EFFECT OF CADMIUM EXPOSURE ON INCREASING RISK OF DIABETES MELITUS THROUGH THE MEASUREMENT OF BLOOD GLUCOSE LEVEL AND LIVER GLUCOKINASE ACTIVITY IN RATS

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***Abstract**: **This present study was undertaken to investigate the effect of cadmium (Cd) exposure on an increasing risk of Diabetes Melitus (DM), through the measurement of blood glucose level and liver glucokinase activity in rats.** The subjects that used in this study are 15 male rats (Rattus novergicus) with normal activity, 2-3 months old, and weighing 300±10 gram. The research subjects the divided into 3 groups; P0 group are given commercial fed rats diets only; P1 are given commercial fed rats + Cd with a concentration of 3 mg/l in drinking water for 1 day (acute); and P2 are given commercial fed rats+Cd with a concentration of 3 mg/l in drinking water for 4 weeks (subacute). The results of this present study shows that treatment with Cd significantly increase the levels of blood glucose (P < 0,05). The result also showed that treatment with Cd can increase the Km value of liver glucokinase, and it means Cd exposure can decrease the affinity between glucose and glucokinase. The present study demonstrated that Cd exposure could increase the risk of DM through increased the blood glucose and decrease the activity of liver glucokinase.*

***Keywords**: Cadmium, glucose metabolism, glucose, glucokinase*

INTRODUCTION

The development of information technology and industrial has changed behavior, way of life, and the environment. The changes can happen in a form of different food consumption, less physical activity, and more environmental pollutions. Subconsciously, these changes contribute to shifting of disease pattern, from communicable disease to non-communicable disease, such as Diabetes Mellitus (DM).[1]

Diabetes Mellitus is one of increasing non-communicable disease in number every year. Based on WHO, it was predicted that people with Non Insulin Dependent Diabetes Melitus (NIDDM) is increasing from 8.4 million in 2000 to approximately 21.3 million in 2030.[2] Based on Riset Kesehatan Dasar (Riskesdas) in 2010, the prevalence of DM in South Kalimantan reaches 1.0% (range 0.3-1.7%). Six regions/cities with higher prevalence than province prevalence, such as Banjarmasin, Banjarbaru, Barito Kuala, Tapin, Banjar, dan Hulu Sungai Selatan.[3]

Diabetes mellitus happens due to carbohydrate metabolism disturbance characterized by increasing glucose concentration in blood (hyperglycemia). This disturbance may be caused by several factors, such as genetics, diet, way of life, also environmental factor, such as heavy metal exposure. One of heavy metal that has been known can cause the metabolism disturbance is cadmium (Cd).[4-5] This was based on research by Akinloye et al[6] which stated that there is positive correlation between blood level of Cd and random blood glucose (r=0,378; p<0,001). Some population studies also stated correlation between the increasing blood and urine level of Cd and type II DM prevalence.[7-11]

Cadmium is a kind of heavy metal produced from accumulator industries, solute pesticides, and mining activities.[12-13] South Kalimantan is famous for its natural resources, especially from mining sectors. In South Kalimantan, Cd level, because of mining activities and coal transportation, has started to pollute the environment.[14] A research by Rahman[14] stated that few kinds of shrimp and *rajungan* in Takisung and Batakan Beach area have been polluted by Cd (range 0.213 mg/kg). Another research by Dini et al[15] there were found Hg, Pb, and Cd in DAS Barito area above normal level. This was predicted caused by coal unloading and transportation which pass this river.

Besides in water organisms, Cd also found in vegetables. A research by Widowati[16] showed that Cd was found in *genjer*, water *kangkung*, and water *selada* each with concentration of 4,91 µg/L; 9,28 µg/L; dan 5,91 µg/L. In the research also concluded that Cd level negatively correlates to vitamin C and vitamin A. This means the increasing level of Cd causes decreasing level of vitamin C and vitamin A.

