

INFLUENCE OF ADMINISTERING WATERMELON RIND WATER EXTRACTS (*Citrullus vulgaris* Schard) ON GLUCOSE LEVEL OF MALE WHITE RATS (*Rattus norvegicus*) INDUCED WITH STREPTOZOTOSIN

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ABSTRACT

Watermelon rind water extract contain of citrulline. Citrulline is a precursor of generating Nitric Oxide (NO). NO possibly reduced the level of blood glucose in streptozotosin-induced diabetic rats. This study was a laboratory experimental study completely randomized design. A number of 30 male albino Wistar rats weighting 100 – 200 gr were divided into six groups (N=5), with group 1 served as normal control group. The other groups were given streptozotosin to induced diabetes mellitus. The rats in control diabetic group were given CMC 0,5%, the others were given watermelon rind extracts for 8 days with different doses for each group. The results showed that serum glucose levels in posttest control group was significantly different, from that group of the watermelon rind extract 250 mg/kg bw/day (p=0.000), groups of watermelon rind extract 500 mg/kgbw/day (p=0.000) and groups of watermelon rind extract 1000 mg/kg bw/day (p=0.000). The level of triglyceride in posttest control was significantly different from that group of the watermelon rind extract 250 mg/kg bw/day (p=0.000), groups of watermelon rind extract 500 mg/kgbw/day (p=0.000) and groups of watermelon rind extract 1000 mg/kgbw/day (p=0.000). The conclusion, the watermelon rind water extract can be used to reduced glucose serum level (250, 500, 1000 mg/kg bw) with the optimal dose was 500 mg /kg bw/day with the optimal dose was 1000 mg/kg bw/day.

Keywords: Watermelon rind; streptozotosin; diabetes mellitus; glucose

ABSTRAK

Ekstrak air kulit buah semangka mengandung citrulline. Citrulline adalah prekursor untuk menghasilkan Nitric Oxide (NO). NO kemungkinan menurunkan tingkat glukosa darah pada tikus diabetes yang diinduksi streptozotosin. Penelitian ini merupakan penelitian eksperimental laboratorium dengan rancangan acak lengkap. Sebanyak 30 ekor tikus Wistar albino jantan dengan bobot 100-200 gr dibagi menjadi enam kelompok (N=5), dengan kelompok 1 sebagai kelompok kontrol normal. Kelompok lain diberi streptozotosin untuk menginduksi diabetes mellitus. Tikus kelompok diabetes kontrol diberi CMC 0,5%, tikus lainnya diberi ekstrak kulit buah semangka selama 8 hari dengan dosis yang berbeda untuk tiap kelompok. Hasil penelitian menunjukkan bahwa kadar glukosa serum pada kelompok kontrol posttest berbeda nyata, yaitu kelompok ekstrak kulit buah semangka 250 mg/kg bb/hari (p=0,000), kelompok ekstrak kulit buah semangka 500 mg/kgbb/hari (p=0,000) dan kelompok ekstrak kulit buah semangka 1000 mg/kgbw/hari (p=0,000). Kadar trigliserida pada posttest control berbeda nyata dengan kelompok ekstrak kulit buah semangka 250 mg/kg bb/hari (p=0,000), kelompok ekstrak kulit buah semangka 500 mg/kgbb/hari (p=0,000) dan kelompok ekstrak kulit buah semangka ekstrak kulit buah semangka 1000 mg/kgbb/hari (p=0,000). Kesimpulan ekstrak air kulit buah semangka dapat digunakan untuk menurunkan kadar glukosa serum (250, 500, 1000mg/kg bb) dengan dosis optimal 500 mg/kg bb/hari dengan dosis optimal 1000 mg/kg bb/hari.

Kata kunci: Kulit buah semangka; streptozotosin; diabetes mellitus; glukosa

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INTRODUCTION

On 2025, Diabetes Mellitus is predictically reached 350 million that is a metabolic disorder of blood glucose which isn't commonly used, it caused hyperglycemia (Dods 1996, Sacks 2001). It caused a complication,

microvascular (retinopati, nefropati, neuropati) macrovascular (heart ischemia, stroke, atherosclerosis) and both complicitaion (Payne 2002). Hyperglycemia in especially Type 2 DM (Non Insulin Dependent Diabetes Mellitus (NIDDM) that was 95%-98% of diabetics was caused of the ability decrease of insulin in pheriperal

tissue (insulin resistance) and beta cells dysfunction that caused the disability of pancreas to produce a sufficient insulin that replaced insulin resistance which was consequently followed by insulin deficiency (Sacks, 2001).

Insulin decreased blood glucose level by increasing glucose uptake of muscles and adipose tissue, increased glucose oxidation and glycogen synthesis. Insulin also inhibited adipose tissue lipolysis, glycogenolysis and gluconeogenesis in the liver and increased lipogenesis (Jakus, 2000).

