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Levels of Oxidative Stress in Rats Treated with Calorie Restrictive Treatment and 50% Sucrose Solution

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Abstract

This study aims to analyze the level of stress oxidative with treatment calorie restrictions and administration of sucrose solution. This study was a laboratory experimental study with 12 male white rats (Wistar strain) as subjects for caloric restrictive treatment and 12 rats for 50% sucrose solution. Where each group of rats is determined by the dependent and independent variables. Using the pre-posttest control design, the dependent variable of caloric restriction, and the independent variable malondialdehyde. The dependent variables used were body weight using a scale, fasting blood glucose using a glucometer, and MDA using a UV-Vis spectrophotometer. The independent variable used was the administration of 50% sucrose solution. The data were statistically analyzed by T-test analysis. There was no significant decrease in MDA levels in the caloric restriction treatment rats ($p = 0.060$), besides that there was a significant difference in body weight in both the control and sucrose groups ($p < 0.05$). There was no significant difference in fasting blood sugar in the control group ($p > 0.05$) and there was a significant difference in the sucrose group ($p < 0.05$). There was no significant difference in serum MDA levels in either the control or sucrose groups ($p > 0.05$). There is an effect of calorie restriction on MDA levels. Also, administration of 50% sucrose solution for 4 weeks had a significant effect on the rats' body weight and blood glucose but did not have a significant effect on serum MDA levels.

Keywords: Oxidative stress; calorie restriction; sucrose solution; malondialdehyde.

INTRODUCTION

Oxidative stress is part of the tissue damage in people that reflects the unevenness between the creation of receptive oxygen species (ROS), which are responsive oxidizing compounds, and the strengthening protection of cells. Free radicals or what are known as Reactive Oxygen Species or ROS are molecules that contain one or more unstable electrons because one or more electrons in their outer orbit are unpaired the body needs one of them as a defense against infectious agents (Jakubczyk *et al.*, 2020). Oxidative stress plays a role in different human diseases, including malignant growth, cardiovascular disease (CVD), lung, nervous, kidney, and liver disease, and, surprisingly, physiological maturation processes. Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of reactive oxygen species or what is often called ROS and antioxidants. Specific reactive oxygen (ROS) leads to an imbalance between pro-oxidants and antioxidants in cells and tissues. In a healthy state, the existence of pro-oxidants is balanced with antioxidants, but in certain circumstances, this balance is disturbed, where this condition is referred to as oxidative stress. This state refers to the modification of lipids, proteins, and DNA. Such as modification or damage to a molecule called oxidative damage. Prooxidants can increase oxidation.

Excessive creation of free extremities in the body can cause oxidative stress. One sign of oxidative stress in humans is the degree of malondialdehyde (MDA) which is the result of lipid peroxidation in the body caused by free radicals. According to research, a 30% calorie restriction for 15 days can reduce MDA levels, which is a marker of oxidative stress in the body (Khoubnasabjafari *et al.*, 2015). Oxidative stress plays a role in different human diseases, including malignant growth, cardiovascular disease (CVD), lung, nervous, kidney, and liver disease, and, surprisingly, physiological maturation processes. Reactive Oxygen Species (ROS) are produced through several mechanisms, one of which is when the body experiences excess glucose (hyperglycemia). Factors that cause hyperglycemia are diet, stress, infection, and consumption of certain drugs. In planning a meal pattern, the emphasis is on diet in terms of the 3 M's (meal schedule, type of meal, and amount of meal).

Excessive consumption of sugar can cause hyperglycemia and increase the production of free radicals resulting in oxidative stress. Oxidative stress is

accompanied by lipid peroxidation in cell membranes so that malondialdehyde (MDA) is formed as a result of lipid peroxidation (Gawel *et al.*, 2004). Thus, MDA can be used to determine the degree of oxidative damage caused by lipid peroxidation (Gawel *et al.*, 2004). The Relationship between level stress oxidative with treatment calorie restrictive and sucrose solution 50% has not been studied yet up to date. This study aims to determine whether there is an effect of caloric restrictive treatment and administration of 50% sucrose solution on levels of oxidative stress.

