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Research Article

The Potential of Bilih Fish (*Mystacoleuseus padangensis* Blkr) Flour as a Zinc Source to Control Blood Glucose and Impact on Oxidative Stress in a Diabetic Rat Model

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Abstract

Background and Objectives: Bilih fish is a potential local food of West Sumatera, Indonesia and it is high in zinc. Fish flour, including that of Bilih fish, is a processed fish product that has not been primarily utilized for food. The levels of zinc in fish flour range from 12.83-22.92 mg. Hence, Bilih fish flour can serve as an alternative food source of zinc for people with diabetes mellitus, who usually exhibit low serum levels of zinc. This study aimed to analyze the effect of Bilih fish flour on the levels of blood glucose, MDA and SOD in diabetic rat models. **Materials and Methods:** This study used a Randomized Complete Design (RCD). Twenty-four white male rats of the Sprague-Dawley strain were placed into the following four groups: Normal and diabetic rats that were fed either the standard feed or Bilih fish flour with a zinc dose of 27 mg kg^{-1} of feed and 13.5 mg kg^{-1} of feed. A single dose of Streptozotocin (STZ) (40 mg kg^{-1}) was used to induce diabetes in the rats. The intervention lasted for 14 days. The data obtained were subjected to Wilcoxon analysis to compare the blood glucose levels before and after the intervention. The differences in MDA and SOD levels between groups were determined with one-way ANOVA followed by Duncan's new multiple range test. The level of statistical significance was set at $p<0.05$. **Results:** The intervention with Bilih fish flour resulted in a decrease in blood glucose levels. Intervention with Bilih fish flour with a dose of zinc of 0.54 mg lowered blood glucose as much as 38.95% and a dose of zinc of 0.27 mg lowered blood glucose as much as 32.45%, which was a significant decrease ($p<0.05$). The levels of MDA in rats that received intervention with Bilih were 9.87±2.88 μmol L^{-1} for the D-P1 group and 11.88±10.5 μmol L^{-1} for the D-P2 group, which were both lower compared to the control diabetic rats (D) (14.35±6.4 μmol L^{-1}). The levels of SOD in rats that received intervention with Bilih with the high zinc content were higher compared to the diabetic rats with standard feed but this increase was not significant ($p>0.05$). **Conclusion:** Bilih fish flour with a high zinc content lowered blood sugar levels but did not decrease the oxidative stress levels based on MDA and SOD levels in a diabetic rat model. MDA was not decreased and SOD was not increased significantly compared to diabetic controls.

Key words: Bilih fish, blood glucose, malondialdehyde, oxidative stress, superoxide dismutase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Uncontrolled diabetes over long periods of time can cause a variety of complications/disruptions that are either acute or chronic. Various studies have demonstrated the crucial role of zinc and the effect of zinc deficiency in relation to the incidence of diabetes mellitus in various populations and age groups. Indonesia is the country with the fourth highest number of people with diabetes mellitus (DM) in the world, after India, China and the United States, with 8.4 million sufferers in 2000; this number is estimated to reach 21.3 million by the year 2030[1].

There are several reasons to suspect that abnormal zinc metabolism plays a role in the pathogenesis of diabetes mellitus and its complications[2]. Various experiments and clinical studies have reported that zinc deficiency may be a predisposing factor for glucose intolerance, insulin resistance, diabetes mellitus, atherosclerosis and coronary artery disease[3,4].

Oxidative stress plays an important role in the pathogenesis of diabetes and its complications and zinc is a key structural component of many antioxidant enzymes such as superoxide dismutase, which is vital for intracellular and extracellular antioxidant defenses[5]. An increase in oxidative stress has been observed in diabetic patients, indicating the increased production of free radicals. Free radicals may play a role in causing diabetes mellitus and its related complications[6].

The levels of malondialdehyde (MDA)-induced lipid peroxidation measured as the MDA content were found to be increased in STZ-induced diabetes models but were significantly reduced by zinc supplementation[7,8]. Zinc supplementation led to a reduction in lipid peroxidase, as measured by the TBARS content, which was monitored in the plasma of people with type 2 diabetes[9]. Zn supplementation can increase the activity of superoxide dismutase (SOD) and decrease the concentration of lipids in diabetic rats[10].