Lipogenesis enhancement occurred due to excessive release of adipose tissue (lipolysis) and uptake reduction of skeletal muscle so that more free fatty acid entered the liver. The liver response would increase the synthesis of triglycerides, VLDL and cholesterol ester synthesis (Murray et al., 2003) which are subsequently secreted into the circulation. DM management covered : dietary habit management, regular physical exercise and the use of pharmacological therapy, by optimizing the use of herbal ingredients (Subroto, 2006) that was a watermelon rind (*Citrullus vulgaris* Schard) which contained active compound of citrulline 24,4 mg/g dry weight (Rimando & Perkins-Veazie 2005). Citrulline played a role as formation precursors of NO, which NO directly involved in an insulin secretion regulation with causing membrane depolarization and increasing the concentration of Ca^{2+} intracellular. NO in the cell would caused a retention of K^{+} intracellular which generated membrane depolarization. Subsequently it opened Ca^{2+} channel, so that Ca^{2+} influx increased and then an insulin secretion. Furthermore, it stimulated glycolysis in hepar. As a result, it decreased blood glucose level (Laffranchi, 2002) by increasing the bond of insulin and insulin receptor. Therefore it caused translocation of GLUT 4 on the surface of membrane cell that optimally generated a carriage of glucose. (Murray et al., 2003). In order to prove Anti-diabetic effect of watermelon rind, it would conduct an experimental research by using diabetic mice which given a streptozotocin injection with a single dosage of 50 mg/kgBW.

MATERIALS AND METHODS

Laboratory experimental research method used Completely Randomized Design. A research sample used a healthy male white rats (*Rattus norvegicus*) averaging 1 – 2 months old with 100 – 200 gram weight. The sample used 30 streptozotocin-induced mice (STZ, 2-deoksi-2-(3-(methyl-3-nitrosoureido)-D-glucopyranosa) from MP Biomedicals, LLC with catalog number of 100557, then made as DM2. The

independent variable is giving watermelon rind water extract of red seedless watermelon. The dependent variable is glucose serum level measured by enzymatic method (GOD – PAP) in mg/dl. The dosage of giving Watermelon rind water extract is a control group without watermelon extract which only given water, the treatment group with a dosage of 250 mg/kgbw/day, 500 mg/kgbw/day and 1000 mg/kgbw/day.

The analytical data performed to know the differences of lowering blood glucose level which given watermelon rind extract between groups is one-way analysis of variance (ANOVA) (with assumption of homogenous data and normal distribution) with a margin of error 5%. If there is a meaningful difference, Least Significant Difference (LSD) is used due to know the difference between treatments.

RESULTS

LSD test was performed to observe the blood glucose level difference between groups. The difference described that there were meaningful differences ($p < 0.05$) for the glucose level between positive control groups and watermelon rind extract groups with dosage 250 mg/kg bw/day ($p = 0.000$), 500 mg/kg bw/day ($p = 0.000$) and 1000 mg/kg bw ($p = 0.000$).

Table 2 shows that there are meaningful differences between the extract groups of 250 mg/kg bw and the extract groups of 500 mg/kg bw ($p = 0.002$) and also the extract groups of 1000 mg/kg bw ($p = 0.003$). It also shown that there are meaningful differences between groups. The extract group of 500 mg/kg bw is not meaningfully different from the extract group of 1000 mg/kg bw ($p = 0.872$).

DISCUSSION

The influence of streptozotocin injection

In this research, the injection of STZ 50 mg/kg bb to an experimental animal with intraperitoneal single dosage, in all groups which were experienced DM, the blood glucose level was increased to over 250 mg/dl. It was meaningfully higher than the groups was not injected STZ and followed by the enhancement of triglyceride level. It was based on the research conducted by Cattopadhyay and Bandyopadhyay, (2005) and Joeliantina, (2008) on a research which used material of herbs as anti-diabetic. The dosage of STZ used to induce DM was a single dosage of 50 mg/kg bb and to show the result of hyperglycemia on the third day and the seventh day. By using this dosage, it assumed that

there was DM type 2, because the diabetic control groups could stay live until the third day and the seventh day. By this dosage, it was suspected DM Type 2, because diabetic control groups could stay live until the fiftenth day and the sulfonylurea treated groups could respond well, although without administering insulin (Cattopadhyay & Bandyopadhyay, 2005). Administering STZ caused the changes of DNA pancreatic β cell that there was a DNA fragmentation through DNA alkylation (Szkudelski, 2001). STZ also produced ROS that caused DNA fragmentation and cell damage. The formation of superoxide anions results from STZ action in mitochondria and an enhancement the activity of xantin oksidase. It shown that it was obstacles of STZ on the Krebs Cycles. Therefore, it decreased the consumption of mitochondria oxygen. This effect strongly limited the production of ATP Mitochondria and reduced nucleotida in pancreatic β cell (Szkudelski, 2001).

