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EFFECTIVENESS OF GLUTATHIONE ON SERUM MALONDYALDEHIDE (MDA) LEVELS AND PANCREATIC HISTOPATOLOGY IN RATS (RATTUS NORVEGICUS) TYPE 2 DIABETES MELLITUS MODEL

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Abstract

Diabetes mellitus (DM) is one of the chronic health problems in the world. Excessive hyperglycemia will form free radicals, which will react with pancreatic cell membranes to form malondialdehyde (MDA) which can cause atrophy of the islet of Lengerhans as well as cell damage and death. Glutathione (GSH) is an antioxidant that can reduce free radicals. This study aims to assess the effectiveness of GSH in improving MDA levels and pancreatic histopathology to male rat in DM type 2 model. Twenty-five male Wistar norvegicus rats were divided into four groups: K-N (ad libitum feed + 5 mL distilled water), K- (STZ + 5 mL distilled water), K+ (STZ + metformin 5 mg), KP1 (STZ + glutathione 200 mg), KP2 (STZ + glutathione 200 mg + metformin 5 mg). The results show that the serum MDA levels of rat were found to be p=0.056 (p>0.05); Histopathological checking of the pancreas in the K-N group did not reveal any abnormalities or damage, compared to other groups which found several fatty vacuoles, hydropic degeneration, edema and congestion. It was concluded that the addition of glutathione to male mice Wistar norvegicus model of diabetes mellitus was not significant in reducing MDA levels and improving the pancreatic organ of experimental animals.

Keywords: Diabetes Mellitus, Glutathione, Malondyaldehide, Histopatologi Pankreas.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by an increase in blood glucose levels over a long period of time, which can cause several complications such as complications in the heart, blood vessels, eyes, kidneys and nerves [1].

The International Diabetes Federation (IDF) reported that in 2019, DM sufferers in the world increase of 51% and it is estimated that the number of DM sufferers in the world will increase from 463 million to 700 million in 2045 [2]. Based on data from the Indonesian Ministry of Health in 2018, it was stated that the prevalence of DM in Indonesia had increased from 5.7% in 2007 to 6.9%, around 9.1 million people in 2013.

Excessive hyperglycemia and hyperinsulinemia will form free radicals or reactive oxygen species (ROS), which is caused by insulin resistance, which can result in increased damage to the structure and morphology of the islet of Langerhans and the

cells around them. Free radicals will later react with pancreatic cell membrane components such as phospholipids, unsaturated fatty acids, and proteins, which can cause a lipid peroxidation reaction to form malondialdehyde (MDA). This condition causes decrease of the islet of Langerhans (atrophy), so that the structure and boundaries of the islet of Langerhans have begun to merge with the surrounding acinar cells. This indicates cell death or damage due to oxidative stress caused by the presence of free radicals [3].

Malondialdehyde (MDA) is one of the final products of lipid peroxidation; this compound is formed because the degradation of hydroxyl free radicals towards unsaturated fatty acids, which will later be transformed into highly reactive free rakilla [4]. Oxidative stress in tiksu can cause malondialdehyde levels to increase. Therefore, malondialdehyde can be used as a biomarker for oxidative stress [4].

Providing additional antioxidant in conditions of oxidative stress will help the body fight oxidant molecules originating from inside and outside the body, thereby helping to reduce or prevent oxidative damage [5]; [6].

Antioxidants are inhibitors that can be used to inhibit autooxidation. Antioxidants are really needed by the body to help protect the body from free radical attacks [7]. Antioxidants can be enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, vitamins, and beta-carotene, as well as several other compounds such as flavonoids, albumin, bilirubin, ceruplasmin, and others. These compounds function to capture oxidant compounds and prevent from occurring chain reactions [8].

One of antioxidant that can be used is glutathione (GSH) which is an enzymatic antioxidant that can detoxify hydrogen peroxide and lipid hydroperoxide by reducing glutathione [9], as well as preventing the formation of new free radicals or converting free radicals that have been formed into less reactive molecules [10]. The condition of glutathione itself can decrease in diabetes [11].

However, there is a lack of research regarding the effectiveness of glutathione on serum malondialdehid (MDA) levels and histopatology pancreas in male rat (Rattus norvegicus) models of type 2 diabetes mellitus, so researchers are interested in conducting this research.

MATERIALS AND METHOD

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 and pancreatic histipatologic examination of experimental animals and Hasanuddin University Medical Research Center (HUM-RC) Laboratory, Hasanuddin University Hospital Makassar to examination serum of MDA 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 Veterinary Medical Hasanuddin University

Makassar with aged 2-3 months and weighed 150-200 grams, 20 rat were divided into 4 groups, namely normal control group (K-N), negative control group (K-), positive control group (K+), treatment group 1 (KP-1), and treatment group 2 (KP-2).