Cadmium that enters the environment then cause biotransfromation and bioaccumulation processes in living organisms. This results in the accumulation of Cd, which subsequently leads to biomagnification or increased concentration within the food chain.[17] Cd concentration is estimated to increase 15 times per two trophic in the food chain.[18] The process will have an impact primarily for human health.[17] WHO estimates a Cd exposure limit of inhalation ie 0.5 mg / m^3 for 8 hours, and 1.5 mg / m^3 exposure is known to be harmful to humans. In addition, the WHO data also suggest that acute exposure of Cd orally with a concentration of 15 mg / KgBW may cause gastrointestinal disturbance, and at doses of 20-30 mg / KgBW may cause more serious disruption in humans.[19]

The increased risk of DM due to Cd exposure begins from the Cd absorption process through the respiratory and digestive

systems. After that, Cd will experience accumulation inside the organs, especially in the liver. The accumulation is suspected because the liver is the metabolic center of various xenobiotic compounds. This has been proven by Josthna *et al*[20] who revealed that oral administration of Cd in mice can cause Cd to accumulate in some organs, especially in the liver and kidneys.

Metal cadmium (Cd) accumulation in the liver will cause damage to hepatocytes. This has been demonstrated by Radosavljevic *et al*[21] which states that mice that get exposed to Cd with dose of 2.5mg/kgBW intraperitoneally cause changes in histologic images of the hepatocytes. In these cells occur degeneration, necrosis, and mild congestion. Fahim *et al*'s study also proved mice that get exposed to Cd with dose of 2,284 mg / kgBW in intraperitoneally had liver damage characterized by elevated levels of aspartate aminotransferase and alanine aminotransferase enzymes.[22]

Damage to hepatocytes will lead to disruption of various metabolic pathways such as carbohydrate metabolism. This is due to the ability of Cd in disturbing the balance of glycogen synthase enzyme, so that glycogen is widely broken into glucose. On the other hand, Cd also decreases the glucokinase activity that converts glucose to glucose-6-phosphate in the liver. Both of these mechanisms can cause increased levels of glucose in the blood.[23-24] This is supported by Al-Rikabi and Jawad[25] research, which states that rabbits exposed to Cd at a dose of 10 mg / kgBW for 35 days resulted in elevated blood glucose levels ($p < 0.05$)

Nowadays, the impact of Cd exposure to increased risk of DM through measurement of glucose levels and glucokinase enzyme activity has not been widely disclosed. Therefore, this study will examine the impact of Cd exposure to increased risk of DM through measurement of blood glucose levels and liver glucokinase enzyme activity.

RESEARCH METHODS

This research was pure experimental (true experimental) with Post Test Only design with Control Group Design. While the design of this study using Completely Randomized Design (CRD) using three treatment groups, among others:

- P0: Negative control group, i.e. mice fed only commercial feed.
- P1: Treatment group, i.e. commercially fed and Cd mice with concentrations of 3 mg / L in drinking water for 1 day (acute).
- P2: Treatment group, i.e. mice fed with commercial feed and Cd with concentration 3 mg / L in drinking water for 4 weeks (sub-acute).

At the each end of the exposure period, blood glucose levels and glucokinase enzyme activity in the liver of white rats will be measured.

The population in this study were male rats (Rattus novergicus), Sprague-Dawley strain, healthy and had normal activity, about 3-4 months old with weight 300 ± 10 gram. This rat was obtained at the Veterinary Research and Investigation Center Banjarbaru. The sample used in this study were 5 samples per group, so the total sample size in this study was 15 samples.

Rat blood samples were taken through the heart. The anesthetized rat was shed on the surgical board on all four limbs. The chest cavity was dissected and the blood in the heart as much as 2 ml was taken using a 3 cc syringe. Blood present in the syringe is then used to measure blood glucose levels.

After surgery, the liver was removed and washed with a 20% TCA, then fixed in a phosphate buffer solution (pH 7). Then the liver was cut into small pieces and mashed up to form a liquid with a mortar. Next the

solution was taken 5 ml and centrifuged 3500 rpm for 10 minutes. The above layer is taken 200 μL for experiment purposes.

