alternative for food sources of zinc in people with diabetes mellitus, which usually tends to have low zinc levels in the serum.

This study aimed to analyze the effect of Bilih fish bars (BFB) and fish flour (BFF) on blood glucose levels, MDA, and serum SOD levels in diabetes mellitus rat models.
The Effect of Bilih Fish Bars in Diabetic Rat Model

MATERIALS AND METHODS

The research was conducted at five laboratories; i.e., the IPB Animal Hospital, Histology Laboratory Department of Anatomy, Physiology, and Pharmacology of the Faculty of Veterinary Medicine IPB, IPB Primate Animal Study Center Laboratory, Physiology Laboratory of the Faculty of Medicine of Brawijaya University Malang, and Bogor SIG Laboratory.

Bilih fish flour (BFF) was made from fresh Bilih fish obtained from the fisherman in Lake Singkarak as the material for making Bilih fish bars (BFB). White male rats of the Sprague-Dawley strain at 8–10 wk of age, weighing 150–200 g, were obtained from Indoanilab Bogor. STZ was obtained from Sigma (USA).

The diabetic rat model was following the study of Elnovriza et al. (5). The rats used as a model, before being induced with Streptozotocin (STZ) adapted for 1–2 wk for acclimation, and it has given feed with standard feed. After rats were induced intraperitoneally with a single dose of Streptozotocin (STZ) at 40–70 mg/kg to get a diabetic rats model, it was still given a standard feed as long as their blood glucose levels were still below 150 mg/dL (8.3 mmol/L). If the rats have a blood glucose level >150 mg/dL can be used as the sample in this study and given feed according to the intervention group. Feed and drinking water were given ad libitum in the form of pellets according to the treatment of about 20–30 g.

The feed formulation in this study followed Elnovriza et al. (5) study. This study used that the Bilih fish flour contains 27 mg/kg of zinc. The feed formula of fish bars also uses the same dose of zinc. The feed formula is calculated using the AOAC standard. The formula was made in the form of pellets and used as an intervention feed-in diabetic model rats.

This study employed a completely randomized design (CRD) with 40 rats. That rats were grouped into the following five treatments with each treatment represented by eight rats: 1) N: normal rats, standard feed, 2) DMS: diabetic, standard feed, metformin, 3) DMFB: diabetic, metformin, BFB, 4) DFB: diabetic, BFB, and 5) DTI: Diabetic, Bilih fish flour (BFF). The intervention lasted for 30 d, based on a study by Aly and Mantawi (7).

The level of MDA was determined using the thiobarbituric acid (TBA) test (8). The level of SOD was determined using the methods developed by Kotan et al. (9).

Table 1. Changes in rat blood sugar before and after 30 d of intervention.

| Treatment | Before intervention | After intervention | p  | Δ Blood Glucose |
|-----------|---------------------|--------------------|----|-----------------|
| N (n=8)   | 110.06±15.36        | 112.50±7.81        | 0.634 | 2.44±4.99     |
| DMS (n=8) | 335.00±137.66       | 275.92±207.56      | 0.981 | -59.08±32.08  |
| DMFB (n=8)| 333.25±178.98       | 243.50±151.32      | 0.002* | -89.75±31.56  |
| DFB (n=8) | 334.00±165.84       | 250.69±165.14      | 0.076 | -83.31±39.01  |
| DTI (n=8) | 309.75±159.89       | 243.00±133.32      | 0.001* | -66.75±21.86  |
| p         | 0.013**             | 0.220              |    |                 |

*p* Paired t-test, the difference was significant at *p*<0.05.

**ANOV A test continued with Duncan’s Multiple Range Test, significant at *p*<0.05. The number followed by the same superscript letter in one column shows that between treatments are not significantly different (*p* > 0.05).

N: normal rat; DMS: diabetes, metformin, standard feed; DMFB: diabetes, metformin, BFB; DFB: diabetes, BFB, and DTI: diabetes, BFF.