METHODOLOGY

Study design

This research is a laboratory experimental study using the pre-posttest control design. The subjects of the study were 12 male white rats (Wistar strain) for the caloric restriction treatment and 12 rats for the administration of sucrose solution where each of them would be divided into several groups of variables and studied for 4 weeks. The study was conducted from December to January at the Pharmacology and Biochemistry Laboratory of the Faculty of Medicine, YARSI University, and was approved by the YARSI University Research Ethics Commission with number 324/KEP-UY/BIA/X/2021.

The experimental animal

The research subjects were male rats weighing ± 130 grams and aged 8 weeks. The group given the calorie restriction treatment will be divided into the dependent variable studied is calorie restriction, the independent variable is malondialdehyde, while the group given 50% sucrose solution will be grouped into the dependent variable used is body weight using a scale, fasting blood glucose using a glucometer, and MDA using UV-Vis spectrophotometer. The independent variable used was the administration of 50% sucrose solution. Control variables will be determined for each group, namely the age of the rats, male sex, the shape, size, and placement of the cages are made the same, and the type, quantity, and quality of food and drink are the same. Data distribution was determined by the Shapiro-Wilk test. Paired t-tests were used to determine between-group differences in mean MDA. The difference is considered significant if $p < 0.05$.

Preparation of 50% sucrose solution

A total of 50 grams of sucrose was dissolved in 100 ml of distilled water, then given to rats orally (fed) at a dose of 1.5 cc/head twice a day (09.00 and 15.00) for two months.

Administration of 50% sucrose solution

After being acclimated for one week, the treatment group was given standard feed, drink ad libitum, and 50% sucrose solution until the end of the study.

Blood sampling

Wipe the eyes of the rats with cotton that has been moistened with 70% alcohol, then give an anesthetic, namely Ketamine-Xylazine (0.15 ml/100 grBB) or Ketamine 100-200 mg/kgBB + Xylazine 10 mg/kgBB (Flecknell, 1993), because the body weight of the mice used was 200 grams, the dose used was 0.3 ml. Furthermore, blood samples were taken using a syringe of as much as 3 ml and then placed in an EDTA tube to measure malondialdehyde (MDA).

Blood glucose measurement

Glucose levels were measured 3 times at the beginning, middle, and end of the study. Blood glucose levels of experimental rats were determined using the glucose oxidase biosensor method, using a blood glucose test meter glucoDr. Before being analyzed, the animals were fasted for 8 hours. Blood was taken from the tip of the rat's tail which was previously cleaned with 70% alcohol, then the tip of the tail was pricked with a small needle. The blood that comes out is then touched on the glucometer strip. After 11 seconds, the blood glucose level will be read on the GlucoDr screen and expressed in mg/dL units.

Measurement of Malondialdehyde (MDA) levels

Malondialdehyde (MDA) levels of rats were measured using thiobarbituric acid (TBA) which will form a pink MDA-TBA product and measured using a spectrophotometric method. To obtain serum, centrifugation was carried out at 3000 rpm for 20 minutes. For every 100 microliters of serum, 2.45 ml of TCA (trichloroacetic acid) and 2.45 ml of TBA (thiobarbituric acid) were added, then heated at 100°C for 20 minutes. Then the mixture was centrifuged at 8000 rpm for 10 minutes. The absorbance of the supernatant obtained was determined using a blank based on 2.45 ml TCA and 2.45 ml TBA on the standard curve.

Statistical analysis

Calculation of data normality was carried out by the Shapiro-Wilk test. The test will reject Ho if the p-value $\leq$ 0.005 (5%). This test was carried out provided that the number of samples was less than 30. The results of this test showed that the p-value of Shapiro Wilk on the variables body weight, glucose, and MDA had a normal distribution of data in both groups (p-value > 0.05), so on these three variables to do a different test using a paired t-test.

RESULTS

Experimental animals were randomly divided into 2 groups consisting of 6 animals. The calorie restriction treatment group and the control group were given standard feed. This research was conducted in the pharmacology laboratory for 10 days, in December 2021.

To find out whether there was an effect, on glucose, MDA, and body weight in the treated rats, a different test was performed, namely the t-test with the variables of glucose, MDA, and body weight. Before the different test is carried out, the data normality test is first performed. The results of the data normality test using SPSS 26.0 are presented in Table I. Calculation of data normality was carried out by the Shapiro-Wilk test. The test will reject Ho if the p-value ≤ 0.005 (5%). This test was carried out provided that the number of samples was less than 30. The results of this test showed that the p-value of Shapiro Wilk on the variables body weight, glucose, and MDA had a normal distribution of data in both groups (p-value > 0.05), so these three variables do a different test using a paired t-test.

