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EFFECTIVENESS OF GLUTATHIONE ON BLOOD GLUCOSE AND SERUM NUCLEAR FACTOR KAPPA BETA (NF-KB) LEVELS IN RATS (RATTUS NORVEGICUS) TYPE 2 DIABETES MELLITUS MODEL

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Abstract

Diabetes mellitus is widely known as a non-communicable disease which is a global problem because its incidence continues to increase every year throughout the world. Nuclear Factor Kappa Beta (NF-kB) is a protein in the cytoplasm that has an important role in cellular regulation in the body. Oxidative stress is a condition caused by increased production of free radicals. The use of antioxidants in diabetes mellitus sufferers is known to be effective in reducing the emergence of complications that appear. This study aims to assess the effectiveness of GSH in improving blood glucose and NF-kB levels in rat model of type 2 DM. Twenty-five male Wistar norvegicus rats were divided into four groups, namely K-N (ad libitum feed + 5 mL distilled water), K- (STZ + distilled water 5 mL), K+ (STZ + metformin 5 mg), KP-1 (STZ + glutathione 200 mg), KP-2 (STZ + glutathione 200 mg + metformin 5 mg). The results showed that blood glucose levels before treatment were p=0.394 (p>0.05); after p=0.006 (p<0.05); while the results of the NF-kB examination of mouse serum showed p=0.0891 (p>0.05). It was concluded that the addition of GSH to male Wistar norvegicus type 2 DM rat was significant in improving blood glucose levels, but not significant in improving rat serum NF-kB levels.

Keywords: Diabetes Mellitus, Glutathione, Blood Glucose, Nuclear Factor Kappa Beta.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by increased blood glucose levels, which over time can cause damage to the heart, blood vessels, eyes, kidneys and nerves [1].

The prevalence of diabetes mellitus worldwide has been reported by 537 million people with an age range of 20–79 years. This number is expected to increase to around 643 million in 2030 and 783 million in 2045. Diabetes mellitus has caused over 6.7 million deaths in 2021 [2].

Type 2 diabetes mellitus is a chronic metabolic disorder and its prevalence continues to increase globally. Type 2 diabetes mellitus is known to be associated with oxidative stress. This affects chronic vascular diseases such as atherosclerosis, hypertension and diabetic nephropathy [3]; [4]; [5]; [6]; [7]; [8].

The condition of hyperglycemia in diabetes mellitus has a very influential effect on the blood vessel endothelium because the existence of auto-oxidation process of glucose to form free radicals which ultimately results in macro and microvascular dysfunction. This condition will cause complications which will further increase the morbidity and mortality number in diabetes mellitus sufferers [9].

Nuclear Factor Kappa Beta (Nf-kB) is a protein in the cytoplasm that has an important role in cellular regulation in the body. These cellular regulations include immunity, inflammatory responses, oxidative stress, cytokines, ultraviolet radiation, oxidized Low *Density Lipoprotein* (LDL), bacterial and virus development processes, cell proliferation, cell differentiation, apoptosis and free radicals [10].

Oxidative stress is a condition caused by increased production of free radicals or reduced antioxidants or both. In relation to this condition, it is known as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [11].

Providing antioxidants is an effort to inhibit the production of intracellular free radicals or increase the ability of defense enzymes against free radicals to prevent the emergence of oxidative stress and vascular complications related to diabetes [12].

Glutathione (GSH), which is an enzymatic antioxidant, is capable of detoxifying hydrogen peroxide and lipid hydroperoxide by reducing glutathione [13], as well as preventing the formation of new free radicals, or converting free radicals that have been formed into less reactive molecules. GSH-PX status decreases in diabetes [14].

The use of antioxidants in diabetes mellitus sufferers is known to be effective in reducing the emergence of complications that appear. This is supported by various studies that prove the benefits of antioxidants related to the pathological process of diabetes mellitus because oxidative stress conditions [9].

However, there is a lack of research regarding the effectiveness of glutathione on blood glucose levels and NF-kB levels in mice (Rattus norvegicus) models of type 2 diabetes mellitus, so researchers are interested in conducting this research.

MATERIALS AND METHODS

Location and research design

This research was carried out at the Integrated Laboratory, Faculty of Veteinary Medicine, Hasanuddin University Makassar, to carry out maintenance, treatment, and blood glucose testing of experimental animals and Hasanuddin University Medical Research Center (HUM-RC) Laboratory, Hasanuddin University Makassar to examination serum of NF-kB using enzyme linked immunosorbent assay (ELISA) with a kit insert Bioassay Technology Laboratory (BT Lab, China). The design of this research is laboratory experimental research with a post-test control group design.