Bilih fish (*Mystacoleuseus padangensis* Blkr)is a potential local food in West Sumatera Province, Indonesia, which has a nutrient content, especially zinc. Fish flour, including that of Bilih fish, is a processed fish product that has not been primarily utilized for food. The present study demonstrated that the zinc content of fresh Bilih fish is 4.76 mg/100 g, whereas the zinc levels in Bilih fish flour range from 12.83-22.92 mg. Therefore, Bilih fish could potentially serve as an alternative food source of zinc in people with diabetes mellitus, who usually exhibit low serum levels of zinc. This study aimed to analyze the effect of Bilih fish flour on the levels of serum blood glucose, MDA and SOD in a rat model of diabetes mellitus.

MATERIALS AND METHODS

Time and place: This research was conducted between September and December 2017 at the animal hospital of Bogor Agricultural University, laboratory of histology and physiology laboratory of the Department of Anatomy, Physiology and Pharmacology of Bogor Agricultural University and Saraswanti Indo Genetech Laboratory, Bogor, Indonesia.

Materials: Bilih fish flour was made from fresh Bilih fish obtained from the fisherman in Lake Singkarak. White male rats of the Sprague-Dawley strain at 8-10 weeks of age, weighing 150-200 g, were obtained from Indoanilab Bogor. STZ was obtained from Sigma (USA).

Generation of the diabetic rat model: A rat model of diabetes was generated in rats weighing 150-200 g, aged 8-10 weeks. The rats were fasted overnight and then injected intraperitoneally with a single dose of Streptozotocin (STZ) (40 mg kg^{-1}). The STZ was dissolved in freshly prepared 50 mM sodium citrate buffer (pH 4.5). STZ-treated rats were selected for further study if their blood glucose levels were >150 mg dL^{-1} (8.3 mmol L^{-1}) and/or statistically higher compared to the control rats. On the first day after injection, rats were provided normal food and 10% sucrose water. On experimental day 2, the 10% sucrose water was replaced with regular water[11].

Determination of the supplementation dose: The doses of zinc supplements used in this study were based on the Council for Responsible Nutrition supplementation recommendations, i.e., 30 mg[12]. Because this is the dose recommended for humans, the dose used for the rat models in this research was based on the conversion factor for human to rats, i.e., 0.018[13].

The rats consumed an estimated 20 g of feed, meaning that every 20 g of feed should contain 0.54 mg of Zn or 27 mg kg^{-1}. These doses were used in the D-P1 group. The D-P2 group received half of the dosage of D-P1, i.e., 13.5 mg kg^{-1} and this dose is still greater than the basic zinc requirement of a rat, which is 12 mg kg^{-1} [14].

Study design: This study employed a completely randomized design (CRD) including 24 rats. That rats were grouped into the following 4 treatments with each treatment represented by 6 rats: (1) Control (N): normal rats, standard feed, (2) Diabetes (D): diabetes, standard feed, (3) treatment 1 (D-P1): Diabetes and Bilih fish flour containing a zinc dose of

27 mg kg^{-1} and (4) treatment 2 (D-P2): Diabetes and Bilih fish flour containing a zinc dose of 13.5 mg kg^{-1}. The intervention lasted 14 days based on a study by Yoshikawa *et al.*[15].

The level of MDA was determined using the thiobarbituric acid (TBA) test[16]. The level of SOD was determined using the methods developed by Misra and Fridovich[17]. Blood glucose was measured using a Gluco-Dr Biosensor.