Glucose levels in the treatment group and control group

In Table II it is known that the average glucose before and after the calorie-restrictive treatment in the control group was 83.3 mg/dL and 115 mg/dL and in the treatment group, it was 79.5 mg/dL and 199.8 mg/dL. From the results of the t-paired test in the control group, the p-value of glucose before and after the calorie-restrictive treatment was 0.169 (p> 0.05), which means that there was no significant change between the beginning and end of the control group, while in the treatment group, the value of p = 0.001 was obtained. This means that there is a significant change in glucose levels.

Malondialdehyde levels in the control and treatment groups

Furthermore, the malondialdehyde was measured. As shown in Table III, the average malondialdehyde at the beginning and the end of the treatment in the control group is 1.241 nmol/ml and 1.265 nmol/ml. From the results of the paired t-test, a p-value of 0.102 (p > 0.005) was obtained, which means that there was no significant change between the start and end of treatment in the control group. Whereas in the treatment group, it was known that the average malondialdehyde was 1.262 and 1.232 at the beginning and end of treatment in the treatment group. From the results of the paired t-test, it was obtained a p-value of 0.060 (p> 0.005), which means that there was no significant change between the beginning and the end of the treatment in the treatment group.

Body weight values before and after treatment

Changes in body weight values at the start and end of treatment were found and shown in Table IV. Based on the table, it was obtained that the p-value was 0.165 in the control group and 0.058 in the fasting treatment group, which was greater than the 5% significance level. It could be interpreted that there was a difference in rat body weight between the group that was given standard feed and the group that was fed a caloric-restrictive treatment, which means the caloric-restrictive treatment did not significantly affect the rat's body weight.

The treatment group was given 50% sucrose solution using a sonde every day, as well as the control group which was given standard feed. Furthermore, both groups measured body weight, blood glucose, and malondialdehyde (MDA), which is a marker of oxidative stress. The study was conducted in the FKUY pharmacology and biochemistry laboratory for 4 weeks, a period from December 2021 – January 2022.

Statistical analysis to see changes in the variables above uses the Anova Repeated Measure test and paired T-test. The variables tested in this study included 3 (three), namely: body weight, fasting blood sugar, and MDA.

Before carrying out the Anova Repeated Measure test and the paired T-test, the data normality test was first performed. The results of the data normality test using SPSS 26.0 are presented in the following table:

- MDA levels in the control and treatment groups

Based on the results of the study or the T-test, it was stated that there was no significant difference between the treatment (fasting) and control groups on MDA levels p>0.05. However, based on the average MDA value in the fasting treatment group, it was 1.262 at the beginning of the treatment and 1.232 at the end of the treatment. In the control group, the average was 1.241 at the beginning of the treatment and 1.265 at the end of the treatment, which tended to decrease in the treatment group compared to the control group. This is also in line with research conducted by Chausse *et al.* (2015). According to Trepanowski *et al.* (2011) that calorie restriction can lead to general health improvements, preventing degenerative diseases, cognitive deficits, and premature aging.

- Glucose levels in the control and treatment groups

Based on the T-test table in table, the average glucose level in the treatment group was 79.5 mg/dl at the beginning of treatment and 199.83 at the end of treatment. In the control group, it was found that the average at the beginning of the study was 83.3 mg/dl and 115 mg/dl at the end of the study. Changes in glucose levels at the beginning and end of the treatment in the control group showed p=0.169 and p=0.001 in the treatment group, where there were no significant changes in the control group and there were significant changes in the treatment group. It was found in this study that the

treatment group experienced an increase in glucose levels more than the control group.

This is following research conducted by Bolli *et al.* (2021) that there is a dawn phenomenon in normal individuals after calorie restriction. Muscle glycogen is a source of glucose 1-phosphate which can be rapidly used for glycolysis within the muscle itself. Liver glycogen functions to store and transport glucose to maintain blood glucose levels between meals. The liver concentration of glycogen is about 450 mmol/L glucose equivalent after a meal, which decreases to about 200 mmol/L after an overnight fast; after 12-18 hours of fasting, liver glycogen is almost completely depleted (Murray *et al.*, 1995).

