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Effect of intermittent fasting on fasting blood glucose, sirtuin 1, and total antioxidant capacity in rat models of diabetes mellitus

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ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycaemia. DM therapy is mainly purposed to control blood glucose levels by adjusting diet and reducing body fat, which can be implemented with calorie restriction (CR) by intermittent fasting (IF), a diet that alternates periods between eating and fasting. Sirtuins, proteins activated by CR, can regulate glucose metabolism, regulate insulin secretion, and protect cells from oxidative stress, so IF is considered to be an opportunity for DM management.
Objective: This study is to determine effects of IF on Fasting Blood Glucose (FBG) levels, sirtuin 1 (SIRT1) activity, and total antioxidants (TAOC) in rat models of Wistar with DM.
Methods: This experimental study applied a post-test control group design involving 24 Wistar rats which were divided into 4 groups: K1 (rats with DM without IF), K2 (rats with DM treated with metformin 45 mg/kg BW, K3 (rats with DM treated with IF), and K4 (normal rats treated with IF). The diabetes condition were induced with intraperitoneal injection of streptozotocin (STZ). The rats received IF treatment, fasted for 16 hours and ate window open for 8 hours. This treatment was conducted for 14 days. The FBG levels were measured by using a glucometer, while SIRT1 activity and TAOC were measured by using the ELISA method.
Results: The statistical analysis using the Kruskal Wallis test for the FBG levels indicated that there was a significant difference among the four groups ($p = 0.000$). The ANOVA test for SIRT1 activity revealed that there was a significant difference among the four groups ($p = 0.001$). The Kruskal Wallis test for TAOC pointed out that there was no significant difference among the four groups ($p = 0.529$).
Conclusion: The IF method using a 16:8 regimen reduced the FBG levels and increased the sirtuin 1 activity, but it was not proven to increase the TAOC in the rat models of Wistar with DM.

INTRODUCTION

Diabetes mellitus is a chronic condition caused by increased blood sugar levels. This is associated with abnormalities of carbohydrate, fat and protein metabolism that occur due to insulin deficiency, namely: abnormalities in insulin secretion, in insulin action or in both. The occurrence of insulin deficiency can be attributed to various factors, including the impairment of pancreatic β-cells due to external influences, reduced glucose receptors in the pancreatic gland, and damage to insulin receptors in peripheral tissues. The DM is classified into 4 types: type 1 DM, type 2 DM, gestational DM, and specific types of DM related to other causes.[1,2,3] Various epidemiological studies demonstrate that there is a trend of increasing the incidence and prevalence of DM sufferers throughout the

world. The World Health Organization (WHO) forecasts a rise in the number of individuals with type 2 DM in Indonesia, projecting an increase from 8.4 million in 2000 to 21.3 million in 2030.[3]

Prevention and treatment of DM is to avoid complications of micro- and macroangiopathy. The extent of complications correlates with the condition of hyperglycaemia which causes tissue damage. Hyperglycaemia is also involved in the formation of free radicals, by accelerating the formation of reactive oxygen compounds. These molecular modifications result in an imbalance between antioxidant defences and increased production of free radicals (known as oxidative stress). One of the prevention efforts to reduce free radical products due to hyperglycaemia is through regulating the amount of calorie intake.[4,5]

In DM therapy, regulating blood glucose levels is the primary objective, which can be performed with pharmacological and non-pharmacological measures. Pharmacological therapy is done by giving drugs to lower blood sugar levels such as metformin, sulfonylureas, thiazolidinediones, and insulin injections. Non-pharmacological actions can be performed by increasing physical activity, regulating diet and reducing the body fat; one of which is CR.[4,6] Metabolic CR can serve as a potential avenue for therapeutic interventions in managing insulin resistance and type 2 DM. Sirtuins, a class of histone deacetylase enzymes that rely on Nicotinamide Adenine Dinucleotide $(NAD)^+$ for their activity, can be activated through the CR. There are 7 groups of sirtuins, namely sirtuin 1 to sirtuin 7, and sirtuin 1 is currently being studied a lot. The sirtuin 1 is one of metabolic regulators, that can regulate glucose and lipid metabolism through deacetylase activity. Sirtuins are involved in insulin signalling, oxidative stress and inflammatory defence, and insulin secretion regulation in pancreatic cells.[6,7] One method of CR is IF, which is a pattern of eating alternating periods between eating and fasting. The IF can improve metabolic parameters such as weight loss, blood pressure, lipids, and blood glucose. By improving these parameters, IF can reduce the risks of cardiovascular disease.[8,9,10]