Table 2. Total zinc consumption, MDA levels, and rat SOD levels after 30 d of intervention with BFB and BFF.

| Treatment | Zinc consumption (mg) | MDA (μmol/L) | SOD (U/mL) |
|-----------|-----------------------|--------------|------------|
| N (n=8)   | 0.22±0.02             | 8.279±0.51   | 0.82±0.37  |
| DMS (n=8) | 0.25±0.04             | 7.631±0.44   | 0.87±0.41  |
| DMFB (n=8)| 12.94±1.62            | 8.761±0.94   | 0.72±0.19  |
| DFB (n=8) | 12.72±2.00            | 8.236±0.46   | 1.40±0.76  |
| DTI (n=8) | 13.31±1.90            | 8.266±0.66   | 1.01±0.56  |
| p         | 0.000*                | 0.048*       | 0.085      |

* ANOV A test continued with Duncan’s Multiple Range Test, significant at *p*<0.05. The number followed by the same superscript letter in one column shows that between treatments are not significantly different (*p* > 0.05).

N: normal rats; DMS: diabetes, metformin, standard feed; DMFB: diabetes, metformin, BFB; DFB: diabetes, BFB, and DTI: diabetes, BFF.
Blood glucose was measured using a Gluco-Dr Biosensor. Blood glucose measurements were carried out from tail veins (lateral veins) of rats using Gluco-DR Biosensor, which was done every five days.

The data were analyzed using IBM SPSS Statistics Version 21. Differences in blood glucose before and after the intervention were analyzed using the Paired t-test. Differences between groups were determined by one-way ANOVA, followed by Duncan’s new multiple range test. The level of statistical significance was set at $p<0.05$.

The present study was approved by the Ethical Committee for Animal Research LPPM of Bogor Agricultural University with the number 68-2017 IPB dated 18 July 2017.

RESULTS

In rats induced with STZ, the most significant decrease in blood sugar was in the DMFB group and the lowest in the DMS group. However, based on different tests of blood sugar levels in rats before and after the intervention, only DMFB and DTI treatments were significantly different. The lowest blood glucose reduction was from a group of diabetic rats with a standard feed. At the end of the intervention, all groups had an average blood glucose level not significantly different from normal rats even though they had not returned to normal.

The most significant Zn consumption during the intervention was found in the DMFB group and the smallest in the control rat group. Consumption of Zn in groups given BFF or BFB feeds differed significantly ($p<0.05$) from normal rats and diabetic rats who consumed the standard feed.

The results of the study showed that zinc consumption was associated with a decrease in blood glucose. It was seen that the higher the consumption of zinc, the more significant the decrease in blood glucose from the model rats used, besides the use of anti-hyperglycemic agents that play a role in decreasing blood glucose.

Oxidative stress in this study was measured with MDA levels and serum SOD levels after 30 d of intervention. In a diabetic condition characterized by the characteristic hyperglycemia, followed by an increase in the levels of free radicals in the body where one of them increases levels of malondialdehyde, which is the end product of lipid peroxidation (10).

MDA levels in this study were found to be highest in the DMFB group, while the lowest was found in the DMS group, and the effect of treatment was statistically significant ($p<0.05$). In this study, it was found that giving BFF and BFB can reduce MDA levels that arise due to diabetes conditions and can increase SOD levels as antioxidants.

Based on the results of the study, it can show that the treatment of BFB in diabetic model rats with the administration of the hypoglycemic agent (metformin) increases oxidative stress based on MDA levels. The antioxidant defense of SOD is not comparable with the continuous production of ROS in diabetes; it can be seen from SOD levels decreasing in DMFB treatment, while those given BFB alone and BFF alone can suppress the increase in MDA and it is not significantly different compared to normal rats.

The SOD levels in this study showed that the lowest decrease in blood glucose was in the DMFB group while the highest SOD levels were found in the DFB group. Giving metformin together with the administration of BFB does not increase SOD levels better than just giving metformin. While it was seen that the administration of BFB and BFF alone could increase SOD levels, even higher than the control group and DMS were given metformin alone. This result is in line with the MDA levels of each treatment group, where the higher the MDA level, the lower the SOD level.

DISCUSSION

Blood glucose can decrease with zinc supplementation in diabetic rats. Zinc plays a vital role in the regulation of insulin and carbohydrate metabolism. Zinc is needed as a co-factor for intracellular enzymes involved in protein, lipids, and glucose metabolism. Zinc supplements for type-2 diabetics have a beneficial effect in improving glycemic control (11).