The synergistic action of NO and ROS also played a role in an occurrence of DNA fragmentation. NO and ROS could form peroxinitrite that could cause DNA damages. DNA damages after administering STZ would activate poly ADP ribosylation. This process decreased

NAD cellular and ATP. Moreover it inhibited insulin synthesis and secretion (Szkudelski, 2001). Therefore, STZ was also NO donor as well as watermelon rind. However, NO that resulted from STZ was followed by the formation of high ROS which caused the damage of DNA pancreatic β cell. NO from watermelon rind played a role as a mediator that functions to increase insulin secretion and GLUT 4 translocation to the plasma membrane.

The role of watermelon skin extract in reducing serum glucose levels

The result of variance analysis test between groups on the serum glucose level showed a meaningful difference result $p=0,000$. LSD Test also showed that the positive control groups were meaningfully difference to watermelon rind extract groups with dosage 250 mg/kg bw/day, 500 mg/kg bw/day and 1000 mg/kg bw/day and the dosage was also meaningfully difference. The result of test showed that administering watermelon rind extract influenced the degression of serum glucose level. It was because watermelon rind contains citrulline.

Table 1. Difference of blood glucose level with $p<0.05$ between positive control groups

Dependent Variable	F _{count}	Sig.
Glucose Level	47.474	0.000*

Table 2. Differences between the extract groups

Dependent Variable	(I) Group	(J) Group	Average Difference (I -J)	Standard Error	Sig.
Glucose	Positive Control (K 3) N = 5	Extract 250 mg	301.80*	27.990	0.000
		Extract 500 mg	403.60*	27.990	0.000
		Extract 1000 mg	399.00*	27.990	0.000
	Extract 250 mg (K 4) N = 5	Positive Control	-301.80*	27.990	0.000
		Extract 500 mg	101.80*	27.990	0.002
		Extract 1000 mg	97.20*	27.990	0.003
Extract 500 mg (K 5) N = 5	Positive Control	-403.60*	27.990	0.000	
	Extract 250 mg	-101.80*	27.990	0.002	
	Extract 1000 mg	-4.60	27.990	0.872	
Extract 1000 mg (K 6) N= 5	Positive Control	-399.00*	27.990	0.000	
	Extract 250 mg	-97.20*	27.990	0.003	
	Extract 500 mg	4.60	27.990	0.872	

Citrulline functions as a precursor to NO formation, where NO was directly involved in the regulation of insulin secretion by causing membrane depolarization and the enhancement of intracellular Ca²⁺ concentration. NO, which was inside the cell, would cause retention of intracellular K⁺ which caused membrane depolarization, then opened Ca²⁺ channel so that Ca²⁺ influx increased, insulin secretion occurred. Furthermore it stimulated glycogenesis in the liver thereby reducing blood glucose levels. (Laffranchi 2002).

Administering watermelon skin extract dose of 250 mg/kg bw/day was significantly different from the extract group 500 mg/kg bw/day and group 1000 mg/kg/day. This is likely due to the mechanism of increasing insulin secretion by NO. The more NO that was produced from the watermelon rind, the higher the insulin produced and ultimately would reduce serum glucose levels. In this case the work of NO in accordance with the work of oral anti-diabetic drugs in the sulfonylurea class is to increase insulin secretion (Suparman.2003). A dose of 500 mg of watermelon rind water extract did not differ from a dose of 1000 mg, meaning that the administration of 500 mg had the same effect as administering of 1000 mg. This was likely due to the role of NO which caused GLUT 4 translocation on the surface of cell membranes.

Intracellular GLUT 4 translocation stimulated by NO actually started from insulin binding to receptors in the extracellular part. This bond stimulated the formation of several phosphorylation reactions that were very important for insulin work. Auto-phosphorylation of the tyrosine group from the receptor protein stimulated by insulin would strengthen the work of the tyrosine kinase enzyme, which then phosphorylates some intracellular proteins including IRS-1. Phosphorylation of IRS-1, results in secondary signals that connect the insulin receptor to trans-membrane glucose transport. Furthermore, phosphoinositol-3 kinase activation was needed for stimulation of glucose transport by insulin and was needed to induce GLUT 4 translocation to the plasma membrane (Larsen et al, 2003). At a dose of 500 mg, NO had been maximal in increasing the sensitivity of insulin receptor through GLUT 4 translocation to the plasma membrane, which caused signal transduction. In this case, NO mechanism of action was similar to oral antidiabetic drugs grouped in thiazolidinediones (TZDs) or glitazones as insulin sensitizers. The mechanism was to increase glucose uptake in peripheral tissues and decrease liver glucose production (Dagogo et al 1997). To know exactly the mechanism of action of NO as a compound that could reduce blood glucose levels, further research could be carried out by examining HOMA B and HOMA R in experimental animals made with DM.

CONCLUSION

Watermelon water rind extracts of 250 mg/kg bw/ day, 500 mg/kg bw/day, and 1000 mg/kg bw/day could reduce serum glucose levels in male white rats injected with a single dose of streptozotocin 50 mg/kg given intraperitoneally with the optimal dose of watermelon rind water extract dose of 500 mg/kg bw/day.

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