Based on this background, this study was conducted to investigate effects of CR using the IF method on fasting blood glucose levels, sirtuin 1 activity and antioxidant level in rat models of Wistar strain with DM.

METHOD

Research design

This study was an experimental laboratory design research methodology, employing a post-test control group design approach. This study was conducted at the Integrated Research Laboratory, Universitas Yarsi, in March-April 2023.

Population and sample

The study focused on male rats of *Rattus norvegicus* Wistar strain as its population samples. The samples were collected randomly, ensuring that they met the inclusion and exclusion criteria. Its inclusion criteria were *Rattus norvegicus*, Wistar strain, male, 12-14 weeks old, 175-200 grams of body weight, healthy condition, and >200 mg/dl of FBG levels after STZ induction. The exclusion criteria were unhealthy rats during the adaptation period and dead rats during the treatment. The animals used in this study were obtained from a provider institution, CV Dunia Kaca, Karanganyar, Central Java. This study began with the selection of experimental animal samples adhering to predetermined criteria for the inclusion and exclusion criteria. Furthermore, the rats were acclimatized for 6 days. A sample of 24 rats were divided into 4 groups, randomly. Group 1 (K1) was rats induced by STZ and without IF treatment. Group 2 (K2) was rats induced by STZ and given metformin 45mg/kg without IF treatment. Group 3 (K3) was rats induced by STZ and given IF treatment. Group 4 (K4) was normal group of rats given IF treatment. The injection of STZ was given on the 7th day at a dose of 35 mg/kgBW by intraperitoneal injection. On the 9th day, the FBG levels were examined, and the rats were considered diabetic if the levels were more than 200 mg/dL. On the 10th day the rat group began to receive treatments. On the 23rd day, termination was performed, and blood plasma was collected. The FBG levels were measured with a glucometer, SIRT1; and TAOC were measured with the ELISA method.

Intermittent fasting

Diet with alternating eating patterns in periods between eating and fasting may result in CR. The IF regimen is typically categorized into three frequently employed approaches, namely time-restricted feeding, alternate-day fasting, and modified fasting.[11,12] This study applied a

time restricted feeding (TRF) regimen with a 16:8 protocol, and then the rats fasted for 16 hours, and the feeding window was opened for 8 hours. The fasting started at 4:00 pm to 8:00 am. The feeding window was opened from 8:00 am to 4:00 pm. When the feeding window was opened, the rats were given standard feed.

The FBG measurements

The FBG measurement used a glucometer (Nesco®, Taiwan). Before the measurement, the rats were fasted for ten hours. Blood samples were obtained through the incision of the rats' tails for 1-2 cm long, and then the blood was dripped into the glucometer.

Sirtuin 1

The measurement of sirtuin 1 activity were performed by using the ELISA (Enzyme-linked immunosorbent assay) method (Fine Test®, Wuhan, Catalog Number: ER1338). The rat blood was taken from the aorta. Previously, to induce anaesthesia, the rats were injected intraperitoneally with ketamine at a dose of 50 mg/kg and xylazine at a dose of 10 mg/kg and then were placed on a surgical tray in a supine position. The surgery was performed from the lower abdomen to the thoracic cavity. The blood was drawn directly by using a syringe to take blood samples intracardially in the amount of 3-4 ml, and 1.5-2 ml were inserted into the tube and then were centrifuged for 15 minutes; next, the plasma was taken for testing. These results were read with a spectrophotometer with an absorbance of 450 nm.