Giving a combination of zinc and metformin together is an effective and safe alternative treatment because it has superior antihyperglycemic efficacy and provides additional benefits compared to the administration of metformin alone in rats with type 2 diabetes (12). Metformin works through several different mechanisms, including reducing serum glucose levels without increasing insulin secretion, primarily through non-pancreatic pathways. Metformin is often called an insulin sensitizer because it increases the effect of insulin (13).

In this study, it was seen that all treatments in diabetic model rats showed a decrease in blood glucose both given metformin, BFB and BFF, and BFB and metformin simultaneously even though it seemed that the decrease in blood glucose occurred through different mechanisms. The biggest decrease in blood glucose is seen in the treatment of BFB and metformin (DMFB). According to Adachi et al. (14), the effect of metformin occurs through increasing glucose use by peripheral tissues that are affected by activated protein kinase (AMPK), the main cellular regulator for lipid and glucose metabolism. Activation of AMPK against hepatocytes will reduce the activity of Acetyl Co-A carboxylase by inducing oxidation of fatty acids and suppress the expression of lipogenic enzymes (14).

In the previous study, MDA levels of diabetic rats given only a standard feed without antihyperglycemic drugs, and fish flour was $14.35 \pm 6.40$ (mmol/L) (5). The MDA levels of rats in this study were lower than that value, it meaning oxidative stress that occurs because diabetes in this study can be corrected by the treatment given either by using Bilih fish or by giving metformin. The MDA levels of rats in this study were lower than those of diabetic rats which was equal to $8.5 \pm 0.76$ (mmol/L) in non-diabetic control rats. $18.3 \pm$
1.26 (mmol/L) in diabetic rats and 9.5 ± 0.86 (mmol/L) in diabetic rats treated with 100 mg/kg methanolic from T. apiculata leaf extract (15).

The decrease in MDA levels in this study seems to be in line with the increase in SOD levels. The higher SOD levels in the treatment of BFF and BF1 are thought to be due to zinc having an antioxidant effect, either as zinc itself or as Zn-Cu SOD. Studies with diabetes induced by STZ and alloxan show that the zinc protection effect involves an antioxidant mechanism, both zinc itself as an antioxidant and zinc induction from MT (16).

Oral hypoglycemic agents may be useful for glycemic control, at least in the early stages of diabetes, but metformin does not seem to be effective in ultimately preventing the development of ROS-mediated organ damage (17). It is shown that although the MDA levels in DMS group rats were the lowest, however, the pancreatic beta-cell damage was seen to be the greatest in immunohistochemical staining results using anti-insulin antibodies in the pancreatic organs.

This study shows that giving fish bars and metformin at the same time does not have a synergistic relationship and enhances each other’s effects, but has potentially opposite effects, which were not examined in this study. Possible mechanisms that the interaction between food and medicine can be in the form of chelate or complex, which causes absorption that is inadequate and antagonistic (18).

Oxidative stress contributes to beta-cell dysfunction, and many studies have shown that antioxidant therapy has the potential to eliminate oxidative stress. Proper administration of antioxidants may have a significant impact on the treatment of beta-cell failure during diabetes (19). Zinc reduces oxidative stress by participating in the synthesis of antioxidant enzymes and acts as an enzyme catalyst, which plays a role in lipid, carbohydrate, and protein metabolism (20). Zinc indirectly neutralizes free radicals by detoxifying ROS and inhibits the formation of free radicals by increasing the activity of antioxidant enzymes. Zinc is also present in the antioxidant enzyme superoxide dismutase (21).

The antioxidant ability of Zn is related to diabetes mellitus, such as increasing SOD status by acting as a structural component of SOD protecting pancreatic beta-cells from damage caused by ROS (22). Antioxidants function to neutralize free radicals that cause damage and death of pancreatic beta-cells and protect pancreatic beta-cells from damage. By reducing the formation of free radicals, the body can regenerate damaged cells through the mechanism of new cell formation so that the number of pancreatic beta-cells gradually becomes.

CONCLUSION

Bilih fish bars and Bilih fish flour lowered blood sugar levels and the oxidative stress caused by diabetes in this study can be corrected by using Bilih fish based on MDA and SOD levels in a diabetic rat model.

Disclosure of state of COI

No conflicts of interest to be declared.

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