Statistical analysis: The data were analyzed using IBM SPSS Statistics Version 21. Differences in blood glucose before and after the intervention were analyzed using the Wilcoxon 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

Zinc intake and feed consumption: There were significant changes in the zinc intake of the STZ-induced rats in all groups ($p<0.05$). Feed consumption in the normal rats during the intervention remained relatively stable, whereas the diabetic rats exhibited a daily increase in feed consumption. The average feed consumption early in the intervention was 9.44 g, which increased to 14.18 g by the end of the intervention. The highest consumption of Zn during the intervention was observed in the D-P1 group and the lowest consumption of Zn was observed in the group of normal rats (N) (Table 1).

Blood glucose: Rat blood glucose measurements were performed every three days in the lateral vein using the Gluco-DR Biosensor. The blood sugar of the control group was normal and tended to be stable. Based on the results after the 14-day intervention, Bilih fish flour significantly lowered the blood sugar levels in rats (Table 2). The group that received 0.54 mg of zinc (27 mg kg^{-1}, D-P1) exhibited the most profound decrease in blood sugar (D = 140 mg dL^{-1}) compared to the other diabetic groups.

Intervention with Bilih fish flour with a zinc dose of 0.54 mg (D-P1) lowered blood glucose as much as 38.95% and a zinc dose of 0.27 mg (D-P2) lowered blood glucose as much as 32.45%, which was a significant decrease ($p<0.05$). Although, the blood glucose levels of the diabetic rats did not decrease to normal levels, it is expected that a longer intervention with Bilih fish flour would further lower the blood glucose levels of these rats (Fig. 1).

Malondialdehyde (MDA): MDA is one of the primary biomarkers of free radical-mediated oxidative stress and lipid damage[18]. After the intervention, the MDA levels of the D-P1 and D-P2 groups approached those of normal rats (N). Even the MDA levels of group D-P1 were lower than that of the diabetic control rats, although the difference between groups

Table 1: Total zinc consumption and the levels of MDA and SOD in rats after the 14-day intervention with Bilih fish flour, n=6

	Mean±SD		
Treatments	Zinc consumption (mg)	MDA (μmol L^{-1})	SOD (unit mL^{-1})
N	1.80±0.09[a]	10.85±2.88[a]	11.80±3.18[a]
D	2.16±0.27[b]	14.35±6.40[a]	9.75±3.17[a]
D-P1	5.63±0.33[d]	9.87±2.88[a]	10.77±1.95[a]
D-P2	2.85±0.13[c]	11.88±10.50[a]	10.77±3.37[a]
p	0.000*	0.737	0.703

N: Normal rat, D: diabetes, standard feed, D-P1: Diabetes, Bilih fish flour with 0.54 mg of zinc (27 mg kg^{-1}), D-P2: Bilih fish flour with 0.27 mg of zinc (13 mg kg^{-1}). *Statistically significant difference. Means with the same letter in one column are not significantly different

Table 2: Blood glucose in rats before and after the 14-day intervention, n=6

	Blood glucose±SD (mg dL^{-1})			
Treatments	Before	After	D Blood glucose±SD	p-value
N	113.50±16.23[a]	104.17±8.70[a]	-9.30±16.19[a]	0.249
D	448.92±127.19[b]	397.33±104.56[c]	-51.58±168.82[a]	0.753
D-P1	359.42±208.43[b]	219.42±90.36[b]	-140.00±132.20[a]	0.028*
D-P2	359.83±208.77[b]	243.08±101.78[b]	-116.75±129.27[a]	0.046*
p	0.011**	0.00**	0.326	

N: Normal rat, D: diabetes, standard feed, D-P1: Diabetes, Bilih fish flour with 0.54 mg of zinc (27 mg kg^{-1}), D-P2: Bilih fish flour with 0.27 mg of zinc (13 mg kg^{-1})
N: Normal rat, D: diabetes, standard feed, D-P1: Diabetes, Bilih fish flour with 0.54 mg of zinc (27 mg kg^{-1}), D-P2: Bilih fish flour with 0.27 mg of zinc (13 mg kg^{-1}).
*Statistically significant difference. **Means with the same superscript letter in one column are not significantly different