Research sample

The sample in this study was male Wistar rats (Rattus norvegicus) which kept and bred in the animal laboratory, Faculty of Medical Hasanuddin University Makassar with aged 2-3 months and weighed 150-200 grams, 20 mice were divided into 4 groups, namely normal control group (K-N), negative control group (K-), positive control group (K+), treatment group 1 (KP1), and treatment group 2 (KP2). The sample size was determined based on Federer's formula. The inclusion criteria for the sample were healthy, active, 2-3 months old, male, weight >200 grams.

The inclusion criteria in the research sample are rats that are in good health, move actively, are 2 to 3 months old, are male, and weigh more or equal to 200 grams, while the exclusion criteria in this research sample are rats that are pregnant, weigh less than 150 grams, hair baldness or loss, anatomical abnormalities, rats that are sick or have health problems during the adaptation period, and rats that die during adaptation or research.

Work Procedures

Pre-intervention stage

Adaptation of experimental animals

Male rat were adapted in a cage for 2 weeks to adapt their way of life and food. Every day the rat's health is checked and their body weight is measured every week. After the adaptation period, the rat were placed individually in cages and given standard feed of 5-10 g/day and drinking water provided ad libitum. After that, routinely clean the cage and keep the rat's cage environment so that it is not damp at a room temperature of around 28-32°C, and provide sufficient light.

Taking rat blood samples

Before treatment, blood samples were taken from the rats to be used to examine the rats blood glucose levels and serum NF-kB levels before treatment. 2-3 mL of rat blood samples were taken through the eyes which were collected in an anticoagulant tube, then the blood samples were centrifuged for 15 minutes at a speed of 2000 rpm, after centrifugation was carried out, 300 µl of serum in the tube was pipetted using a clinipet, then put into an eppendorf tube, then the sample was aliquoted in a refrigerator at -20°C for 2-3 months.

Intervention stage

Streptozotocin induces to make diabetes mellitus in rat

Rat were fasted for 12 hours before streptozotocin (STZ) induction. Streptozotocin is dissolved in 0.01 M citrate buffer, pH 4.5 and prepared in fresh condition for use within 10-15 minutes. Streptozotocin injection was given intraperitoneally and the dose was determined based on the rat's body weight.

The dose of STZ given is 45 mg/kg BW [15]. Streptozotocin injection is carried out only once to induce diabetes mellitus [16].

Observation of blood glucose levels after streptozotocin induction

After STZ injection, the rat's blood glucose level was measured using a glucometer by cutting the tip of the rat's tail about 1 mm using scissors, then dropping the rat's blood onto the glucometer strip and recording the value that appeared on the glucometer display. Observation of blood glucose after STZ injection is day 0 and day 3. On that day, the percentage of diabetes in rat’s was observed. Normal diabetes is 75-150 mg/dL, mild diabetes is 150-200 mg/dL, moderate diabetes is 200-400 mg/dL, and severe diabetes is >400 mg/dL [17]; [16].

Intervention in a rat model of diabetes mellitus

The K-N group is the group that was only given 5-10 g/day of diet + aquades at a dose of 5 mL/head (morning and afternoon) for 14 days, the K- group was the group that was induced by STZ and given 5-10 g/day of diet. + aquades at a dose of 5 mL/head

(morning and afternoon) for 14 days, the K+ group is the group induced by STZ and given the intervention of 5 mg metformin/head/day for 14 days, the KP-1 group is the group induced by STZ and given the intervention of 200 mg glutathione/head/day for 14 days, KP-2 group is a group that was induced by STZ and intervened with glutathione 200 mg/head/day and metformin 5 mg/head/day for 14 days. After the 15th day, blood was drawn from all treatment groups, then serum NF-kB levels and blood glucose levels.

Post intervention stage

Blood glucose levels examination

Blood glucose levels of rats were measured using a glucometer by cutting the tip of the rats tail about 1 mm using scissors, then the rats blood that came out was dripped onto the glucometer strip and the value that appeared on the glucometer display was recorded. Blood glucose checks were carried out every day during the treatment.

Serum NF-kB levels examination

Blood samples of 3 mL of experimental animals were centrifuged for 15 minutes at a speed of 2000 rpm. After centrifugation, 200 µl of serum was pipetted using a micropipette into an eppendorf tube, the serum samples that had been obtained were checking to NF-kB ELISA Kit (BT Lab, China) according to the manufacturer's instructions. Optical density values were read using an ELISA reader with a wavelength of 450 nm.