The sample size was determined based on Federer's formula. 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 rat to be used to examine the rats blood glucose levels and serum MDA 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 rats body weight. The dose of streptozotocin given is 45 mg/kg BW [12]. Streptozotocin injection is carried out only once to induce diabetes mellitus [13].

Observation of blood glucose levels after streptozotocin induction

After streptozotocin 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 streptozotocin injection is day 0 and day 3. On that day, the percentage of diabetes in rat 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 [14]; [13].

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 KP1 group is the group induced by STZ and given the intervention of 200 mg glutathione /head/day for 14 days, KP 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 malondialdehyde (MDA) levels were examined and histopathological examination of the pancreas was carried out.

Post Intervention Stage

Serum malondialdehyde 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 MDA 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.

Pancreatic histopatological examination

The pancreas organ is checked using hematoxylin and eosin (H&E) staining, which will be checked under a microscope with 100x and 400x magnification. The parameters used to determine the presence of damage to the pancreatic islet of langerhans are vacuolization, congestion, regularity of the shape of the islet of langerhans and the availability of pancreatic beta cells. Measurement of damage to islet langerhans was carried out by calculating the area of islet langerhans that was damaged compared to the area of islet Langerhans as a whole using the ImageJ application [15].

Statistical 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 Ethics

Research ethics after approval by the Health Research Ehics Commission Hasanuddin University Medical Faculty – Hasanuddin University Hospital, with Letter Number 698/UN4.6.4.5.31/ PP36 / 2023.

RESULTS

Rat Serum MDA Levels

The research data on Mouse serum MDA levels was tested for normality on all groups of treated mouse. The results showed that the normality of the data on mouse serum MDA levels for all treatment groups, both before and after, was obtained as normally distributed results with a value of p=0.854 (p>0.05). Furthermore, to assess differences in mouse serum MDA levels between groups both before and after treatment, a statistical test was carried out using the One Way Anova test, which can be seen in Table 1.

Table 1: Serum MDA Levels Before and After Treatment

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
MDA before	Between Groups	.727	4	.182	2.166	.110
	Within Groups	1.678	20	.084		
	Total	2.405	24			
MDA After	Between Groups	.693	4	.173	2.746	.057
	Within Groups	1.262	20	.063		
	Total	1.956	24			

Based on statistical results, it was found that the p value for rat serum MDA levels before treatment was p=0.110, this shows that there was no significant mean difference between groups before treatment. Meanwhile, statistical results found that the p value of serum MDA levels after treatment was p=0.057, this shows that there was no difference in the mean between groups after treatment. Next, to find out the comparative value of serum MDA levels between treatment groups, proceed using the paired T test, which can be seen in Table 2.

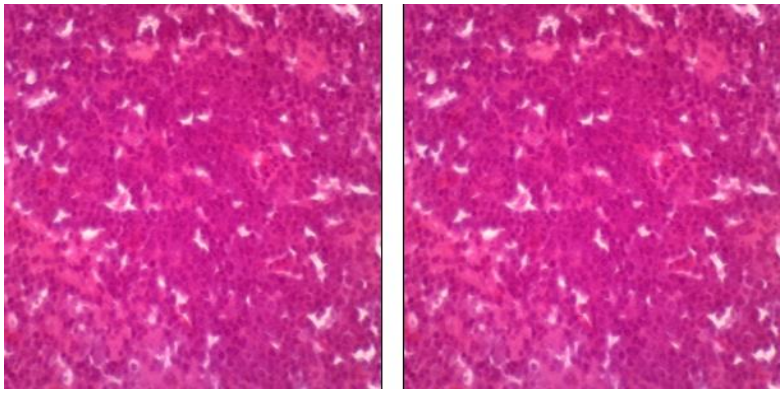
Table 2: Comparison of Serum MDA Levels Before and After Treatment

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	.17360	.43245	.08649	-.00491	.35211	2.007	24	.056

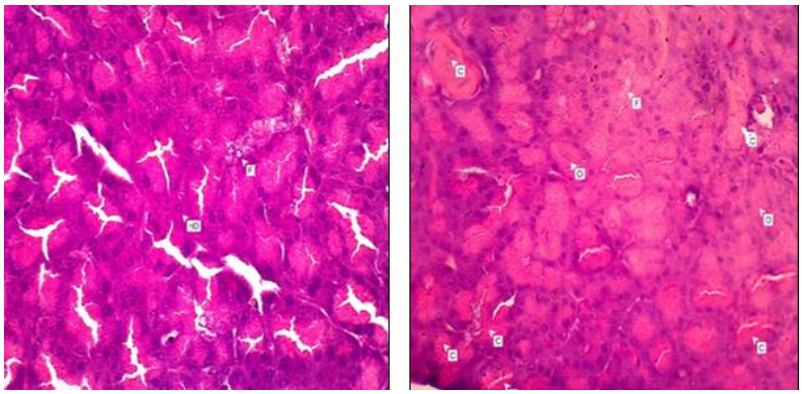
Based on the statistical results of the comparison test of serum MDA levels before and after treatment, it was found that the value of p=0.056 (p>0.05), this shows that there was no statistically significant difference in the serum MDA levels of rat before and after treatment in all treatment groups.

