Role of SOD and GPx in Mitigating Oxidative Stress in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic disease characterized by an increase in blood glucose levels, which leads to oxidative stress as a consequence. Streptozotocin works by damaging pancreatic beta cells, resulting in hyperglycemia. This chronic hyperglycemia can increase the production of free radicals. Moreover, streptozotocin can cause significant oxidative stress by damaging cellular DNA, lipids, and proteins. The excessive free radicals can deplete antioxidant reserves like SOD and GPx, as they are utilized to neutralize the free radicals. The aim of this study is to understand the role of SOD and GPx in reducing oxidative stress in streptozotocin-induced diabetic rats. Spectrophotometric analysis was utilized to quantify SOD and GPx levels in rat tissues, providing insights into the extent of oxidative damage. The results revealed that while the levels of SOD showed no significant difference after streptozotocin induction, but there was a noticeable decrease in SOD levels. In contrast, GPx levels decreased significantly following induction. In conclusion, this study underscores the relevance of monitoring SOD and GPx levels as biomarkers for assessing oxidative stress in diabetic conditions and guiding therapeutic interventions.

Keywords: Diabetes Mellitus, Streptozotocin, Superoxide Dismutase, Glutathione Peroxidase

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or a combination of both. Currently, DM is emerging as a global health threat. Various epidemiological studies have shown an increase in the incidence and prevalence of type 2 DM worldwide. The World Health Organization (WHO) predicts a significant surge in the number of type 2 DM patients in the

coming years. In Indonesia, WHO estimates that the number of type 2 DM patients will increase from 8.4 million in 2000 to approximately 21.3 million in 2030. Predictions from the International Diabetes Federation (IDF) also indicate that during the period from 2019 to 2030, the number of DM patients in Indonesia will rise from 10.7 million to 13.7 million by 2030 (Perkeni, 2021).

Diabetes mellitus is characterized by a state of chronic hyperglycemia. This leads to an increased production of free radicals, triggering oxidative stress. Oxidative stress is a condition where the free radicals generated through biochemical reactions damage cell membranes and cause various functional disorders in the body. Free radicals in the body can cause damage to DNA, carbohydrates, proteins, and lipids (Anita, 2019).

In DM, there is an increase in the production of Reactive Oxygen Species (ROS). Physiologically, the presence of ROS in the body is balanced by endogenous defense mechanisms that produce antioxidants to neutralize free radicals. However, when ROS production exceeds the body's defense capacity, oxidative stress occurs. In this condition, the significant increase in ROS activates antioxidant enzymes to neutralize ROS, ultimately reducing the amount of antioxidants in the body. Previous studies have shown that oxidative stress in rats results in a significant decrease in SOD and GPx enzyme levels in serum, liver, testes, and heart (Volpe, 2018).

The aim of this study is to analyze the role of antioxidant enzymes, particularly Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx). This study will evaluate the changes in SOD and GPx levels in body tissues and identify the relationship between increased free radical production and decreased antioxidant enzyme activity in diabetic animal models.

This research is motivated by the rising incidence and prevalence of type 2 diabetes mellitus, which poses a global health threat, including in Indonesia. Given the significant role of oxidative stress in the pathogenesis of diabetes and its related complications, this study focuses on further understanding the body's defense mechanisms against free radicals, specifically through the activity of the antioxidant enzymes SOD and GPx. By understanding the roles of these enzymes in chronic hyperglycemia conditions, this study aims to provide new insights that could be beneficial in developing therapeutic strategies to prevent or mitigate the adverse effects of oxidative stress in diabetic patients.

LITERATURE REVIEW

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action, or both (Perkeni, 2021). The pathogenesis of hyperglycemia can be broadly attributed to eleven factors, collectively known as the "Egregious Eleven." These include decreased insulin secretion by pancreatic beta cells, reduced incretin hormone levels, increased lipolysis, enhanced glucose reabsorption by the kidneys, reduced glucose uptake by muscles, neurotransmitter dysfunction, increased hepatic glucose production, insulin resistance in adipose tissue, excessive glucagon effects, insulin resistance in the intestines, and decreased amylin effects. All these factors contribute to elevated blood glucose levels that are difficult to control in individuals with type 2 diabetes (Schwartz et al., 2016).

Diabetes Mellitus (DM) can be diagnosed through blood glucose and HbA1c testing. Blood glucose testing typically includes fasting blood glucose, oral glucose tolerance test (OGTT), and random blood glucose. HbA1c (glycated hemoglobin) provides information about average blood glucose levels over the past 2-3 months. The use of HbA1c for DM diagnosis has the advantage of offering a more comprehensive overview of long-term blood glucose management, while blood glucose levels are more useful for determining acute conditions or short-term glucose regulation (Wang et al., 2023).