Blood glucose levels are measured using a Blood Glucose Test Meter of the Easy Touch brand. The tool is set according to the Easy Touch Glucometer Test Strip code used, and then the blood sample is dropped onto a strip connected to the glucometer. Then left for 6 seconds and read the scale seen on the screen. Blood glucose levels are expressed in units of mg / dl.[26]

Glucose concentration (100 mM, 200 mM, 300 mM, 400 mM, and 500 mM) of each concentration was taken 3 mL and added 3 mL phosphate buffer (pH 7). Next it will be mixed until becoming homogeneous. As much as 1 mL of homogenate was added to each mixture, then measured the glucose level [G0]. After 20 minutes, the glucose of each mixture was measured again [G1] by Duboie's hydrolytic method. The rate of glucose oxidation by glucokinase (v) is expressed in changes in glucose concentration per minute.[27]

Glucokinase activity is measured by measuring the value of Michaelis-Mentens (Km) concentration, i.e. by making a linear graph between 1/[G] with 1/v. Graph obtained is the equation of straight line with slope = Km/V max and intercept = 1/V max.[28]

RESULTS AND DISCUSSION

This study was conducted to determine the effect of Cd exposure to increased risk of DM, by measuring blood glucose levels and liver glucokinase enzyme activity of white rats. The mean blood glucose level in each treatment group was expressed in mg / dl and presented in Figure 1.

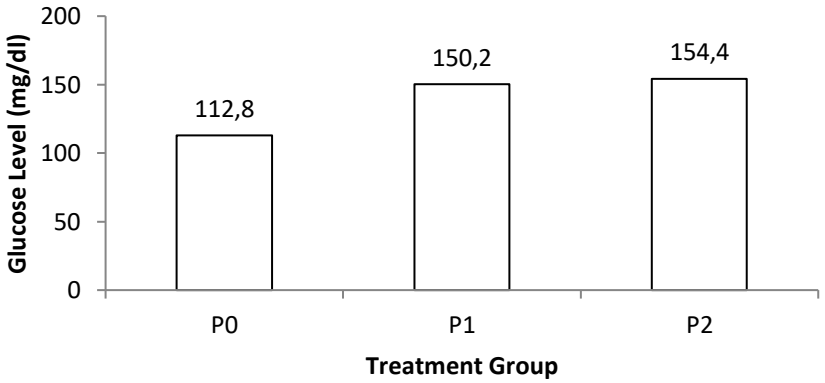

Figure 1. Mean blood glucose levels of white rats in three treatment groups

The results of the study in Figure 1 show that the average glucose levels in the P0, P1 and P2 groups were respectively 112.8; 150.2; and 154.4 mg / dl. The mean blood glucose level was highest in group P2 and lowest in group P0. The results also showed that the mean blood glucose levels in the two treatment groups (P1 and P2) were higher than the control group (P0).

The data of blood glucose measurement was then tested for normality using Shapiro Wilk test. The test results obtained $p > 0.05$ ($p = 0.200$). The results of the test show that the data of the measured mean blood glucose levels are normally distributed. Further data of measurement result of blood glucose level was done by homogeneity test using Levene test. The test results obtained $p > 0.05$ ($p = 0.207$). The results of these tests indicate that the data measured the mean blood glucose levels are homogeneous.

The result of normality test and homogeneity of mean blood glucose level showed that the data was normal and homogenous. Furthermore, to determine the effect of Cd exposure to blood glucose level, it will be tested using One Way Anova test. The results showed that Cd exposure resulted in significant differences in blood glucose levels between treatment groups ($p = 0.035$; $p < 0.05$).

Furthermore, it will be tested using Post Hoc Tukey HSD to know the differences between each group. The result of the analysis showed that there was significant difference of blood glucose level between group P0 with P1, and P0 with P2, whereas between group P1 and P2 analysis showed a not significant difference. The results of the analysis are presented in Table 1.

Table 1. Conclusions of Post Hoc Tukey HSD test blood glucose levels of white rats in some treatment groups

Group	*P* value	Conclusion
P0 – P1	0,043	Significantly different
P0 – P2	0,045	Significantly different
P1 – P2	0,959	Not Significantly different

The results showed that Cd exposure in both acute and subacute was able to increase blood glucose levels of white rats significantly. The results of this study are in line with the results of another study which states that administration of CdCl2 with a dose of 5 mg/kgBW intragastrically for 30 days proved to increase blood glucose levels significantly ($p < 0.05$) (5). Other studies also suggested that administration of CdCl2 at a dose of 1 mg/100 gr BB orally for 45 days was shown to significantly increase blood glucose levels ($p < 0.01$).[29]

The study of Al-Attar[29] concluded that *Oreochromis niloticus* that exposed to Cd 9.3 ppm for 1, 4, and 7 days were shown to significantly increase blood glucose levels of the fish. Asagba and Obi[30] studies also mentioned that mice exposed to CdSO4 in concentrations of 0.3 mg/l for 1 month were found an increase blood glucose levels significantly ($p < 0.05$).

