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Blood Glucose Level Lowering Effect of Alkaline Reduced Water (ARW) in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Alkaline Reduced Water (ARW) is beneficial to prevent oxidative stress disease such as diabetes. The aim of the study is to determine the effect of ARW on reducing blood glucose level in diabetic rats induced streptozotocin. Fifteen male Wistar rats were divided into 3 groups. Group 1 (normal) nondiabetic rats, group 2 diabetic rats (negative control) received single intraperitoneal of streptozotocin (65mg/kg), group 3 was diabetic rats treated with ARW 20 mL/kg/day, orally for 28 days. Blood samples were collected through retro-orbital method before and after the experiment. Measured parameter were body weight and blood glucose concentration. Data were analyzed using one-way analysis of variance test followed by Dunnet multiple comparisons post hoc test. Administration of ARW for 28 days resulted in significant ($p < 0.05$) lowering of blood glucose. In conclusion, the results show that ARW can lower elevated blood glucose against streptozotocin-induced diabetes in rats.
Keywords: *ARW; body weight; blood glucose levels; streptozotocin-induced diabetic rats*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome with a characteristic feature of increasing blood glucose concentration, impaired metabolism of carbohydrates, proteins, and fats, which is associated with deficiency of insulin secretion (Widowati, 2010). Drinking water is not only for thirst release but also has physiological functions for the body. Water molecules contain of dissolved minerals and it affected the function of cells, organs and organ systems (Hirokawa *et al.,* 2006).

Alkaline Reduced Water (ARW) is an electrochemically reduced water, activated using electrolysis method (Bari *et al.,* 2003), rich in hydrogen, has negative oxidation-reduction potential to neutralize reactive oxygen molecules (Shirahata *et al.,* 1997; 2007; 2012) and prevent oxidative stress disease such as diabetes, cancer and neurodegenerative disease (Shirahata *et al.,* 2012). Shirahata (2002) stated that active hydrogen in water reduction as an ideal antioxidant because oxidized molecules is not form after the reduction process, as in the organic antioxidant type like vitamin C, vitamin E, and polyphenols. Li *et al.,* (2002) reported that intracellular ATP levels and glucose-stimulated production of insulin were respectively increased 2-3.5 times and 2-4 times after the administration of reduced water. Previous study suggested that reduced water stimulate glucose uptake by muscle cells and adipocytes to prevent type 2 diabetes (Oda *et al.,* 1999). Therefore, this study aims to determine the effect of ARW on reducing blood glucose levels in streptozocin-induced diabetic rats.

METHODOLOGY

Materials

The ARW was prepared by using an electrolyzing device with following physical properties: pH 10.34 ± 0.51 with redox potential (ORP) value 170.60 ± 1.37 mV. The pH and ORP were measure ed using pH (HI98107, Hanna Instrument, USA) and ORP (Kedida CT-8022, China) meter.

Animals

Fifteen male rats of Wistar strain were used in this study weighing 150-200 gram, aged 2-3 month. Rats were maintained under controlled temperature (20-26°C), light/dark cycle, accessible for water, and food with sufficient stainless steel cage ventilation. The protocol was approved by the Animal Ethical

Committee Lembaga Penelitian dan Pengujian Terpadu (LPPT) UGM at number 00101/04/LPPT/X/2017, before the initiation of the study. All the animals were kept individually in the stainless steel cage for 14 days for acclimatization before the ARW administration. After 14 days, the animals were administrated with Streptozotocin (Nacalai Tesque, Kyoto, Japan) at a dose of 65 mg/kg intraperitoneally (i.p). After 72 hr, animals considered diabetic with blood glucose levels > 200 mg/dL and included in the study (Vijayakumar *et al.,* 2006).

Methods

Grouping animals

Fifteen streptozotocin-induced diabetic rats were divided into three groups, five rats each group. Group 1 (normal) nondiabetic rats, group 2 diabetic rats (negative control) received single intraperitoneal of streptozotocin (65mg/kg), group 3 was diabetic rats treated with ARW 20 mL/kg/day, orally for 28 days. Animals in group 2 and 3 were considered diabetic with blood glucose levels > 200 mg/dL after 72 hours administration of STZ.