The TAOC measurements

The TAOC activity was measured by using the ELISA method (Elabscience®, USA, Catalog Number: E-EL-R1102). The rat blood samples were collected from the aorta by using the same procedure as the SIRT1 blood sample. About 1.5-2 ml of blood sample was inserted into the tube and then was centrifuged for 20 minutes; next, the plasma was taken for testing. These results were read with a spectrophotometer with an absorbance of 520 nm.

Data analysis

Data of FBG levels, SIRT 1 activity and TAOC were presented in the form of tables and graphs. Then the data were tested for their normality with the Shapiro-Wilk test, and their homogeneity was tested with the Levene test. For normal and homogeneous data, the ANOVA and Least Significant Difference (LSD) tests were performed to analyse mean differences among the groups. If the data were not normal and not homogeneous, non-parametric analysis using Kruskal Wallis was performed; if $p < 0.05$, it was concluded that there were differences between the groups. The analysis of the data was performed by using IBM SPSS Statistics software version 20.

Ethics

The entire process in this study complied with ethical principles in accordance with the permission of the ethical committee of Research Institute of Universitas Yarsi, with a letter No. 344/KEP-UY/BIA/XII/2022.

RESULT

Fasting blood glucose levels

Intraperitoneal STZ induction was performed on 7th day. After that, observations were made of the clinical signs that appeared after the STZ induction. On the 9th day, the FBG levels were measured. The results of the FBG levels after STZ induction indicated that there was an increase in the FBG level of > 200 mg/dl in the STZ-induced group; the rats were considered diabetic if their FBG levels were more than 200 mg/dL. In the non-induction group, the blood glucose was within normal limits (Table 1).

Table 1. The FBG levels of the rats after the STZ induction on the 9th day

Groups	n	FBG (mg/dL)
K1	6	237.67 ± 31.37
K2	6	329.67 ± 93.28
K3	6	334.83 ± 60.02
K4	6	97.16 ± 16.26

K1: DM group without IF; K2: DM group given metformin; K3: DM group with IF; K4: normal group with IF; FBG: fasting blood glucose; STZ: streptozotocin; IF: intermittent fasting; DM: diabetes mellitus.

The second measurement of FBG levels was carried out on the 23rd day of the study before the rats were terminated. From the measurement results, it was found that the DM group with IF indicated the lowest FBG (153.67 mg/dl) and that the DM group given metformin (341.17 mg/dl) and the DM group without IF (315.33 mg/dl) indicated the highest FGB; meanwhile, the normal group with IF had FBG levels within normal limits (116.42 mg/dl). The results of data analysis revealed that the data were normally distributed and the variance was not homogeneous. The result of the Kruskal Wallis test pointed out that the FBG levels were significantly different (p = 0.000). The descriptive analysis and the results of the analysis are presented in Figure 1. The results of the Mann-Whitney test were significantly different. It could be concluded that IF was able to reduce the FBG levels in the DM group with IF, compared to the DM group without IF (p = 0.004) and the DM group with metformin (p = 0.004).

