Single Low Dose Streptozotocin (STZ) to Increase Serum Triglyceride Levels of Rats

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Abstract. Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to relative or absolute lack of insulin. Insulin resistance and insulin deficiency are relatively related to lipid changes because insulin plays an important role in regulating lipid metabolism. The study aimed to analyze the effect of single low dose streptozotocin (STZ) on serum triglyceride levels of rats. Experimental with posttest only control group design was used in this study. A total of 12 male Sprague dawley rats who visited LPPT 4 Gadjah Mada University at Yogyakarta was selected as research samples. Rats were assigned to two experimental group (control group and diabetic group). Control group (n=6) was given intraperitoneal injection with phosphat buffer saline (PBS), and diabetic group (n=6) was given intraperitoneal injection with single low dose STZ (30mg/kgBB). Triglyceride levels was analyzed using GPO-PAP methods after 3 days STZ-induced rats. Data analysis used Independent-T Test (p<0.005). Serum triglyceride levels in control group 56,33 mg/dl were actually lower than in diabetic rats 265,33 mg/dl. Serum triglyceride levels was significantly differences between control group and diabetic group (p=0.042). Single low doses STZ-induced diabetic rats has an effect on increasing serum triglyceride level of rats.

1. Introduction
Streptozotocin [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] is synthesized by Streptomyces achromogenes and a nitrosourea analogue [1] [2]. High STZ affinity for pancreatic β-cell membranes causes selective toxic effects on pancreatic β-cells [2]. Effects of STZ induction on pancreatic β-cells through the Glut-2 transporter and causes aklylation of the DNA. Activation of PARP leads to NAD+ depletion, cellular ATP decreased and inhibition of insulin production [1] [2]. Macrophages are the first cells to infiltrate the pancreatic β-cells, and cytokine production affects the development of diabetes [1].

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to relative or absolute lack of insulin [1]. Glucose and lipid metabolism are linked to each other in many ways [3]. Dyslipidemia is a metabolic disorder associated with diabetes characterized by quantitative and qualitative lipids and lipoproteins [4]. Pathophysiological mechanisms for diabetic dyslipidemia are
still unclear, insulin resistance and insulin deficiency are relatively related to lipid changes because insulin plays an important role in regulating lipid metabolism [4][5].

Diabetic dyslipidemia can be characterized by hypertriglyceridemia, decreased concentration of cholesterol-HDL (High-Density Lipoprotein) and an increase in low density low-density lipoprotein (LDL) [6]. Hypertriglyceridemia is a dominant lipid disorder due to insulin resistance and plays an important role in determining the lipid profile in diabetic dyslipidemia. Increased triglyceride levels occur due to increased production and decreased clearance of lipoproteins rich in triglycerides during fasting and non-fasting. Increased triglyceride levels can increase glyceride transporters so that they can increase VLDL levels [4].

The present study was designed to investigate the effects of single low doses streptozotocin (STZ) on serum triglyceride levels of rats.

2. Materials and Methods

2.1. Experimental Animals

Experimental with posttest only control group design was used in this study. A total of 12 Sprague dawley rats who visited LPPT 4 Gadjah Mada University at Yogyakarta was selected as research samples. Rats are kept in Biomedical Laboratories Faculty of Dentistry at University of Jember, and serum triglyceride levels was analyzed in Bioscience Laboratories Teeth and Mouth Hospitals at University of Jember. The inclusion criteria of this study were male rats, 3-4 months of age, body weight range 200-300 gram, and active motion. Exclusion criteria were dead at the time of the study. Rats were assigned to two experimental group (control group and diabetic group). Induction of diabetic rats was done according to the method described previously with slight modification diet. The control group received standard feed (Rat Bio), while the diabetic group received modified feed for 6 weeks and had free access water ad libitum. Control group (n=6) was given intraperitoneal injection with phosphat buffer saline (PBS), and diabetic group (n=6) was given intraperitoneal injection with single low dose STZ (30mg/kgBB) at the end of the sixth week.

2.2. Induction of Diabetes Mellitus

Diabetes was induced using streptozotocin (STZ), which was freshly prepared in 0.1 M phosphat buffer saline (PBS) with pH 4.5 after overnight fasting and were given 10% glucose solution to drink during the first 24 hours after STZ induction to overcome hypoglycemia.