Histopatological Examination of Pancreatic Organs

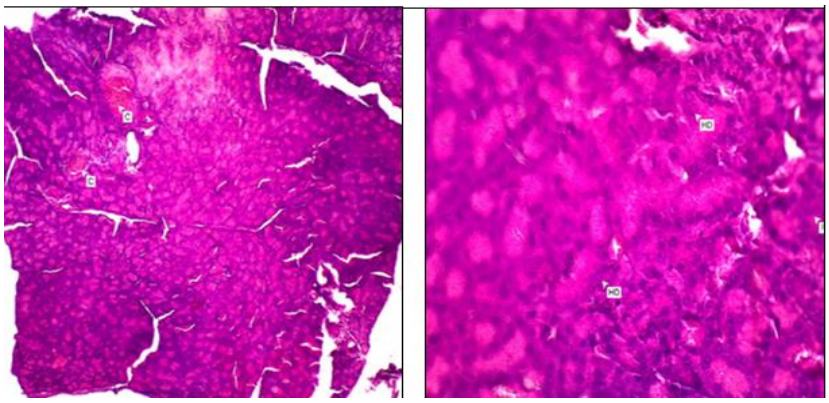
Histopathological observations of the rat pancreas using the Hematoxylin-Eosin staining method. Hematoxylin and eosin are essences that are often used to color tissue so that it is easier to observe with a microscope. The principle of this coloring is the acidic cell nucleus will attract alkaline substances so that it turns blue. The alkaline cytoplasm will attract acidic substances so that it turns red. The group of treated rat that had diabetes mellitus had a histopathological picture of the pancreas that was different from the group of mouse without treatment, which could be seen based on the results of histopathological observations of the pancreas organ (Figure 2).



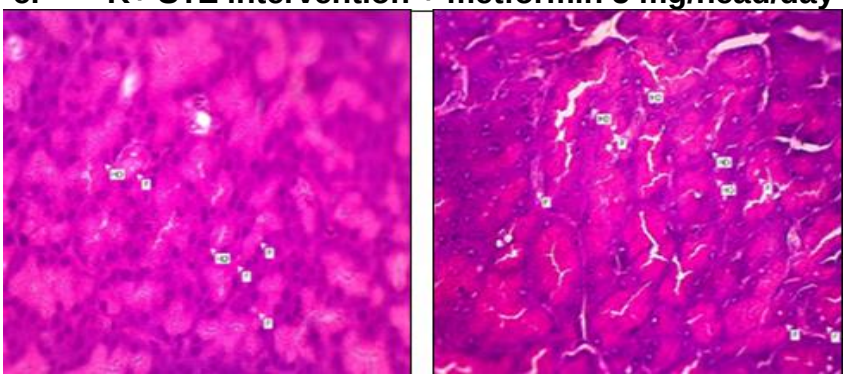
a. K-N without intervention



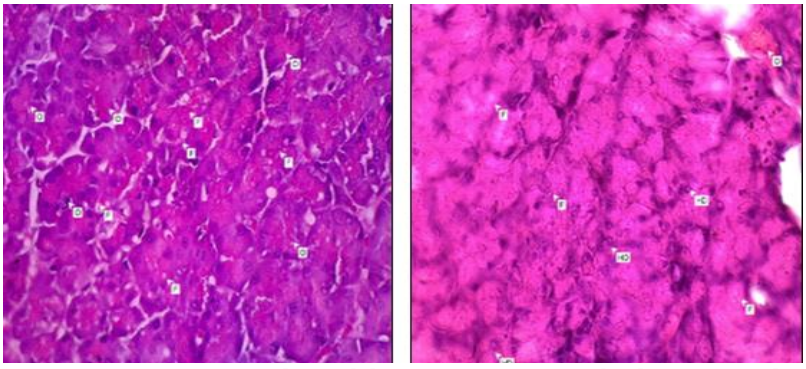
b. K- STZ intervention



c. K+ STZ intervention + metformin 5 mg/head/day



d. KP-1 intervention STZ + glutathione 200 mg/day/head



e. KP-2 STZ + glutathione + metformin intervention

Figure 1: Histopathological Picture of Pancreas Organs of Treated Rats at 400x Magnification with HE Staining, with Information (C) *Congestion*, (F) *Fatty Vacuole*, (H) *Hydropic Degeneration*, (O) *Oedema*

DISCUSSION

Cigarette smoke is a pollutant for humans and the environment. Cigarette smoke contains free radical compounds, which will cause oxidative stress. In conditions of oxidative stress, free radicals cause peroxidation of cell membrane lipids and damage cell membrane organization, characterized by increased levels of malondialdehyde (MDA) and an increase in the number of macrophages in the lungs [16].