Experimental period

Animals were treated for 28 days. Body weight were weighing every day with scales (Triple Beam, Ohaus, USA), results were expressed in gram. Blood samples were collected through retro-orbital, measured on 0, 14^{th} and 28^{th} days of experimental period by spectrophotometer using Diagnostic Systems Kit (DiaSys, Holzheim, Germany). The results were expressed in mg/dL.

Data Analysis

The values were as Mean±Standard Error of Mean. Data were analyzed with one-way analysis of variance test using the SPSS statistical program and followed by Dunnett's multiple comparisons *post-hoc* test. Differences were considered significant at $p< 0.05$.

RESULT AND DISCUSSION

Body weight

The body weight measurement before and after the study were shown in table I. The body weight of the negative control diabetic rats (Group-2) treated with single dose intraperitoneally streptozotocin was found 160.00 ± 6.08 g and it significantly ($p<0.05$) decreased compared with the normal control group (Group-1). In the streptozotocin + ARW treated group (Group-3) the body weight was significantly increased ($p<0.05$) to 189.20 ± 5.89 g. Intraperitoneal administration of STZ caused significant diabetogenic response such as reducing body weight. Weight loss were observed after administration of STZ, it represented in excess of urine and loss of water in the cell due to muscle breakdown possibility in hyperglycemic rats (Malekinejad *et al.,* 2012). The administration of ARW orally for 28 days has potential to prevent weight loss in diabetic rats and enhanced body weight as compared with the untreated diabetic rats.

Blood glucose

Administration of single dose STZ intraperitoneally caused significant increase of blood glucose levels as compared with normal rats (Figure 1). Result were shown in Table I. A single dose STZ (40-70 mg/kg) is commonly used to cause direct toxicity to pancreatic beta cells, rapidly resulting in diabetes with greater blood sugar levels (Marcel Dekker, 2001; Rossini and Like, 1976). The blood glucose level (mg/dL) in normal control rats (Group-1) was found 74.90 ± 1.66. The blood glucose concentration (mg/dL) of negative control diabetic rats (Group-2) after induced with single dose of STZ was increased significantly ($p<0.05$) to 248.83 ± 3.55. Administration of streptozotocin + ARW orally for 28 days significantly ($p<0.05$) decreasing the blood glucose concentration to 160.71±4.65 mg/dL.

Our findings revealed that administration of ARW at dose 20 mL/kg/day has potential effect of reducing blood glucose levels in STZ-induced diabetic rats. Previous study reported that ARW can accelerate the uptake of glucose by muscle cells and adipocytes and effective in preventing type 2 diabetes (Oda *et al.,* 1999). Another study suggested that Nordenau water and Hita Tenryosui Water reduce glucose tolerance and caused pancreatic damage in type 2 diabetes (Osada *et al.,* 2010). Study findings using alloxan models suggested that oxidative damage can be suppressed by ARW (Yupin Li *et al.,* 2011). Oxidative stress has been implicated in the development of many pathophysiological conditions including diabetes (Rentoukas *et al.,* 2012; Shishehbor and Hazen, 2004). Moreover, ARW contains active hydrogen atoms with high reduction capability to neutralize ROS and has

Table I. Effect of ARW in body weight and blood glucose of streptozotocin-induced diabetic rats

Groups	1	2	3
BW (day 0)	192.80 ± 7.59	177.40 ± 5.73[b]	179.40 ± 5.23[b]
BG (day 0)	72.06 ± 1.69	246.53 ± 3.03[a]	231.82 ± 10.03[a]
BW (day 28)	221.00 ± 9.06	160.00 ± 6.08[a]	189.20 ± 5.89[a]
BG (day 28)	74.90 ± 1.66	248.83 ± 3.55[a]	160.71 ± 4.65[a]

BW : body weight (g); BG: blood glucose (mg/dL), values represented are Mean ± S.E.M (n=5). Group 2 was compared with group 1, and group 3 was compared with group 2; *[a] p<.05; [b] p>.05*

an antioxidant potential in reducing blood glucose concentration (Kim *et al.,* 2007). Finally, the findings of this study show ARW intake for 28 days has potential to prevent body weight loss and reduce blood glucose concentration in streptozotocin-induced diabetic rats.

CONCLUSION

ARW has effected in increasing body weight and lowering elevated blood glucose levels against streptozotocin-induced diabetic rats.

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