2.3. Modified Feed

The control group received standard feed (Rat Bio) which contained (60% carbohydrate, 20% protein, 4% fat, 4% crude fiber, 12% calcium, and 0.7% phosphorus). The diabetic group received modified feed, with a composition of 50% Rat Bio, 25% Wheat Flour, 2% Cholesterol, and 5% pork oil.

2.4. Determination of Body Weight

Body weight of rats was determined using a digital scale and was monitored every week.

2.5. Determination of Serum Triglyceride Levels

Blood samples were collected in eppendorf tube from the orbital sinus of rats using microcapillary tubes. Serum triglyceride levels was analyzed using GPO-PAP methods after 3 days STZ-induced rats.

2.6. Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 22. Data are expressed as the means ± standard deviations (SD). Independent-T Test analysis was used to compare the differences among groups, p < 0.05 was considered statistically significant.
3. Results and Discussion
On the third day after STZ-induced, then blood glucose measurements using GPO-PAP method. Diabetic group had high blood glucose levels compared to the control group. Intraperitoneal induction of diabetes with single low dose STZ 30 mg/kg body weight is able to increase blood glucose of rats significantly. Low-dose STZ is used to make type 2 diabetes models [7]. Intraperitoneal injection of STZ can cause damage to pancreatic cells [8]. Oxidative damage to the pancreatic system due to STZ producing oxygen radicals in vivo [5]. STZ induction of pancreatic β cells causes a decrease in insulin levels in the blood and an increase in the concentration of glucose in the blood. Glucose oxidation is affected by STZ, reducing insulin biosynthesis and secretion. STZ enters pancreatic β cells through GLUT2 glucose transport causing decreased GLUT2 expression resulting in decreased sensitivity of peripheral insulin receptors, and causes increased insulin resistance and blood glucose levels [9]. Acute triphasic responses can be observed in rats after STZ injection, (a) initial hyperglycemia at 2–4 hours due to liver glycogen mobilization without an increase or with a concurrent decrease in serum insulin; (b) hypoglycemia can be observed 6-10 hours after injection due to an increase in serum insulin levels; and (c) permanent hyperglycemia from the next 24 hours, characterized by polyuria, glycosuria, hyperglycemia, and decreased pancreatic insulin levels. Metabolic changes occur within the first 2-8 weeks after STZ injection [2].

Serum triglyceride levels was analyzed using GPO-PAP after 3 days STZ-induced rats. Serum triglyceride levels in control group were highest at 108 mg/dl, whereas in the diabetes group 583 mg/dl (Table 1). Serum triglyceride levels in the control group obtained 56.33 ± 27.22 mg / dl, while the diabetes group 265.33 ± 189.35 mg / dl. Serum triglyceride levels was significantly differences between control group and diabetic group (p=0.042) (Table 2).

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<tr>
<th>Table 1. Serum Triglyceride Levels of Rats</th>
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<td>Control Group (mg/dl)</td>
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<th>Table 2. Serum Triglyceride Levels of Rats Different Analysis</th>
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<td>Group (mg/dl)</td>
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<td>Control</td>
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<td>Diabetic</td>
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Triglyceride levels remained elevated in diabetic group one week after induction STZ [10]. Oxidative stress is one of the processes of the pathogenesis of complications in diabetes [11]. Changes in insulin sensitivity pathways, increased concentration of free fatty acids and inflammation play a role in the mechanism of overproduction and decreased catabolism of triglyceride-rich lipoproteins from the origin of the intestine and liver. Increased triglyceride-rich lipoproteins are associated with decreased HDL and elevated LDL levels. Hypertriglyceridemia stimulates the enzymatic activity of cholesterol (CETP) transfer ester protein, and causes an increase in HDL and LDL triglyceride levels [4]. Lipid abnormalities in patients with type 2 diabetes plays a central role in the development of atherosclerosis [5].

4. Conclusion
Single low doses STZ-induced diabetic rats has an effect on increasing serum triglyceride level of rats.
5. Acknowledgments
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