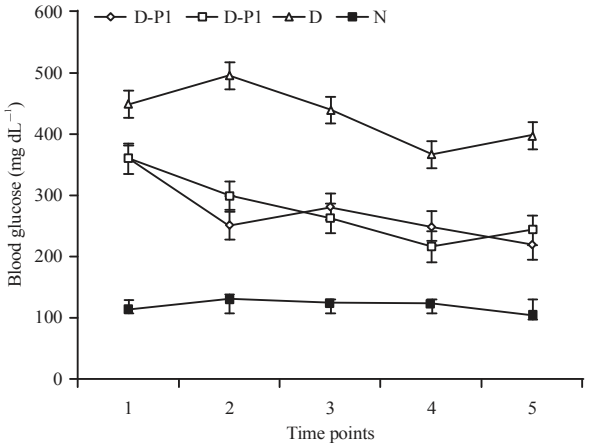

Fig 1: Changes in blood glucose measured every 3 days during the intervention with Bilih fish flour.
N: Normal rat, D: Diabetes, standard feed, D-P1: Diabetes, Bilih fish flour with 0.54 mg of zinc (27 mg kg^{-1}), D-P2: Bilih fish flour with 0.27 mg of zinc (13 mg kg^{-1})

was not significant (Table 1). This finding indicates that Bilih fish flour can attenuate the increased levels of MDA in diabetic rats.

Superoxide dismutase (SOD): This study demonstrated that the 14-day intervention led to a decrease in the levels of SOD compared to the control rats but differences among groups were not significant (Table 1). When compared to the diabetic rats that were given the standard feed (D), the diabetic rats that consumed Bilih fish flour for 14 days maintained higher levels of SOD. The levels of SOD were higher in the D-P1 and D-P2 groups compared with the diabetic control group (D).

DISCUSSION

Diabetes mellitus (DM) affects zinc homeostasis in various ways. Zinc is required as a co-factor for many enzymes that are involved in protein, lipid and glucose metabolism. Zinc may also participate as an integral component of several antioxidant enzymes. Complications of diabetes may be related to an increase in intracellular oxidation and free radicals associated with the decrease in intracellular zinc and zinc-dependent antioxidant enzymes[19].

Blood glucose levels may be decreased by zinc supplementation in people with diabetes. Zn plays a substantial role in insulin regulation and carbohydrate metabolism and is required as a co-factor for the function of intracellular enzymes involved in protein, lipid and glucose metabolism. Zinc supplementation for type-2 diabetics has the beneficial effect of improving glycemic control[19,20]. The control of blood glucose levels is a key step in preventing or reversing diabetes-related complications[21]. In the present study, that rats which consumed Bilih fish flour with a higher zinc content exhibited a greater decrease in blood glucose compared to the rats that consumed less zinc.

Oxidative stress is relatively common in diabetes[22]. Oxidative stress in diabetes is caused by two factors, namely, the increased formation of free radicals and a decrease in plasma antioxidant defense[23]. High levels of blood glucose were shown to lead to the formation of free radicals, to disrupt metabolism and to enhance oxidative stress[24]. Enhanced oxidative stress has been observed in diabetic patients, as indicated by the increased free radical production, lipid peroxidation and diminished antioxidant status. Free radicals may play an important role in the causation and complications of diabetes mellitus[6].

Antioxidants serve to neutralize free radicals, thereby reducing the oxidative stress that damages and kills pancreatic β-cells. Therefore, antioxidants protect pancreatic β-cells. By decreasing the formation of free radicals, the body can regenerate damaged tissue through the formation of new cells such that the number of pancreatic β-cells gradually returns to normal[25,26]. Many studies have demonstrated that antioxidant therapy potently inhibits ROS generation and eliminates oxidative stress. Therefore, antioxidants may have a considerable impact on the treatment of β-cell failure during diabetes[25].

The chronic hyperglycemia that is common in diabetes mellitus promotes oxidative stress through the production of enhanced reactive oxygen species and/or by reducing the activity of the antioxidant defense system[27]. Oxygen-derived free radicals and reactive oxygen species interact with the lipid bilayer of the cell membrane, resulting in lipid peroxidation. Malondialdehyde (MDA), which is a stable end product of lipid peroxidation[28]has the molecular formula $C_3H_4O_2$ and is produced by the oxidation of unsaturated fatty acids by free radicals in the body[6]. MDA is increased in oxidative stress conditions and decreased by the activity of the antioxidant superoxide dismutase[29].

