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The Effect of Addition of Pridot Leaf Extract and Juice of Red Watermelon Albedo on Carbonated Coconut Drink of Blood Glucose Levels of Mice

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Abstract: In this study, the effect of addition of pirdot leaf extract and juice of red watermelon albedo on carbonated coconut drink of blood glucose levels was done by giving samples orally to mice. This study used 30 white male wistar mice, which were divided into 6 treatment groups that containing 5 mice in each group i.e: normal untreated mice given with aquades (negative control), diabetic untreated mice given with aquades (positive control), normal mice treated with 0.6 mL per 27 g bw per day of carbonated coconut drink, diabetic mice treated with 0.6 ml per 27 g bw per day of carbonated coconut drink, normal mice treated with 0.6 mL per 27 g bw per day of commercial soda, and diabetes mice treated with 0.6 mL per 27 g bw per day of commercial soda. The treatment was carried out for 14 days and observations made on blood glucose levels and body weight of mice. The results showed that giving of carbonated coconut drink by adding pirdot leaf extract and juice of red watermelon albedo had a highly significant effect on blood glucose levels of mice. The effect was hypoglycemic effect, which can reduce blood glucose levels on the diabetes group about 6,536%, while on the non-diabetic group about 3,316%. However, the giving of commercial soda provides a hyperglycemic effect that increased blood glucose levels about 19,787% on the diabetic group, while on the non-diabetic group about 12,750%. In addition, giving of carbonated coconut drink by adding pirdot leaf extract and juice of red watermelon albedo had a highly significant effect on the body weight of mice. The effect was weight gain on the diabetes group about 4,171%, while on non-diabetics about 2,564%. However, giving commercial soda had a weight loss effect about 33,750% on the diabetic group, while on the non-diabetic group about 6,884%.

Keywords : blood glucose levels; body weight; carbonated coconut drink; diabetes mellitus

1. INTRODUCTION

Carbonated drinks are a favorite soft drink, which tastes good and refreshing. However, carbonated drinks contain high sugars that is equal to 40-50 g [1]. Increased consumption of soft drinks in the world about 17% per year. This indirectly becomes a trigger factor for various metabolic disorders such as obesity, impaired glucose tolerance, and diabetes mellitus [2].

In 2015, Indonesia ranked seventh in the world as the highest diabetes sufferer, with an estimated number of 10 million. Among all types of diabetes, type 2 diabetes has the highest percentage of 90%-95% [3]. High Fructose Corn Syrup (HFCS) is a type of sweetener commonly used in soft drinks, because it has a fructose sweet taste that is higher than sucrose and glucose [4].

Indonesia is the largest HCFS producer in Southeast Asia with a productivity of 90 million kg per year [5]. Carbonated drinks also contain a mixture of phosphoric acid, sugar, caffeine, coloring, and flavor. Phosphoric acid functions to keep water sterile and prevent bacterial growth. However, added in large enough quantities, which can cause tooth brittleness in 2 days. The caffeine contained in it can also cause damage to gastric ulcers [6].

Seeing these problems, it is necessary to modify soft drinks by replacing synthetic ingredients such as sweeteners and preservatives with natural ingredients, which can prevent diabetes mellitus. These healthy natural ingredients are obtained by utilizing bioactive components, such as antioxidants that are contained in plants.

Green coconut water is known to be a herbal medicine for various diseases. This is because the tannin content is able to remove toxins from the body. Green coconut is distinguished from ordinary green coconut (*C. viridis*) and red fiber green coconut (*C. rubescens*) or wulung coconut [7]. Coconut water has a caloric content of 17.4 kcal, 95.5% water, < 0.1% fat, 0.1% protein, 4% carbohydrate, 5.6% total sugar, and 2.2-3.4 mg/100 mL of vitamin C [8]. The water extract of *P. nitida* seeds using coconut water provides a significant hypoglycemic effect on diabetic albino rats, because coconut water stimulates the active component of *P. nitida* seed extract, so that it stimulated glucose metabolism in cells [9].

This is what underlies this research, to make coconut water as a carbonated coconut drink. Carbonated coconut drink is made by adding a mixture of ingredients that contain bioactive components, such as antioxidants which have a hypoglycemic effect. Materials that contain bioactive components, can be found in plants. The use of plants as medicine has been traditionally applied by the local community. The local community usually utilizes local specialties of the region as traditional medicine, so that it becomes a local wisdom to the area.

The Karo community in North Sumatera has local plants which is used as medicine, namely pirdot plants. Karo people use boiled water from pirdot leaves to treat diabetes [10]. This is caused by the content of bioactive substances in it. Pirdot leaf water extract has antioxidant content with IC_{50} value about 22,9182 μg/mL, total flavonoids about 39,50 mg QE/g, and total phenols about 96,75 mg GAE/g [11].

This research also utilizes watermelon peel waste. Utilization of watermelon peel has not been optimally utilized, whereas watermelon peel has a white layer of flesh that contains citrulline. According to Amir and Borang (2015) the white layer of peel watermelon (albedo) is known as antidiabetic, because it contains citrulline which can reduce the blood glucose levels [12].

This study aims to determine the effect of the comparison of pirdot leaf extract and juice of red watermelon albedo on carbonated coconut drink of blood glucose levels of mice.