Statistic analysis

Normality test used the Shaphiro-Wilk test. The data is normally distributed with a p value > 0.05. Homogeneity test using data Levene's test. Homogeneous data with p value > 0.05. Comparative Test, the analysis used to test the difference of two unpaired group variables (control group and treatment group) is the unpaired T-test with a significance level of 5% (p > 0.05) if the data is normally distributed. In addition, a type of comparability test analysis using Mann-Whitney will be used if the data is not normally distributed.

Research analysis

Research ethics after approval by the Health Research Ehics Commission Hasanuddin University Medical Faculty – Hasanuddin University Hospital, with Letter Number 662/UN4.6.4.5.31 PP36/2023.

RESULTS

Rat blood glucose levels

The previous research data was tested for normality of blood glucos level data for all groups of treated rats. The results showed that the normality of blood glucose level data for all groups of treated rats before and after was normally distributed with a p value >0.05. Next, to find out the comparison value of glucose data among groups of treated rats with a normal distribution of food, continue using the one way anova test, Table 1.

Table 1: Blood Glucose Levels Before and After Treatment

ANOVA						
		Sum of Square	df	Mean Square	F	Sig.
Blood glucose (before)	Between Groups	5239.040	4	1309.760	1.078	.394
	Within Groups	24306.800	20	1215.340		
	Total	29545.840	24			
Blood glucose (after)	Between Groups	304952.000	4	76238.000	5.036	.006
	Within Groups	302758.000	20	15137.900		
	Total	607710.000	24			

Based on statistical results, it was found that the p value for rat blood glucose before treatment was p=0.394, this shows that there was no difference in the mean among groups of rats before treatment. Meanwhile, statistical results found that the p value for blood glucose after treatment was p=0.006, this shows that there was a difference in the mean among groups of rats after treatment. The results of the blood glucose examination before and after treatment for 14 days in each research group can be seen in Figure 1.

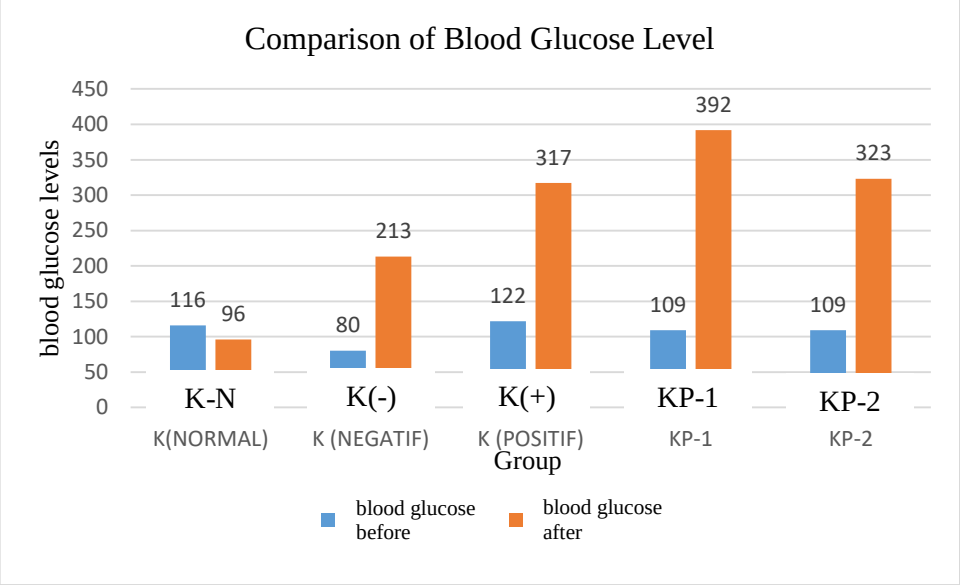

Figure 1: Diagram of the average blood glucose levels before and after treatment for each group.

Based on the picture above, it is shown that the average blood glucose value before the K-Normal group was higher than the blood glucose after treatment, while the K-Negative, K-Positive, KP-1, and KP-2 treatment groups showed higher blood glucose values after treatment than before treatment of experimental rats. Based on the statistical results of the comparison test for glucose levels before and after treatment, it was found that the value of p=0.000, this shows that there is a difference in the average blood glucose before and after treatment in all groups.