- Changes in body weight in treated and controlled rats

In this study, it was found that there was a change in the body weight of the rats at the beginning of the treatment and the end of the treatment. Based on Table V, the average body weight in the control group at the beginning of the treatment was 167g and 194g at the end of the treatment, then in the treatment group, it was obtained an average of 159 g was at the beginning of the treatment and 182 g at the end of the treatment. In calculating the results p= 0.165 in the control group and p=0.058 in the treatment group whereas in the two treatment groups, no significant changes were found. Obtained in this study the weight of the control and treatment group rats experienced weight gain.

Administration of 50% sucrose solution

Average body weight in the control group and 50% sucrose solution

Changes in body weight for 4 weeks of treatment, as shown in Table V. There was an increase in BW (body weight) on days 0, 15, and 30 in the control group and the 50% sucrose solution group. The average weight on days 0, 15, and 30 in the control group was 167.3 grams, 194.3 grams, and 243.3 grams. And the average body weight on days 0, 15, and 30 in the sucrose group were 161.5 grams, 167.8 grams, and 202.8 grams.

Based on Table VI, the p-values (sig/Greenhouse-Geisser) in the control and sucrose groups were 0.031 and 0.011 (P <0.05). So it can be interpreted that there is a difference in the body weight of the rats between the group that was given standard feed and the group that was given an additional 50% sucrose solution on days 0, 15, and 30, which means that sucrose significantly affects the body weight of the rats.

Average fasting blood sugar levels in the control group and 50% sucrose solution

There was an increase in GDP (Fasting Blood Sugar) on days 0, 15, and 30 in the control group, namely 83.3 mg/dL, 115.2 mg/dL, and 134 mg/Ll (See Table VII). Whereas on day 0 the sucrose group was 84.7 mg/dL and decreased on day 15 to 82.5 mg/dl and increased again on day 30, namely 110.2 mg/dL.

Based on Table VIII, the p-value (sig/Greenhouse-Geisser) obtained in the control group was 0.124 (p > 0.05) and the sucrose group was 0.021 (p <0.05). The interpretation is that there is no difference in the blood sugar of the rats on days 0, 15, and 30 in the control group and there is a difference in the group that was given an additional 50% sucrose solution, which means that sucrose affects the blood sugar of the rats significantly.

The average serum MDA level in the control group and 50% sucrose solution

There were changes in MDA (Malondialdehyde) levels on day 0 and day 30 in the control group, namely 1.285 nmol/ml and 1.241 nmol/ml, and in the sucrose group, namely 1.248 nmol/ml and 1.237 nmol/ml (see Table IX).

Based on the results of the paired T-test in Table X, the p-values in the control and sucrose groups were 0.469 and 0.637 (p > 0.05). So it can be interpreted that there was no significant change in MDA between the group that was given standard feed and the group that was given an additional 50% sucrose solution, which means that 50% sucrose did not significantly affect the MDA levels of rats.

DISCUSSION

Weight Change

Both the experimental and control animals experienced an increase in body weight. After the Anova Repeated Measure test, the two groups also had significant differences. In the high-simple carbohydrate diet group, namely sucrose, there was a significant increase in average body weight. Types of carbohydrates, glycemic index, and glycemic load affect blood glucose which can lead to changes in body weight. The higher the glycemic load, the higher the increase in blood glucose. Elevated blood glucose over a long period can increase the risk of obesity. Types of simple carbohydrates tend to have a high glycemic index. This is in line with the Literature Review by Manuel-y-Keenoy and Perez-Gallardo (2012) regarding the metabolic effect of the amount and type of carbohydrates on the risk of obesity and diabetes. Based on this review, it is explained that excess glucose from a high-carbohydrate diet will be stored in the form of glycogen and the rest will be stored in the form of fat in the process of de novo lipogenesis. This is what can increase body weight (Aller *et al.*, 2011).

Based on this research, it can be said that the consumption of simple carbohydrates, one of which is sucrose, can increase body weight. This is following the results from the literature review by Aller *et al.* (2011) concluded that a high-starch diet is more beneficial for health than a high-sugar diet for weight control by reducing body fat (Aller *et al.*, 2011).