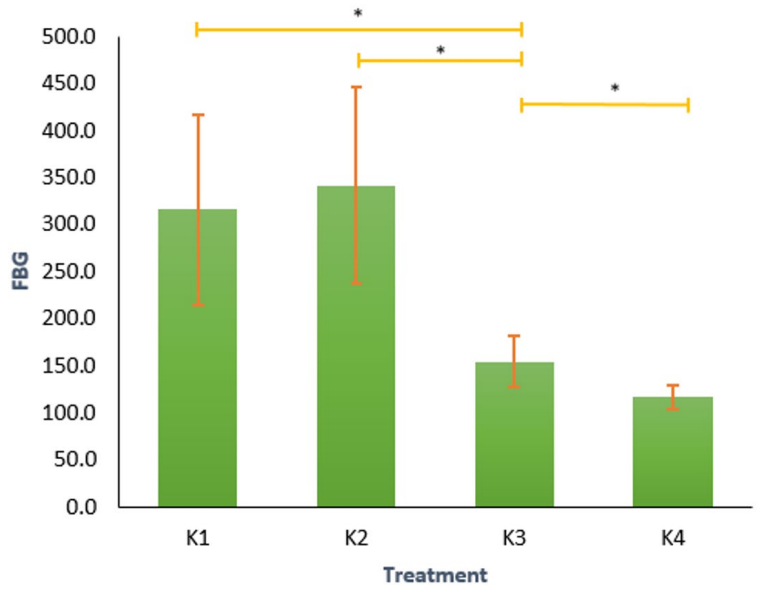

Figure 1. Average FBG value after receiving treatment. The average FBG value in K3 (the DM group with IF) was lower than K1 (DM group without IF) and K2 (DM group given metformin). Meanwhile, K4 (the normal group with IF) had a lower average FBG level than K3. The Mann Whitney test showed significant differences between K3 to K1, K2 and K4. *Significant (p < 0.05); IF: intermittent fasting; FBG: fasting blood glucose; DM: diabetes mellitus

Sirtuin 1

The data analysis results revealed that the data were normally distributed and the variance was homogeneous. The result of the ANOVA test demonstrated that the value of SIRT1 activity was significantly different in the four experimental groups (p = 0.001). The descriptive analysis is presented in Figure 2. The results of the LSD found that the DM group with IF had a significant value against the DM group without IF and DM group given metformin, but it did not have significant value against the normal group with IF. The results of the analysis are presented in Table 4. It could be concluded that the that IF was able to increase the SIRT1 activity in the DM group with IF compared to the DM group without IF (p = 0.001) and the DM group given metformin (p = 0.000).

Total Antioxidant Capacity

The results of data analysis revealed that the data was normally distributed and the variance was not homogeneous. The Kruskal Wallis test indicated that the TAOC value was not significantly different (p = 0.529). The descriptive analysis is presented in Figure 3. From the available data, it could be concluded that there was no significant difference in the average TAOC values in the four experimental groups.

DISCUSSION

The data collected in this study demonstrated a decrease in the FBG level in the DM group with IF compared to the DM group without IF and DM group with metformin. This is related with a study conducted by Furmli et al., IF could

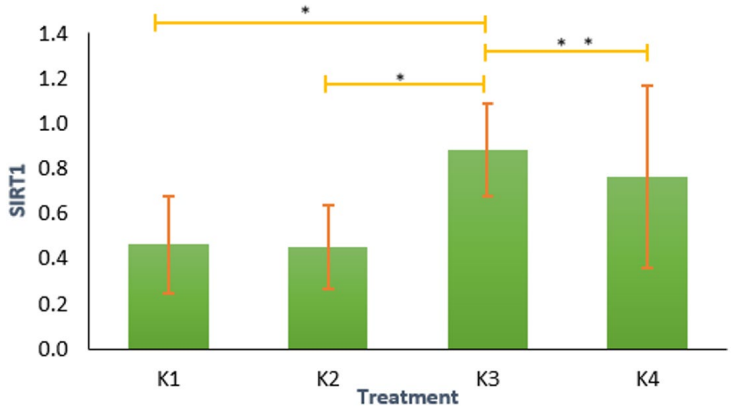

Figure 2. Average SIRT1 value after receiving treatment. The average SIRT1 value increased in K3 (the DM group with IF), and was higher than K1 (DM group without IF), K2 (DM group given metformin) and K4 (the normal group with IF). The LSD test showed significant differences between the K3, ,K1 and K2 and it did not have significant differences between K3and K4. *significant ($p < 0.05$); ** not significant; IF: intermittent fasting; SIRT1: sirtuin 1; DM: diabetes mellitus