Several studies show that oxidative stress plays a role in systemic inflammation, disruption of pancreatic β-cell secretion, disruption glucose use in peripheral tissues and endothelial dysfunction. The emergence of oxidative stress in DM occurs through three mechanisms, they are non-enzymatic glycation of proteins, the polyol sorbitol pathway (aldose reductase), and glucose auto-oxidation [17]. Excessive oxidative stress make abnormalities in glucose metabolism can be controlled by regulating food intake, especially sources of antioxidants [17]; [18].

Malondialdehyde (MDA) is a compound that can describe the activity of free radicals in cells so it is used as an indication of oxidative stress caused by free radicals [19].

Based on the statistical results of the comparative test of serum malondialdehyde levels before and after stiffening, it was found that the value of p=0.056 (p>0.05), this shows that there was no statistically significant difference in serum malondialdehyde levels before and after treatment in all groups. Meanwhile, the statistical results of serum malondialdehyde levels after treatment found a value of p = 0.057, this shows that there was no difference in the mean between groups after treatment.

Several previous studies have shown that in type 2 diabetes melitus (DM) sufferers there is an increase in free radicals. Research conducted in Egypt showed that levels of SOD, MDA, and GSH increased significantly in type 2 DM sufferers [20]. An increase in MDA levels indicates an increase in free radicals because free radicals can cause peroxidation of blood lipids so that MDA levels increase [17]; [20], which indicates oxidative stress occurs in type 2 DM patients [21].

Other studies reveal that there is a moderate positive correlation between temporary blood sugar levels and MDA levels in type 2 DM patients, which means that a temporary increase in blood glucose levels almost always causes an increase in blood lipid peroxidation. This happens because glucose is a very reactive compound.

The aldehyde group in glucose is able to glycate various compounds to form free radicals [22]; [23]. Apart from that, hyperglycemia conditions also trigger glucose autoxidation to produce superoxide radicals and hydroxyl radicals [17]; [24]. Therefore, in hyperglycemia, glucose in the blood immediately reacts by oxidizing the lipids in the blood. Lipid peroxidation will increase if blood glucose levels exceed the hyperglycemia threshold (200 mg/dL) [23]; [25]. In line with research conducted in Malaysia by [23], and Thailand by Likidlilid, et al. 2010 it was stated that HbA1c levels and fasting blood glucose were positively correlated with blood MDA levels [26].

Based on the results of the histopathological checking of the pancreas of treated mouse, it can be seen in Figure 2. It shows that in the KN group (without intervention) there were no abnormalities or damage found in the mouse pancreas, in the K- group (STZ intervention) fatty vacuoles, hydropic degeneration, were found. edema, and congestion, in the K+ group (STZ + metformin intervention) congestion, fatty vacuole, and hydropic degeneration were found, in the KP1 group (STZ + glutathione intervention) vacuole and hydropic degeneration were found, while in the KP2 group (STZ + glutathione + intervention metformin) it was found that there was a number of fat cell vacuolizations in almost all parts and some cells experienced hydropic degeneration, and the tissue was seen experiencing edema.

According to Voronina, et al 2015 vacuolization is a very important indicator of pancreatic acinar cell damage [27]. Furthermore, trypsinogen activation occurs in the endocytic vacuole, therefore, the vacuole can be considered as an organelle that initiates the development of cell damage.

Malondyaldehid and glutathione levels have a strong significant correlation and have a reciprocal relationship, meaning that the higher the MDA level, the lower the glutathione level in the worker's body. This has similarities with other research, which states that there is an increase in MDA levels and a decrease in glutathione levels in cement workers [28]. High MDA levels indicate higher levels of free radicals as well. These benzene radicals can suppress detoxification enzymes, one of which is GSH [28].

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 significant differences in mouse serum malondialdehyde levels before and after treatment in all groups. Histopathology results in the K-N group did not reveal any abnormalities or damage, compared to other groups, which found several *fatty vacuoles, hydropic degeneration, edema and congestion.*

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Conflict of Interest

There is no conflict interest .

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