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The Effect of Giving Alkaline pH Water Combine with Physical Exercise Training on Blood Glucose Level and Glut 4 Quantity in Muscle Cell Membrane of Rat (*Rattus Norvegicus*) Diabetes Mellitus Model

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Abstract
Background: *Metabolic system disorders experienced by DM patients can lead to unmet energy needs so that people easily feel hungry, thirsty, and tired untilbecome the complication causesof other diseases. DM management consists of 5 main pillars, including nutrition fulfillment, physical exercise, medication, education, blood glucose monitoring. Utilize a combination of nutritional fulfillment and physical exercise can optimize the stability of blood glucose levels.* ***Methods:*** *The study design used the randomized post-test only control group design with the number of samples using the Federer formula, 30 male white Wistar strain male rat, divided into five groups. The first group as negative control group, second group as positive control (induce STZ 50mg / kgBB), third group was given alkaline water 20 ml / day, fourth group was done swimming exercise 4 times a week with duration 30 minutes, fifth group was given the combination of two treatments, Where the treatment was carried out for 28 days. Data analysis using MANOVA test.* ***Results:*** *The results of this study were, there was effect of giving alkaline pH water and physical swimming exercise on blood glucose and GLUT 4 with p <0.0001.* ***Discussion:*** *This study shows that combination therapy can reduce fasting blood glucose levels and 2 hours of PP blood glucose level to normal limits. Provision of a single alkaline water therapy, and physical exercise can lower fasting blood glucose levels to normal levels, but have not been able to reduce 2 hours PP blood glucose levels to normal limits. The number of GLUT 4 has not shown any changes to the therapy.*

Keywords: *Alkaline water, Swimming excercise, GLUT 4, Blood glucose level*

I. INTRODUCTION

Metabolic disorders experienced by patients with DM (Diabetes mellitus) can lead to unmet energy needs so that people easily feel hungry, thirsty, and tired (Tierney et al., 2000). Some of these clinical manifestations need to be handled because they can reduce the productivity level of the patient and can lead to other complications, both acute and chronic (Soewondo, 2007). Blood sugar control drugs for DM sufferers continue to be developed but in fact, these drugs are not always adequate and there are a variety of side effects, so it needs a more effective and relatively safe alternative (Ignatio et al., 2013).

Patients with chronic disease DM in Indonesia ranks fifth as the deadly disease in the world in 2014 with the total population of Indonesia affected by DM reached 9.1 million people and 53% of patients in Indonesia did not realize that he was exposed to DM. International Diabetic Federation in 2007, estimates that the total population of Indonesian aged 20 and over suffered DM as much as 5.6 million people in 2001, and that number will increase to 8.2 million by 2020.

Diabetes mellitus as a metabolic disease with characteristics of hyperglycemia occurs due to insulin secretion abnormalities, insulin working resistance, or both. Insulin resistance in patients with DM is marked by a decrease in the GLUT 4 translocation process (Glucose transporters 4) to make insulin ineffective to stimulate tissues glucose uptake. Lack of insulin secretion also triggers little or no insulin bonding with the receptor so that the process of translocation of glucose transporter (GLUT 4) to the cell membrane is impaired (Smeltzer and Bare, 2008; Ganong, 2008).

GLUT 4 trans-location can be triggered by high-hydrogen compounds, alkaline water is high-hydrogen water capable of increasing insulin receptor phosphorylation and triggers GLUT 4 trans-location to cell membranes for glucose absorption (Shirahata et al., 2012). Expression of GLUT 4 also increases in physical exercise conditions. A body if given regular physical exercise will lower the glucose amount in the blood (Harahap et al, 2015). Muscle contractions routinely increase energy consumption resulting in decreased ATP (Adenosine Triphosphate), this causes metabolic changes including stimulating GLUT 4 trans-location to the cell membrane surface then carrying glucose into the cell and decreasing blood glucose level (Sigal, 2004; Rose, 2005).