Table 2. Comparison of SIRT1 based on the LSD analyses

Treatment	Mean difference	Sig.
K1 to K2	0.00860	0.934
K1 to K3	-0.42014*	0.001*
K1 to K4	-0.30126*	0.008*
K2 to K3	-0.42874*	0.000*
K2 to K4	-0.30986*	0.006*
K3 to K4	0.11888	0.257

*Statistically significant ($p < 0.05$); K1: DM group without IF, K2: DM group given metformin 45 mg/kg BW, K3: DM group with IF, K4: normal group treated with IF; IF: intermittent fasting; SIRT1: sirtuin 1; DM: diabetes mellitus: LSD: least significant difference

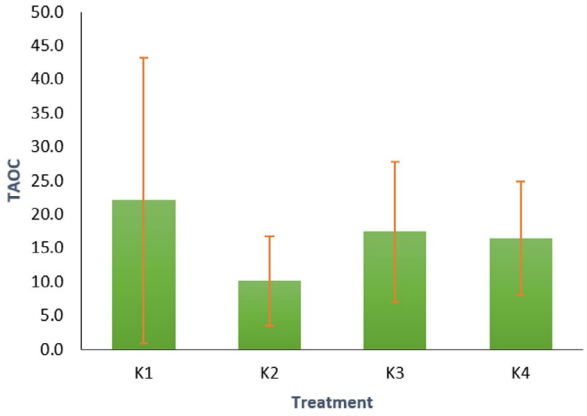

Figure 3. Average TAOC value after receiving treatment. Statistically there was no significant difference in the average values TAOC in the four experimental groups. Descriptively there was a tendency that the TAOC value in K3 (the DM group with IF) was higher than K2 (the DM group with metformin) and K4 (the normal group with IF) but was lower than K1 (the DM group without IF); IF: intermittent fasting; TAOC: Total Antioxidant Capacity; DM: diabetes mellitus

improve glycaemic control in type 2 DM patients as the IF method could reverse insulin resistance, control blood glucose, lose weight, reduce waist circumference, and reduce HbA1c levels.[13] Calorie restriction given in IF method is associated with a decrease in insulin production and elevates AMP-activated protein kinase (AMPK) levels. The AMPK activation due to insulin depletion is reported to improve insulin sensitivity, glucose homeostasis, and further it can improve hyperglycaemia condition and is also beneficial for promoting healthy aging process.[10] The decrease in FBG levels

in the DM group treated with IF was also related to metabolic switch; after several hours of fasting, there was a shift in the utilization of glucose towards fatty acids and ketones as an energy source. Intermittent fasting method can improve insulin deficit and glucose intolerance with the mechanism that involves repair of pancreatic β-cells. Cellular and molecular mechanisms when this period of IF is given can prevent and reverse diabetes by increasing the sensitivity of insulin receptor signalling, so insulin becomes easier to stimulate glucose uptake by muscle and liver cells. After the fasting period, glycogen reserves will decrease, followed by reduced insulin in the blood circulation, and then the process of breaking down fatty acids into energy will occur.[14,15]

In this study, we developed experimental animal models using streptozotocin (STZ), a chemical commonly used to induce diabetes in laboratory animals. The STZ is cytotoxic to pancreatic β-cells. The toxic effect of STZ begins with the uptake of STZ into cells via the low affinity glucose transporter-2 (GLUT-2) found in the plasma membrane of β-cells, hepatocyte cells and renal tubular cells. Streptozotocin has a glucose group, so its toxic effect is more selective on pancreatic β-cells, making it easier for STZ to enter the β-cells' plasma membrane because pancreatic β-cells are more active in taking up glucose than other cells. Methyl nitrosourea group of STZ can cause DNA (Deoxyribonucleic Acid) methylation, which further will trigger DNA damage which ultimately cause pancreatic β-cell necrosis through depletion of cellular energy stores.[16,17] Induction of DM using intraperitoneal STZ can react directly on pancreatic β-cells and also induce changes in blood insulin and glucose concentrations. After entering pancreatic β-cells via the GLUT-2, STZ causes decreased expression of GLUT-2, which will further decrease sensitivity of peripheral insulin receptors, resulting in increased insulin resistance and increased blood glucose levels. STZ also affects glucose oxidation and decreases insulin biosynthesis and secretion which cause a decrease in total plasma insulin.[18]