The samples in this study are rats induced with streptozotocin (STZ), a compound known to trigger the formation of free radicals, which can damage pancreatic islets and contribute to the development of diabetes (Sholikah et al., 2020). Free radicals such as superoxide radicals (O_2), hydroxyl radicals (OH), peroxynitrite (ONOO), and hydrogen peroxide (H_2O_2) are particularly implicated in STZ-induced diabetes (Samarghandian et al., 2017). Increased oxidative stress and the formation of free radicals, particularly hydroxyl radicals, have been observed in STZ-induced diabetic rats (Gille et al., 2002). These free radicals react with nearby cellular molecules to obtain electron pairs, thereby stabilizing themselves while converting the cellular molecules into new free radicals. This perpetuating cycle, if unaddressed, leads to oxidative stress, which can result in inflammation, DNA or cellular damage, and various diseases (Phaniendra, 2015).

Antioxidants are essential substances needed by the body to neutralize free radicals and prevent damage to normal cells, proteins, and lipids. Endogenous antioxidants, produced naturally within the body, include enzymes such as Superoxide Dismutase (SOD) and

Glutathione Peroxidase (GPx). These enzymes are crucial for defending against oxidative damage and maintaining oxidative balance. In the context of STZ-induced diabetes, the imbalance between oxidative stress and antioxidant defenses exacerbates tissue damage and dysfunction (Flieger, 2021). Evaluating the activities of SOD and GPx in diabetic rats provides valuable insights into how oxidative stress influences diabetic pathogenesis. This understanding could inform the development of targeted therapeutic strategies that enhance antioxidant defenses alongside traditional glycemic control measures, potentially mitigating diabetic complications and improving overall disease management.

METHODS

This study employed an experimental design conducted at the Faculty of Medicine, Universitas Prima Indonesia, to investigate the role of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in mitigating oxidative stress in Streptozotocin-induced diabetic rat models. This study was conducted in accordance with the ethical standards set by Animal Research Ethics Committees (Approval No. [070/KEPH-FMIPA/2023]), ensuring that all animal experiments adhered to the principles of laboratory animal care. Male Wistar rats were randomly assigned to control and experimental groups. The experimental group received an intraperitoneal injection of streptozotocin (STZ) to induce diabetes.

Fourteen male Wistar rats, aged 8-12 weeks and weighing 250-300 g, were randomly divided into two groups: Group 1 as the Control Negative (no treatment) and Group 2 as the Control Positive (Streptozotocin-induced). The rats were housed in wire-topped cages at the animal facility of the Faculty of Medicine, Universitas Prima Indonesia, and were acclimatized for seven days with standard pellet feed and water ad libitum.

After the adaptation period, the rats underwent a 12-hour fasting, followed by induction of hyperglycemia using streptozotocin (STZ) at a dose of 40 mg/kg body weight. Three days post-induction, blood glucose levels were measured using a One Touch Basic blood glucose monitoring system. Rats with blood glucose levels $\geq$ 200 mg/dL were considered hyperglycemic and included in the study. The activities of SOD and GPx in rat tissues were measured both before and after STZ induction using specific assays, to evaluate their roles in managing oxidative stress.

SOD activity in blood serum was measured using a modified method. A total of 0.06 ml of blood supernatant was reacted with a mixture consisting of 2.70 ml of 50 mM sodium

carbonate buffer containing 0.1 mM EDTA (pH 10), 0.06 ml of 10 mM xanthine, 0.03 ml of 0.5% bovine serum albumin (BSA), and 0.03 ml of 2.5 mM nitroblue tetrazolium (NBT). Xanthine oxidase (0.04 units) was then added to the mixture. The absorbance produced after 30 minutes of incubation was measured at 560 nm using a spectrophotometer. As a control, phosphate-buffered saline (PBS) containing 11.5 g/L KCl was used. SOD activity (%) is calculated using the following equation: (1-(A/B)) x 100% where: A = absorbance of the sample solution and B absorbance of the control solution.

GPx activity in blood serum was measured using a modified method. A total of 200 µl of blood supernatant was mixed with 200 µl of 0.1 M phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 200 µl of 10 mM reduced glutathione (GSH), and 200 µl of glutathione reductase enzyme (2.4 units). The mixture was incubated at 37°C for 10 minutes. Following the incubation, 200 µl of 1.5 mM NADPH was added and the solution was incubated again at 37°C for three minutes. Finally, 200 µl of 1.5 mM H_2O_2 was added to initiate the reaction. Absorbance was measured using a spectrophotometer at 340 nm between one and two minutes.

Data collection involved recording SOD (Superoxide Dismutase) and GPx (Glutathione Peroxidase) levels before and after induction with streptozotocin. To analyze the data, SPSS version 15.0 was used. Initially, the data were assessed for homogeneity with Levene's test and normality with the Shapiro-Wilk test. For normally distributed data, a two-way ANOVA was performed, and significant results were further analyzed using the Duncan test. For non-normally distributed data, the Kruskal-Wallis test was applied, with significant findings further examined using the Mann-Whitney U test. A p-value of < 0.05 was considered significant. This thorough analysis aimed to evaluate the role of SOD and GPx in mitigating oxidative stress and their implications for diabetic complications and therapeutic strategies in streptozotocin-induced diabetic rat models.