DM management consists of 5 main pillars including nutrition fulfillment, physical exercise, medication, education, and blood glucose monitoring (Smeltzer& Bare, 2008). The contents of nutrients fulfillment can be food and beverage. One of the new breakthroughs are still exposed minimalywhich is use alkaline water. Water is chosen as a relatively safe and effective alternative because the human body contains 60-80% of water that has wide benefits, both at the molecular level and its constituent atoms. When water passes through the blood-brain barriers, water does not experience obstacle and almost no side effects (Lee et al., 2012). Ordinary water can be converted into alkaline water by processed through electrolysis process (Watanabe, 1995).

Based on the five pillars, DM patients tend to consume hyperglycemic drugs as a solution to declining blood glucose levels without or with attention to other pillars but in fact drugs are not always adequate, then the management of DM should remain enforced through notherpillars, for example through a combination of nutrients and physical exercise. The combination can be applied with alkaline water administration and physical exercise to measure the amount of GLUT 4 on the adipose cell membrane and blood glucose levels which in this study will be subjected to rats (Rattusnorvegicus) as a DM type-2 model. The combination of nutrition and physical exercise is expected to strengthen the basis for the development of research related to subsequent modification of water administration, especially for human consumption.

II. METHODS

This research was a pure experimental research, the design used was the randomized post-test only control group design. The research subjects were wistarstrain (*Rattus norvegicus*) male rats previously acclimated, with a weight range between 140-160 grams. Treatment division consists of 5 groups, where the division is using simple random sampling technique. There were 7 groups, each group in which one group was negative control group (non-DM), the second group were positive control group (DM) with 50 mg/kgBW STZ induction as DM type-2 model. The third group were given alkaline water treatment of 20 ml/day orally, the fourth group that was given swimming treatment 4 times a week with duration of 30 minutes, while fifth group was giventhe combination of both treatment. The treatment was done for 28 days.

The provision of alkaline water in this study came from ordinary mineral water that flows into the Kangen Water machine to produce alkaline water with a pH of 9.5 and high alkalinity. Ordinary mineral water was given to the control group. Provision of drinkingwas done by oral using nipple drinker 20 ml/day, it is necessary to ensure that nipple drinker does not leak. The weightless pool treatment given using tubs of 50 cm in diameter and 1 m high, filled with PDAMwater (Government Water Company) as high as ± 75cm and temperature ± 28-29^{0}C.

Blood sampling to measure glucose levels was taken in the veins of the tail by using medical scissors and examined on Glucotest. Intake of gastrocnemius muscle tissue also using medical scissors, the examination using immunohistochemical method using antitatantocyte antibody GLUT 4.

Preparation phase:

1. Wistar adult male white rats that meet the age criteria 2-3 months, healthy, and weight 140-160 grams prepared as many as 35 rats. Weighing using digital scales.
2. Division of rats into 5 groups consist of 7 rats each.
3. Rat placed in a cage made of plastic material covered by wire, in size of 20 x 30 cm as many as 35 cages, so that one cage containing one rat. Each cage is equipped with husk as a base for eat and drink. The white rats cage environment preserved in the natural temperature range (27-27,5^{0}C). The rooms exposure for 12 hours bright and 12 hours dark. Foodwas a regular rat food (*pellets*) and PDAM water (Government Water Company) is supplied daily. Nipple drinker as a drinking media per oral confirmed does not leak.
4. Acclimated for 7 days to adapted with rom the environtment.
5. After 7 days of the acclimation period ended, the rats were fasted for 6 hours to performed STZ 50mg/kgBW induction in the positive control group and the treatment group. PZ induction is given to the negative control group.
6. After 3 days post-induction, the rat was re-fasted for 6 hours to measure fasting blood glucose levels extracted from the tail veins to ensure that the rat had hyperglycemia.
7. Ordinary mineral water is electrolysed by using Kangen Water machine to transformed into alkaline water with processing method once every week to reduce the chance of damage to the water content.