In DM group given metformin, it was reported that the FBG level in this group had increased. Metformin is a biguanide anti-diabetic drugs, working as an insulin sensitizer that can suppress endogenous glucose production in the liver, primarily due to reduction in the rate of gluconeogenesis and an effect on glycogenolysis. In this group, there were differences in diabetic levels that occurred after the STZ induction. A total of 4 rats had severe hyperglycaemia (FBG > 400 mg/dl), and 2 rats had moderate hyperglycaemia (200-400 mg/dl). Many factors can cause this condition, including mechanism of action of the STZ's receptor which is different in each rat. Moderate hyperglycaemia can be caused by DNA damage coded for pancreatic β-cells; STZ releases nitric oxide which acts to release free radicals and trigger damage to pancreatic β-cells. Severe hyperglycaemia occurs because there is DNA damage due to STZ which activates poly ADP (Adenosine Diphosphate)-ribolization which then results in suppression of cellular NAD^+, and then the amount of ATP (Adenine Triphosphate) decreases and finally inhibition of insulin secretion and synthesis occurs.[18] The increase of FBG level in this group was made possible due to the effects of STZ which caused severe hyperglycaemia and damage to pancreatic β-cells resulting in inhibition of insulin secretion and synthesis. This condition caused metformin unable to respond to diabetic therapy as expected.

Metformin is one of main choice in type 2 diabetes therapy. The therapeutic effect of this drug is achieved through multiple mechanism including increased insulin sensitivity, decreased hepatic glucose production, and reduced absorption of glucose in the intestines. However, there are number of factors that can cause metformin failed to lower the FBG levels, such as excessive low insulin level, so that it becomes ineffective in reducing the FBG levels. Another possible mechanism, in chronic insulin resistance (in which the body cells become less sensitive to insulin), cause blood sugar to remain high; even if insulin levels in the blood are already high, metformin cannot lower FBG levels significantly.[19] Another consequence of giving STZ injection is the liver damage. Effects of STZ on the animal models' liver can be divided into two effects, namely acute and chronic effects. Acute effect is observed within 24-48 hours after STZ administration. This effect is characterized by the occurrence of necrosis liver cells, especially in hepatocytes. Liver cells' necrosis can result in decreased liver function, such as increasing bilirubin levels in the blood, decreasing albumin levels in the blood, and disorders in blood coagulation.

The results in this study also revealed that there was an increase in the SIRT1 activity in the DM group with IF compared to the DM group without IF and DM group given metformin. This is related with a study conducted by Hammer et al. which tested the effect of IF for 48 hours in male db/db rats. The rats used were male db/db rats, a genetically modified strain of rats which usually had onset of diabetes at around 10 weeks of age. These rats previously had their blood sugar checked after two measurements of FBG >13.9 mmol/l. The measurement of SIRT1 activity was conducted through the utilization of a histone deacetylase kit. The obtained results were presented by measuring fluorescence at 355 nm and 460 nm excitation and emission of total protein. The results showed that IF induced and increased the expression of SIRT1 mRNA in the liver and retina of rats treated with IF. The IF method has also been shown to increase SIRT1 activity eightfold in the rat retina compared to non-fasting controls.[20]