Increased blood glucose levels due to Cd exposure are suspected through several mechanisms. Cd exposure is thought to trigger pancreatic damage resulting in a decrease in insulin production. This is based on the research of Muayed et al[5], which states that Cd can damage β Langerhans cells in the pancreas. The subsequent decrease in insulin causes the membrane permeability of glucose to be disrupted so that glucose cannot enter the cell. In addition, the decrease in insulin causes glucokinase and amylase, the enzyme that plays a role in the process of glucose metabolism, there is nothing to induce. Thus, the condition causes the build-up of glucose in the blood.[24,31]

Cd exposure can also trigger damages of liver cells that are known to be the occurrence place of carbohydrate metabolism. This is due to the activity of Cd in oxidizing liver cell membranes resulting in lipid peroxidation process.[32] The process is characterized by elevated MDA levels.[33-34] This is based on Suhartono *et al*[35] study which states that Cd exposure may increase MDA levels in rat liver.

Cd can bind to the -SH group present in the Na^+-glukosa cotransporter-1, ie the glucose-carrying protein resulting in a conformational change of the protein.[36-38] This results in reduced activity of glucose-carrying proteins, resulting in extracellular

glucose accumulation.[39] It results in an increase in blood glucose levels.

The second parameter measured was liver glucokinase enzyme activity of white rats. Measurements of liver glucokinase enzyme activity are presented in the table 2.

The result of measurement in table 2 shows that glucokinase Km in group P0, P1, and P2 are 500,630; 6919,121; and 6815,157. The values of V max in groups P0, P1 and P2 were 37,846; 340,451; and 315,214. These results indicate that the values of Km and V max P1 and P2 are greater than P0. The maximum Km and V max values are found in P1 and the smallest at P0.

Table 2. The values of Km and V max liver glucokinase enzymes of white rats in some treatment groups

Groups	Km	Vmax
P0	500,630	37,846
P1	6919,121	340,451
P2	6815,157	315,214

The results showed that Cd exposure was able to influence liver glucokinase enzyme activity of white rats. This is due to the ability of Cd to bind covalently to the -SH group of several enzymes that play a role in the metabolism of carbohydrates such as glucokinase.[40] Glucokinase is an intracellular enzyme that plays a role in the conversion of glucose into glucose-6-phosphate. Glucokinase is known to have an -SH group of 213, 220, 230, 233, 364, 371, and 382.[41-42] Cd amino acids suspected to bind to the cysteine -SH amino acid group.[43] This will result in decreased glucokinase enzyme activity and can increase blood glucose levels. This is in line with the results of this study indicating that the Km values in the treatment group (P1 and P2) are greater than the control group (P0)

The Michaelis-Menten (Km) constant is the substrate concentration required by an enzyme to reach its maximum half-speed. Each enzyme has different Km values for a substrate, and this can show how strong the substrate binds to the enzyme. The higher the Km value means the lower the affinity or the strength of the binding of the susceptible to its enzyme. Based on the results of the study shows that Cd exposure can increase the value of Km. This means that Cd exposure lowers the affinity of the glucokinase enzyme to glucose.[44-45] The decrease in the affinity, may result in the inactivation of the enzyme, so that the glucose bond to glucokinase became weak.[46] This results in the bonding loose, resulting in elevated glucose levels.

CONCLUSIONS

Based on the results of research and discussion, it can be concluded that the exposure of Cd with concentrations of 3 mg/l through drinking water can increase the risk of diabetes mellitus characterized by elevated blood glucose levels and decreased liver glucokinase enzyme activity. In addition, Cd exposure can affect blood glucose levels of white rats and liver glucokinase enzyme activity of white rats. Therefore, there was a significant difference between blood glucose levels of exposed white rats and those not exposed to Cd.

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