Implementation phase:

1. Provision of alkaline water treatment given orally as much as 20 ml/day using nipple drinker. Water is measured using 50 cc syringes.
2. Physical exercise training done for 4 weeks, the exercise done 4 times in a week, with a duration of 30 minutes (10' swim - 5' breaks - 10' swim– 5’ break - 10' swim). Implementation of swimming done every 08.00 am on Monday, Wednesday, Friday, Sunday. Ninety minutes before swimming, rats were fed first.

3. After the 28th day, the mouse is fasted for 6 hours to prepare for fasting blood glucose sampling. Rat were fed after take the fasting blood glucose sampling. Two hours later a blood glucose measurement measured 2 hours PP on the veins of the vein of the tail.
4. Intake of GLUT 4 muscle tissue performed after sampling the blood glucose 2 hours PP. Rats were sacrificed by using ketamine, after death then gastrocnemius muscle tissue was taken and immersed in 10% formalin for at least 1x24 hours, then examined the amount of GLUT 4 by immunohistochemical method using GLUT 4 antirathic monoclonal antibody.

Data collection using blood glucose measurement sheet and GLUT 4 amount. The measurement result is numerical data so that it refers to Nugroho (2014), at the first stage the data is analyzed descriptively as mean and standard deviation. In the next stage the data normality test using Kolmogorov-Smirnov test, and continued with Manova test.

III. RESULTS

Table 1. Mean and standard deviation of fasting blood glucose level

Group	***Mean* ± SD**
K1 (negative control)	95,83±12,70
K2 (positive control)	521,67±11,61
K3 (alkaline water)	96±12,38
K4 (swim exercise)	100±12,39
K5 (combination)	67±12,73
p value	0,000

Table 1 shows that the highest fasting blood glucose level was positive control group, namely group of DMs without treatment, i.e. 521,67 ± 11,61 while the lowest mean of fasting blood glucose level was in group 5 that is group of DM rats with combination treatment i.e. 67 ± 12.73.

The result of LSD test can be seen that the value of fasting blood glucose level on K1 has significant difference with K2 (p = 0,000) and K5 (p = 0,000), but has no significant difference with K3 (p = 0,982) and K4 (p = 0,565). K2 has a significant difference with K3, K4 and K5 (p = 0,000). K3 has a significant difference with K5 (p = 0,000) but with K4 (p = 0,580). K4 has a difference with K5 (p = 0,000).

Table 2. Mean and standard deviation values of 2 hours PP blood glucose levels

Group	***Mean* ± SD**
K1 (negative control)	113,33±9,44
K2 (positive control)	592,33±14,14
K3 (alkaline water)	329,5±8,36
K4 (swim exercise)	585±10,49
K5 (combination)	99,67±11,84
p value	0,000

Table 2 shows that the highest mean of 2 hours PP blood glucose level was positive control group, namely group of DMs without treatment i.e. 592,33 ± 14,14 while the lowest mean of 2 hours PP blood glucose level was found in group 5 that is group of DM rats with combination treatment that is 99,67 ± 11,84.

Based on table 2, p-value = 0,000 is obtained, so there are at least a pair of groups that have a difference average of 2 hours PP blood glucose level. LSD test results showed that 2 hours PP blood glucose levels on K1 had significant differences with K2 (p = 0,000), K3 (p = 0,000), K4 (p = 0,000), and K5 (p = 0.042). K2 has a significant difference with K3 and K5 (p = 0,000) but has no significant difference with K4 (p = 0.261). K3 has a significant difference with K4 and K5 (p = 0,000). K4 has a difference with K5 (p = 0,000).

Table 3. Mean and standard deviation values of GLUT 4 variables

Group	*Mean* ± SD
K1 (negative control)	25,72±0,58
K2 (positive control)	0,28±0,34
K3 (alkaline water)	0,28±0,33
K4 (swim exercise)	0,21±0,23
K5 (combination)	0,41±0,37
p value	0,000

Table 3 shows that the highest average of GLUT 4 was in negative control group, i.e. the group of normal rat without treatment that is equal to 25,72 ± 0,58 whereas the lowest mean of GLUT 4 was in group 4 which is group of DM rats with physical swimming exercise treatment i.e. 0.21 ± 0.23.