The IF is a diet method with alternating eating patterns in periods between eating and fasting with certain regimens so that CR occurs. In this study, the treated rats were fasted for 16 hours, and their feeding window was opened for 8 hours. The increase in the SIRT1 value in the DM group with IF was due to the fact that this group received IF treatment and that CR occurred. The CR is known to induce the expression of SIRT1, SIRT3 and SIRT5 in rats. During the IF period and CR occurrence, total energy, food composition, and fasting duration contribute to the oscillation of bioenergy level sensors such as NAD^+ to NADH (Nicotinamide Adenine Dinucleotide Hydrogen), ATP to AMP (Adenine Monophosphate), and Acetyl Co-A to Co-A. These energy transporters initiate the activations of downstream proteins, which in turn control the functioning of cells. Examples of such proteins include transcription factors like forkhead box O (FOXO), peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α), nuclear erythroid factor 2–related factor 2 (NRF-2), AMPK, and sirtuins. On the other hand, a high-fat diet can interfere with SIRT1 activity in rats through the mechanism of proteolysis, and obesity can reduce SIRT1 expression in humans. Several reports also demonstrate that increasing SIRT1 expression in rats can reduce disease syndromes such as diabetes, neurodegenerative diseases, hepatic steatosis, osteoporosis and inflammation.[21,22,23]

Activation of SIRT1 as the main metabolic regulator can help regulate metabolism of glucose and lipid, while also influencing insulin signalling modulation pathways in DM conditions. In the pancreas, SIRT1 also plays a role in controlling insulin secretion and safeguarding cells against oxidative stress and inflammation. The reduced value of SIRT1 in the DM group without IF treatment due to DM conditions and hyperglycaemia can suppress SIRT1 activity.[22,23]

The findings from statistical analysis of TAOC measurements in this study indicated that there was no significant difference in the mean TAOC value among the four experimental groups. However, it descriptively pointed out that TAOC value in the DM group with IF was higher than the DM group given metformin and the normal group with IF, but was lower than the DM group without IF.

The TAOC can be used as a marker for evaluating factor influential nutrition to disturbance pathophysiology such as DM. This method is based on formation ABTS cation [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] which is broken down by antioxidants in the blood and produces group chromophore coloured by blue greenish that can be measured spectrophotometrically The TAOC serves as parameter for quantifying the overall antioxidant capacity in the body cumulatively from both endogenous antioxidants and exogenous antioxidants, as well as enzymatic and non-enzymatic antioxidants. Types of endogenous antioxidants are enzymatic antioxidants such as copper, zinc, manganese, superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase; while, non-enzymatic types of antioxidants include glutathione, ubiquinol, selenium, lipoic acid, and others. Sources of exogenous antioxidants, for examples, are ascorbic acid (vitamin C) and tocopherol (vitamin E). [4,24]

Streptozotocin used to induce hyperglycaemia in this study could cause oxidative stress on pancreatic β-cells. This hyperglycaemia condition could activate several signalling pathways to dispose of excess glucose. The pathways stimulated by this hyperglycaemia include increased NADH/NAD^+ ratio, hexosamine pathway, protein kinase C activation, polyol pathway activation, formation

of methylglyoxal and advanced glycation products, enediol formation, and endoplasmic reticulum stress. All of these pathways will result in the generation of Reactive Oxygen Species (ROS), accompanied by a decline in the overall antioxidant capacity. Administration of STZ can also cause an increase in malondialdehyde which significantly reduces the activity of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase.[17,25]

During the period of IF, the imbalance of oxidative stress will initiate the activation of the antioxidant system in order to eliminate free radicals. The activation of this antioxidant system includes the body's endogenous antioxidants through cofactors or by exogenous antioxidants originating from food intake.[26] Intermittent fasting is also often associated with preventing oxidative stress in the body. Calories in excessive and unlimited food intake can increase lipid peroxidation. Polyunsaturated fatty acids can readily undergo oxidation, resulting in the formation of free radicals and other reactive compounds, including H2O. Lipid peroxidation serves as a cellular injury mechanism, which can be employed as a reliable marker for assessing oxidative stress in both cells and tissues.[27]

