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 contains 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; streptozotocin; diabetes mellitus; glucose

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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 (retinopathy, nephropathy, neuropathy) macrovascular (heart ischemia, stroke, atherosclerosis) and both compilctaion (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 pheripheral...
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 lipopysis, 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 regulator with causing membrane depolarization and increasing the concentration of Ca2+ intracellular. NO in the cell would cause a retension of K+ intracellular which generated membrane depolarization. Subsequently it opened Ca2+ channel, so that Ca2+ influx increased and then an insulin secretion. Furthermore, it stimulated glyco- genesis 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 differences 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 showed 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 experimented DM, the blood glucose level was increased to over 250 mg/dl. It was meaningfully higher than the groups that was not injected STZ and followed by the enhancement of triglyceride level. It was based on the research conducted by Cattopalidhyay and Bandypadhyay, (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 the diabetic control groups could stay live until the tenth day and the sulfonylurea treated groups could respond well, although without administering insulin (Cattopadhyay & Bandyopadhyay, 2005). Administering STZ caused the canges of DNA pancreatic β cell that there was a DNA fragmentation through DNA alkylation (Szkudelski, 2001). STZ also produced ROS that cused DNA fragmentation and cell demage. 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 Kreb Cycles. Therefore, it decreased the consumption of mitochondria oxygen. This effect strongly limited the production of ATP Mitochondra and reduced nucleotida in pancreatic β cell (Szkudelski, 2001).

The synergistic action of NO and ROS also played a role in an occurance 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 laso NO donor as well as watermelon rind. However, NO that resulted from STZ was followed by the formation of high ROS which caused the demage 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 | Fcount | 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.  |
|--------------------|-----------|-----------|--------------------------|----------------|-------|
| Positive Control   | Extract 250 mg | Extract 500 mg | 301.80*                  | 27.990         | 0.000 |
| (K 3) N = 5        | Extract 1000 mg | Positive Control | 399.00*                  | 27.990         | 0.000 |
| Extract 250 mg     | Extract 500 mg | Extract 1000 mg | -301.80*                 | 27.990         | 0.000 |
| (K 4) N = 5        | Positive Control | 101.80*                | 27.990         | 0.002 |
| Extract 500 mg     | Extract 1000 mg | 97.20*                | 27.990         | 0.003 |
| (K 5) N = 5        | Positive Control | -403.60*               | 27.990         | 0.000 |
| Extract 1000 mg    | Extract 250 mg | -101.80*               | 27.990         | 0.002 |
| (K 6) N = 5        | Extract 1000 mg | -4.60                  | 27.990         | 0.872 |

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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 bw/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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