Based on table 3, p-value = 0,000 was obtained, so there are at least a pair of groups that have a difference in the average number of GLUT 4. LSD test results can be seen that the value of GLUT 4 in K1 has a significant difference with K2, K3, K4, and K5 (p = 0,000). K2 has no significant difference with K3 (p = 0,994), K4 (p = 0,768) and K5 (p = 0,542). K3 had no significant difference with K4 (p = 0,763) and K5 (p = 0,547). K4 also has no difference with K5 (p = 0.368).

IV. DISCUSSION

A. The Effect of Alkaline Water Administration on Blood Glucose Level and GLUT 4 Amount

The results obtained from this study indicate that alkaline water treatment can reduce fasting blood glucose and 2 hours PP blood glucose levels than the DM positive control group. Decreased fasting blood glucose levels in the treatment group had reached the normal blood glucose limit, but 2 hours PP blood glucose levels only decreased and has not reached the normal blood glucose limit. The number of GLUT 4 in the treatment group had not shown a change in the result of GLUT 4 trans-location to the cell membrane, but only seen the features of the more absorbing cytoplasmic resemblethe color of negative control group.

The result of this research is supported by Amitani et al. (2013) that hydrogen can help controling glucose in the blood of diabetic rats by increasing glucose uptake by muscle cells. Research conducted by Jin et al. (2006) about decreased blood glucose levels of rat OLETF model after treated alkaline water for 12 weeks. Shirahata et al. (2012) states that alkaline water containing many hydrogen molecules that can stimulate cell glucose uptake.

The results of this study can be explained that the administration of alkaline water with 9.5 pHcan give a different effect of hypoglycemia between fasting blood glucose and 2 hours PP blood glucose level. This can be due to the expression of GLUT 4 in the muscle cell membrane has not been fully expressed after getting food stimulation. The number of GLUT 4 in the muscle cell membrane of the treatment group was not as much as in the negative control group, but the cytoplasm was almost similar to the negative control group, this could be triggered because the trans-location process was not perfected, yet the process of synthesis of GLUT 4 in the cytoplasm had begun to improve.

B. The Effect of Physical Swimming Exercise on Blood Glucose Level and Number of GLUT 4

Treatment of physical swimming exercise with a dose that has been determined in this study can reduce fasting blood glucose levels to reach the normal limit in rat after intervention for 28 days, but has not been able to reduce the 2 hours PP blood glucose levels. The number of GLUT 4 in the treatment group had not shown any changes in the result of GLUT 4 trans-location toward the cell membrane, the cytoplasmic appearance also looked pale in the positive control group.

Muscle contractions routinely and continuously increase energy consumption resulting a declining in ATP to be broken down into cAMP by the AMPK enzyme, causing metabolic changes including glucose transport (Sigal, 2004). A collaborative recommendation from ACSM (American College of Sports Medicine) and the ADA (American Diabetes Association) in 2010 showed that physical exercise therapy in DM patients should be done by combining moderate intensity aerobic exercise and strengthening exercises. Aerobic exercise is done with moderate intensity of at least 150 minutes (2 hours and 30 minutes) a week, one way to approach this is to do 30 minutes at least five days a week. Reinforcement exercises by moving the leg muscles, hips, back, chest, abdomen, shoulders and arms using weights and performed at least 1 set of 8-12 repetitions per activity. Aerobic activity alone can not provide the full benefits of exercise to individual diabetes (Colberg *et al.*, 2010).