In a previous study conducted by Mulyaningrum et al., who treated rats with IF (Daud's fasting method for 8 weeks), reported that IF could stimulate an increase of the levels of antioxidant Glutathione Peroxidase (GPx). and catalase in the IF group compared to control groups. Intermittent fasting also can increase level of Superoxide Dismutase (SOD), GPx, and catalase levels due to adaptive response mechanisms. The body will convert adipose tissue into free fatty acids during fasting. Ketone bodies will be formed as a result of free fatty acid metabolism through beta oxidation in liver tissue mitochondria, which causes mild oxidative stress. Following this condition, the activation of nuclear factor erythroid 2 related factor (NRF2) will occur. NRF2 serves as a transcription factor for antioxidant and detoxification genes, consequently diminishing oxidative stress.[28]

The increase of TAOC value in DM group without IF could occur because it was influenced by exogenous antioxidants obtained from food intake which this group received food ad libitum during treatment, so that the increase in TAOC value was the cumulative of exogenous antioxidants and endogenous antioxidants. For the measured TAOC value in the DM group with IF, it was possible that it was cumulative only from endogenous antioxidants after receiving IF treatment. The correlation between SIRT1 and oxidative stress conditions in this study was the role of SIRT1 as a redox regulator. Sirtuin 1 would detect imbalanced conditions around it through NAD levels, and then the upregulation of SIRT1 is initiated, making several changes accordingly to alter its activity. The main SIRT1 substrate identified is p53, which is involved in activating antioxidants such as SOD2 and GPx. Another redox transcription factor associated with sirtuin is FOXO3a which induces an antioxidant response through SOD2 and catalase. Another SIRT1 substrate is PGC-1α which is involved in regulating the expression of mitochondrial antioxidants such as SOD2.[29]

Sirtuin 1 has the opportunity to serve as a novel therapeutic agent in the management of diabetes with its various mechanisms. The implementation of CR through IF leads to favourable metabolic outcomes and enhances mitochondrial function, primarily through the activation of sirtuins. Nevertheless, a comprehensive range of experimental and clinical investigations are imperative to ascertain the precise role of sirtuins in the management of DM.[6]

This study has some limitations that a histopathological examination was not elaborated to observe and to prove the degree of damage and improvement after treatment on the target organ, namely the pancreas.

CONCLUSION

It was reported that the IF method with the time restricted feeding using regimen 16 hours of fasting and 8 hours of feeding window could reduce the FBG levels, increase SIRT1 activity but was not proven to increase total antioxidants in the DM-model Wistar rats. It is hoped that in future research, a histopathological examination can be conducted to determine the appearance of the pancreas. The further research can also be elaborated by using other IF protocol variations such as alternate - day fasting or modified fasting. Also, it can be further implemented by testing other possible agents that activate sirtuins and

antioxidants such as curcumin, resveratrol or blueberries.

CONFLICT OF INTEREST

The authors declared that there were no potential conflicts of interest of the research, authorship, and publication of this article.

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AUTHOR CONTRIBUTIONS

H and MS conceived and designed the experiments; MS analysed the experimental data; MS, H and SW performed the experiments, discussed the result and contributed to the manuscript draft.

LIST OF ABBREVIATIONS

ADP: Adenosine Diphosphate; AMPK: Activated Protein Kinase; ATP: Adenine Triphosphate; DM: Diabetes Mellitus; DNA: Deoxyribonucleic Acid; ELISA: Enzyme-linked immunosorbent assay; FBG: Fasting Blood Glucose; FOXO: Forkhead Box Group O; GLUT: Glucose Transporter; GPX: Glutathione Peroxidase; IF: Intermittent Fasting; NAD: Nicotinamide Adenine Dinucleotide; NADH: Nicotinamide Adenosine Dinucleotida Hydrogen; NRF2: Nuclear Factor Erythroid 2–Related Factor 2; PGC-1α: Peroxisome Proliferator Activated Receptor Coactivator 1α; ROS: Reactive Oxygen Species; SIRT: Sirtuin; SOD: Superoxide Dismutase; TAOC: Total Antioxidant Capacity; STZ: Streptozotocin

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