Fasting Blood Sugar

Based on Figure 4.2, the control rat group experienced an increase in blood glucose both on days 0, 15, and 30. Meanwhile, in the treatment group, it decreased on day 15 and increased again on day 30. Based on the Anova Repeated Measure test, there was no difference in rat blood sugar between the control group and the group that was given an additional 50% sucrose solution, which means that sucrose did not significantly affect the rat's blood sugar. Rats can experience a decrease in blood glucose in conditions of an excess energy diet, the satiety center will be activated and reduce epinephrine secretion so that the body's activity in looking for food will decrease. Mice that are given high-calorie feed will experience an increased sense of calm due to reduced stress due to hunger. This feeling of fullness suppresses the rat's desire to find food. This incident will cause rats to become calmer and suppress stress. This is also supported by the physical condition of the rats which are a little passive and the color of the fur is starting to fade.

Furthermore, on the 30th day blood glucose levels increased again because the administration of sucrose increased blood glucose and fat deposits as well as body weight in rats. This is in line with Firdaus *et al.* (2016) that a high-sucrose diet for 3-5 weeks can increase blood glucose levels and hyperinsulinemia and also reduce insulin sensitivity in rats and mice. A high sucrose diet markedly alters insulin-mediated glucose metabolism which can result in insulin resistance in rats. Based on research by Battung *et al.* (2019) concerning the effect of sucrose on blood glucose levels, the result was an increase in blood glucose levels in the intervention with 20 grams of sucrose.

Serum MDA (Malondialdehyde) levels

Malondialdehyde Levels (MDA) are one of the end products of lipid peroxidation. MDA levels will increase under conditions of oxidative stress, in various pathological conditions, and in infections otherwise, MDA levels will be low if the antioxidant defense system is good.

Results The serum MDA profile serves as a marker of cellular damage due to free radicals (Zaetun *et al.*, 2019). Based on research by Zaetun *et al.* (2019) that the higher the radical level, the higher the MDA level that is formed. However, this did not appear in the results of the study when viewed from this because the MDA levels of the control and treatment mice did not increase. It is also possible that there has been no exposure to free radicals in the experimental group of animals. If the experimental animal treatment is carried out for a longer period, the results may have a more significant effect on the presence or absence of cellular damage.

In addition, there is a relationship between high GI (glycemic index) foodstuffs and MDA levels. Foods with high GI values cause higher blood glucose and insulin responses compared to foods with low GI values. Consumption of high-GI foods is absorbed more quickly in the small intestine and has the potential to increase blood glucose.

However, in this study, there was no significant difference in MDA levels in the two groups because the intervention in the form of 50% sucrose solution was only carried out for 4 weeks so the new rats experienced hyperglycemia and entered the starting point of free radical formation so that MDA levels did not increase. This is in line with the study of Suarsana *et al.* (2011) that blood glucose levels in hyperglycemia treatment began to increase in week 4. After that, excess blood glucose for a long time could cause the formation of excess free radicals. These free radicals that are formed can attack the surrounding cells, including pancreatic cells. As a result of the chain reaction these free radicals cause cell damage.

In this study, insulin was still able to offset the increase in blood glucose levels. However, if this increase lasts a long time, insulin will no longer be able to maintain blood sugar levels at a normal level and free radicals will accumulate, thereby increasing MDA levels.

CONCLUSION

Based on the results of the study it can be concluded that there was a decrease in MDA levels in the calorie restriction group, although it was not statistically significant. However, there was a significant increase in blood sugar levels in the calorie restriction group. While the increase in body weight in both groups but not statistically significant. Furthermore, 50% sucrose solution administration for 4 weeks was a significant change ($p < 0.05$) the body weight. For blood glucose level, there was no significant change in the control group ($p > 0.05$) and there was a significant change in the sucrose group ($p < 0.05$). Administration of 50% sucrose solution for 4 weeks did not show a significant change in serum MDA (Malondialdehyde) levels.

For future researchers, it is hoped that they can conduct similar research with more samples and a longer time. The general public is expected to maintain a healthy lifestyle and maintain a diet to avoid various diseases due to increased oxidative stress in the body. Seeing the prevalence of lifestyle-related diseases causing an increase in oxidative stress, it is hoped that doctors can educate the public.

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Diniwati Mukhtar & Azha Azzuna Amsaka wrote the manuscript. Fanny Ratnasari Pd, Aan Royhan & Karina Ajeng Ridwan revised the manuscript. All authors read and approved the final version of the manuscript.

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