The results of this study indicate that the treatment of physical swimming exercise with the frequency, intensity, time, and type that have been planned can give the effect of hypoglycemia by the measurement of fasting blood glucose (without food stimuli) according to normal limits. Measurement of 2 hours PP blood glucose level (food stimulated) in the physical exercise group has not shown good effect of hypoglycemia, it can be cause by the amount of glucose in the blood can not get into the cell because the number of exposed GLUT 4 in the cell membrane is also not optimal. The recommendation from ACSM shows that this 30 minutes duration of physical swimming exercise therapy is an aerobic exercise, so it needsto add little bit of weights tied to the rat tail to maximize the strengthening exercises.

C. The Effect of Alkaline pH Water and Swimming Exercise on Blood Glucose Level and GLUT 4 Amount

The results obtained from this study indicate that the combination treatment can reduce fasting blood glucose and 2 hours PP blood glucose levels to reach the normal limit. The number of GLUT 4 in the treatment group had not show a change in the resultof GLUT 4 trans-location toward cell membrane, only a small percentage increase when compared to the positive control group. In the description of GLUT 4 expression, cytoplasmic color is not as pale as positive control group.

Alkaline water increase insulin receptor phosphorylation through suppression of phosphatase activity of tyrosine protein which is a redox-sensitive protein and activates PI3 kinase and Akt or otherwise known as protein kinase B (Shirahata et al., 2012). Protein kinase B and PI3 bind and undergo phosphorylation, then bind to TBC1D1 and activate RabGDP to release GLUT 4 (translocated) resulting in increased glucose uptake (Campbell et al., 2002). Muscle contraction in physical exercise may transmit signals to the endoplasmic reticulum to increase the concentration of calcium that activates adenylate cyclase triggering ATP changes to cAMP, cAMP activates PKA, then binds to TBC1D1 to increase glucose uptake (Campbell et al., 2002). Insulin sensitivity will increase especially when physical exercise is done one and a half hours after meals (Smeltzer and Bare, 2008). Consumption of 5-30 g of carbohydrates during and within 30 minutes after complete glycogen clearance exercises will reduce the risk of hypoglycemia and enable more efficient muscles glycogen restoration (Colberg *et al.*, 2010).

Combined treatment may have a hypoglycemic effect on fasting blood glucose (without food stimulation) and 2 hours PP blood glucose (food stimulated) with a range of values within normal limits in the negative control group. The number of GLUT 4 exposed in the cell membrane was not maximal yet showed a slight increase in the combination group compared with the positive control group. The number of GLUT 4 will increase if researchers apply recommendations from ACSM associated with aerobic exercise and strengthening.

The single dose of physical swimming exercise administered in this study could not determine the effectiveness of the MWC (maximum work capacity) in each animal, so the effects of fatigue that resulted in acute hypoglycemic when swimming in each of the various rat could not be monitored. In the combination group, 3 rat experienced death while swimming, and a 2 hours PP blood glucose examination was immediately performed and the results from each obtained were 34 mg/dL, 24 mg/dL, and 28 mg/dL.

V. CONCLUSION AND SUGGESTION

A. Conclusion

Provision of alkaline pH water can reduce fasting blood glucose levels to normal limits and 2 hours PP blood glucose levels but have not reached the normal limit. The administer of alkaline pH water can not increase the number of GLUT 4 on DM model rat (Rattusnorvegicus). Physical swimming exercise can decline fasting blood glucose levels to normal levels, but can not decline2 hours PP blood glucose levels, and can not increase the number of GLUT 4 on DM model rat (Rattusnorvegicus). Provision of alkaline pH water and physical swimming exercise can reduce fasting blood glucose levels and 2 hours PPblood glucose levels to normal limits, but can not increase the number of GLUT 4 on DM model rat (Rattusnorvegicus).

B. Suggestion

Measurement of blood glucose level will be better if using spectrophotometer. Swimming interventions should be measured according to each rat MWC (Maximum Work Capacity), so there will be no fatigue to the effect of acute hypoglycemia during the swimming process. Further research is needed by combining moderate intensity aerobic exercise and strengthening exercises as a sports intervention.

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