In this study, although MDA levels did not exhibit significant changes, the group given Bilih fish flour exhibited lower MDA levels compared to the group given standard feed. A previous in vivo study on diabetic rats demonstrated that zinc has a protective effect against diabetes by down regulating oxidative stress[30]. In addition, significant changes in the levels of MDA and lipid-standardized MDA were significantly altered after 3 months of supplementation with zinc and magnesium[31]. In that study, the MDA levels of rats

were significantly reduced by zinc treatment. An increase in lipid peroxidation, measured by the MDA content, indicated an oxidative status and no changes in the serum levels of zinc[8]. In another study, lipid peroxidase was shown to be increased and superoxide dismutase levels were decreased in people with type II diabetes mellitus compared to controls[23].

Zinc supplementation reduces oxidative stress[22]. Zinc is also a component of the antioxidant superoxide dismutase enzymes. Zinc is a secondary antioxidant that serves as a scavenger of free radicals, capturing or detoxifying ROS and increasing the activity of antioxidant enzymes. Zinc indirectly neutralizes free radicals and inhibits the formation of free radicals[29]. Zinc plays an important role in the antioxidant defense system of type 2 diabetic patients by acting as a cofactor of the superoxide dismutase enzymes. Zinc also improves oxidative stress by reducing chronic hyperglycemia and promotes the phosphorylation of insulin receptors by enhancing the transport of glucose into cells[27].

The potential antioxidant effect of Zn in the context of diabetes may be related to several mechanisms. Zn plays a role in the Cu-Zn structural integrity of SOD. In addition, Zn metallothionein complexes in the islet cells provide protection against the immune-mediated attack of free radicals and Zn may also act to protect sulfhydryl groups against oxidation and participate in the inhibition of free radical production in the Haber Weiss cycle by competing with transition metals[9].

In this study, supplementation with Bilih fish flour did not significantly increase SOD levels after 14 days of intervention. These results are in agreement with other studies that found no increase in the activity of SOD in patients with T2DM after supplementation with 30 mg of Zn gluconate[9]. A decrease in Cu-Zn SOD activity in the liver, kidney and erythrocytes was observed 10 days after the induction of diabetes with streptozotocin[32]. In contrast, another study reported that Zn supplementation increased the activity of SOD and decreased the peroxidation of lipids in diabetic rats[10]. Another study on diabetic rats found that treoninate-chelated Zn supplementation increased the activity of SOD and decreased the concentration of MDA in the serum and pancreas, reducing the levels of damaged and the activity of the antioxidant defense system[33]. It was shown that 30 mg of Zn supplementation for 6 months in patients with T2DM resulted in a decrease in plasma lipid peroxidation but did not alter the activity of SOD[34].

CONCLUSION

The 14-day intervention with Bilih fish flour in a diabetic rat model lowered blood glucose levels significantly, although the blood glucose levels did not return to normal. This intervention did not decrease oxidative stress, as indicated by MDA and SOD levels; MDA was not decreased and SOD was not significantly increased compared to diabetic controls.

SIGNIFICANCE STATEMENT

This study demonstrated that Bilih fish flour controlled blood glucose in a diabetic rat model, although there was no impact on oxidative stress. This study reveals that fish, particularly those rich in zinc, may be beneficial for people with diabetes mellitus, which has not been thoroughly explored. Many studies have investigated the benefits of plant food in diabetes mellitus but there has not been much research on the benefits of animal foods, particularly fish, for people with DM. Previous research has shown that fish consumption can reduce the prevalence of metabolic syndrome and reduce the risk of DM. The novel findings of the present study reveal that zinc and perhaps other nutrients contained in fish, particularly Bilih fish, are beneficial for diabetic people. However, the underlying mechanism remains unclear.

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