RESULTS

The study demonstrated that diabetic rats, after successful induction, displayed characteristic symptoms like polyuria and polydipsia. A significant reduction in GPx (Glutathione Peroxidase) activity was observed in the diabetic group (0.48 ± 0.59) compared to the control group (4.39 ± 0.08), indicating a 9.14-fold suppression. This sharp decline highlights the impact of hyperglycemia on the antioxidant defense system, specifically GPx, which plays a critical role in countering oxidative stress. Conversely, SOD (Superoxide Dismutase) levels

showed no significant difference between diabetic and control groups as shown in table 1, suggesting that SOD may be less affected by hyperglycemia compared to GPx.

Table 1. GPx and SOD Activity Levels (Spectrophotometer Results)

Rats	GPx	SOD
Control (-)	4,39 ± 0,08	2,17 ± 1,05
Control (+)	0,48 ± 0,59	2,09 ± 0,61

DISCUSSION

The induction of diabetes in rats was successful, as evidenced by the manifestation of classic diabetic symptoms such as polyuria and polydipsia, which are commonly associated with hyperglycemia. The study demonstrated a significant alteration in the antioxidant defense system, particularly in the activities of Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD).

The expression level of GPx in the hyperglycemic group was significantly lower (0.48 ± 0.59) compared to the negative control group (4.39 ± 0.08), with a p-value less than 0.05, indicating a statistically significant difference. This represents a 9.14-fold suppression of GPx activity in diabetic rats compared to their healthy counterparts. The substantial reduction in GPx activity underscores the profound impact of diabetes on the body's antioxidant defense mechanisms, as GPx plays a critical role in mitigating oxidative stress by neutralizing harmful peroxides.

These findings are consistent with previous studies by Riyanti (2014) and Moussa (2008), which reported decreased GPx enzyme activity in individuals with diabetes. The reduction in GPx levels can be attributed to increased formation of free radicals due to sustained high blood glucose levels, particularly affecting organs like the pancreas that inherently have lower levels of antioxidant enzymes (Suarsana et al., 2011). The diminished GPx activity highlights the heightened vulnerability of diabetic subjects to oxidative damage, contributing to the progression of various diabetic complications.

Contrary to the significant changes observed in GPx activity, the study did not find a notable difference in SOD levels between the positive control group (2.09 ± 0.61) and the negative control group (2.17 ± 1.05), with a p-value greater than 0.05. This suggests that SOD activity remains relatively resilient in the early stages of hyperglycemia-induced oxidative stress.

SOD is a crucial endogenous antioxidant enzyme that plays a vital role in combating oxidative stress by dismutating superoxide radicals into less harmful molecules (Younus, 2018). The stability of SOD levels in this study may be due to the duration of diabetes induction not being sufficient to produce significant changes. Diabetes mellitus develops progressively, and longer exposure to hyperglycemic conditions may be required to observe substantial alterations in SOD activity.

Similar studies have highlighted the dynamic nature of antioxidant enzyme activities over time in diabetic conditions. Yang et al. (2012) observed that in STZ-induced diabetic rats, oxidative markers such as O_2^- and H_2O_2 increased significantly, with antioxidant enzymes like SOD and GPx initially showing compensatory increases before decreasing as diabetes progressed. At eight weeks post-STZ induction, increased GPx protein levels were noted, but by sixteen weeks, both GPx and SOD levels had substantially decreased compared to controls. This temporal decline indicates that while antioxidant defenses may initially respond adaptively to oxidative stress, their capacity diminishes over time, leading to increased oxidative damage.

Further studies corroborate these findings, emphasizing the significant rise in oxidative stress markers and concomitant decrease in antioxidant enzyme activities in diabetic models (Ağgül et al., 2020; Alaofi, 2020). The impaired function of SOD and GPx contributes to the imbalance between oxidants and antioxidants, exacerbating cellular damage and fostering the development of diabetic complications.

Understanding the differential impacts of diabetes on key antioxidant enzymes is crucial for developing targeted therapeutic strategies. Interventions aimed at enhancing the activities of SOD and GPx have shown promise in reducing oxidative damage and improving glycemic control. For instance, natural compounds like resveratrol have been demonstrated to upregulate these enzymes, thereby mitigating oxidative stress and protecting against diabetic complications (Salazar, 2021). Maintaining or restoring antioxidant enzyme activity could be pivotal in delaying the onset and progression of diabetes-related disorders (Sani et al., 2014).

CONCLUSION

The study highlights a pronounced suppression of GPx activity and a relatively unchanged SOD activity in STZ-induced diabetic rats, indicating a selective vulnerability within the antioxidant defense system under hyperglycemic conditions. This imbalance contributes to

increased oxidative stress and underscores the need for therapeutic approaches that bolster antioxidant defenses. Future research should focus on long-term studies to elucidate the progression of antioxidant enzyme activities over time and explore potential interventions that can effectively enhance these critical defense mechanisms in diabetic patients.

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