Rat serum NF-kB levels

Previous research data was tested for normality of data on the serum NF-kB levels of all groups of treated rats. The results showed that the normality of serum NF-kB data for all groups of treated rats before and after was normally distributed with a p value >0.05.

Table 2: Serum NF-kB Levels Before and After Treatment.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
BEFORE	Between Groups	2.123	4	.531	1.230	.330
	Within Groups	8.628	20	.431		
	Total	10.751	24			
AFTER	Between Groups	3.284	4	.821	2.371	.087
	Within Groups	6.924	20	.346		
	Total	10.209	24			

Based on Table 2, the statistical results found that the p value for rats serum NF-kB before treatment was p=0.087, this shows that there was no significant mean difference among groups before treatment. Meanwhile, statistical results found that the p value of serum NF-kB after treatment was p=0.330, this shows that there was no difference in the mean among groups after treatment. Next, to determine the comparison value of serum NF-kB among groups of treated rats with a normal distribution of food, continue using the paired T test, Table 3.

Table 3: Comparison of Serum NF-kB Levels before and After Treatment

Paired Samples Test								
	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
BEFORE vs AFTER	-.02560	.92125	.18425	-.40587	.35467	-.139	24	.891

Based on the statistical results of the comparison test of serum NF-kB levels before and after treatment, the value of p=0.891 was found, this shows that there was no statistically significant difference in serum NFkB levels before and after treatment in all groups.

DISCUSSION

Giving streptozotocin to rat causes diabetes mellitus so that it can increase blood glucose levels after giving streptozotocin. Based on the statistical results of comparative tests on glucose levels before and after treatment, it was found that the value of p=0.000 (p<0.05) (Table 1), this shows that there was a difference in mean blood glucose before and after treatment in all groups of rats. The research results of Rosyadi, et al 2018 show that blood sugar levels in rat will increase within 24 hours after giving a single dose of streptozotocin of 40 mg/kg body weight to rat intraperitoneally [18]. Based on statistical tests, it shows that in the KP1 and KP2 treatment groups there were differences in blood glucose levels before and after treatment with respective values of p=0.014; p=0.016 (p<0.05). This is because glutathione functions as an antioxidant which plays a role in reducing oxidative stress in hyperglycemic rat after streptozotocin intervention.

Streptozotocin (STZ) can affect blood glucose levels through three mechanisms, namely: 1) Blunting or loss of the first stage insulin response, so that insulin secretion is delayed and fails to restore prandial blood sugar within a normal time, 2) Decreased insulin sensitivity in response to glucose so that it causes hyperglycemia, 3) Fails to

stimulate a normal insulin response [19]. Giving Sucrose increases the symptoms of STZ-induced diabetes by increasing blood glucose and fat deposits as well as body weight in rat [20].

High blood gluose levels in DM sufferers cause various changes in the body. One of the detrimental processes is called an oxidation reaction which causes an increase in the formation of dangerous substances called free radicals. Increased oxidative stress causes type 2 DM sufferers to require large amounts of exogenous antioxidant intake to inhibit oxidative damage in the body [21]; [22]. Blood glucose regulation is also indicated to be related to GSH levels [23]. Glutathione is a major intracellular antioxidant and plays a key role in reducing the effects of oxidative stress [24].

Meanwhile, the results of the examination of rat serum Nf-kB showed that there was no significant difference between serum NF-kB levels before and after treatment with a value of p=0.891 (p>0.05) (Table 3), and the statistical results did not show any significant differences serum NF-Kb levels after treatment with p value = 0.330 (p> 0.05) (Table 2). NF-kB is a pro- and anti-apoptotic trigger of pancreatic beta cells with a greater pro-apoptotic tendency than its anti-apoptotic role. In diabetes, NF-Kβ activity will trigger pancreatic beta cell dysfunction resulting in progressive apoptosis in these cells [25]. Based on these things, it can be seen that the ability of glutathione to improve NF-kB levels and how NF-kB levels can influence blood sugar reduction in the treatment group cannot be proven in this study. A situation that shows the insignificance of the results cannot be said to be a failure, but rather is a mechanism that cannot be explained in this research. Limitations in this research are the procedures for handling experimental animals, as well as laboratory environmental conditions which can also influence the research results.

CONCLUSION

There were no differences in mean blood glucose levels before the intervention in all groups, and there were no differences in mean blood glucose levels between treatment groups after the intervention. No significant differences were found between serum NF-kB levels before and after treatment.

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Conflıct of Interest

There is no conflict interest

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