2. MATERIALS AND METHODS

2.1. Making of carbonated coconut drink

Wulung coconut shells peeled to obtain the water. Wulung coconut water is put into a stainless steel pan and heated at 80ºC for 10 minutes in a water bath, then cooled. Wulung coconut water (150 mL) was added with a mixture of pirdot leaf extract (90 mL) and juice of red watermelon albedo (10 mL) so that the final product volume is 250 mL (3:2), then adding 0.32% stevia sugar (0.8 g), 0.15% citric acid (0.375 g), and 0.05% sodium benzoate (0.125 g). The mixture is stirred until the material dissolves, and stored in the refrigerator for 1 hour at 10ºC, then injected carbon dioxide gas using a soda maker. Carbonated coconut drink is packed in a glass bottle with a seal. Before

packaging, the bottles used are washed and sterilized at 100ºC for 30 minutes, through the boiling process.

2.2. Animal experiment

Thirty (30) white male wistar mice, 2-3 months old, weighing 20-30 g (the average weight of mice in this study was 27 g were purchased from the animal house of the Department of Biology, Faculty of Math and Science, University of Sumatera Utara. They were housed in cages with different compartments and fed ad libitum with aquades and top feeds and were acclimatized for a 7 days under standard laboratory conditions of relative humidity, adequate ventilation and ambient room temperature.

2.3. Experimental design

The animals divided into six treatment groups containing five mice in each group, the treatment was carried out for 14 days, and the observations are measurements of blood sugar levels and body weight of mice.

K_1 : Normal untreated mice given with aquades (negative control)

K_2 : Diabetic untreated mice given aquades (positive control)

K_3 : Normal mice treated with 0.6 mL per 27 g bw per day of carbonated coconut drink

K_4 : Diabetic mice treated with 0.6 mL per 27 g bw per day of carbonated coconut drink

K_5 : Normal mice treated 0.6 mL per 27 g bw per day of commercial soda

K_6 : Diabetic mice treated with 0.6 mL per 27 g bw per day of commercial soda

2.4. Induction of hyperglycemia

Diabetic mice were induced experimentally by administration of streptozotocin (STZ) in a citrate buffer of pH 4,4. The animals were fasted for 16 hours, but allowed free access to water. A dose of 50 mg/kg body weight streptozotocin was administered by intraperitoneal injection while still fresh, the dose was found to be most optimum in inducing diabetes in a separate study.

Seventhy two hours (3 days) after injection, blood glucose was determined by use of glucose analyzer model (Autocheck) with glucometer strips. Mice with blood glucose levels above 200 mg/L, were considered diabetic and suitable for use in the study. If the blood glucose levels had not reach > 200 mg/dL, then the mice are re-injected with the same dose of streptozotocin.

2.5. Blood sampling and in vivo hypoglycemic asays

The tail of the mice was sterilized with 10% alcohol nibbed and a drop of blood squeezed into the glucometer strip. Blood glucose was determined by use of glucose analyzer model (Autocheck) with glucometer strips.

2.6. Data analysis

Data collected was entered in Microsoft Excel, claned and then analysed using Statistical Package of Social Sciences (SPSS). The results were expressed as Mean (x) ± standard deviation (SD) of the number of animals used in a group. Analysis was done using ANOVA and determination of the notation was obtained from the transformation of the square root. The significant level was consindered at $P < 0,01$.

3. RESULTS AND DISCUSSION

3.1. Blood sampling

Administration of aquades, carbonated coconut drink, and commercial soda for 14 days had a highly significant effect ($P < 0.01$) on the percentage change of blood glucose levels of mice. Measurement of glucose levels of mice (Table 1) was carried out on day-0, which was the diabetic mice group was induced by STZ 50 mg/kg, then on day-8 which was the beginning of the treatment because the STZ induced diabetes group had blood glucose levels above 200 mg/dL, then the blood glucose level of mice was measured on the day-22 which was where each group of mice had been given treatment for 14 days. The results of the measurement of average blood glucose level for each group for 22 days are presented in Table 1.

Table 1. Results of measurements of blood glucose levels of mice for 22 days

Group	Blood glucose levels (mg/dL)			Percentage change of blood glucose levels (%)
	Day-0	Day-8	After treatments	
			Day-22	
	x±SD	x±SD	x±SD	
K_1	74.4 ± 4.450	112.8 ± 18.281	122.8 ± 20.130	$8.867 \pm 1.318^{c,C}$
K_2	70.8 ± 3.962	222.0 ± 4.301	248.0 ± 6.164	$11.707 \pm 1.376^{d,D}$
K_3	73.4 ± 2.881	113.2 ± 3.493	109.4 ± 2.966	$-3.346 \pm 0.673^{b,B}$
K_4	68.2 ± 4.494	229.2 ± 8.136	214.2 ± 7.014	$-6.536 \pm 0.594^{a,A}$
K_5	75.2 ± 6.181	109.8 ± 7.050	123.8 ± 7.981	$12.750 \pm 0.354^{e,E}$
K_6	70.6 ± 5.030	232.4 ± 4.336	278.4 ± 6.189	$19.787 \pm 0.452^{f,F}$

The (+) sign represents an increase; The (-) sign indicates a decline

Table 1 shows that the groups of mice K_1, K_2, K_5, and K_6 experienced an increase in blood glucose levels, while the groups of mice K_3 and K_4 experienced a decrease in blood sugar levels, after being treated for 22 days.

Decreased blood glucose levels in mice, obtained from the calculation of the difference between blood sugar levels before being given treatment with blood sugar levels after giving treatment. K_1 of mice group with negative control treatment experienced an increase in blood sugar levels, but it was still in normal condition, that's from 112.8 mg/dL to 122.8 mg/dL (Table 1). Blood glucose levels in normal mice of 70-140 mg/dL [13].

K_2 of mice group also experienced an increase in blood sugar levels but above the normal condition, that's from 222.0 mg/dL to 248.0 mg/dL (Table 1). This was due to the STZ effect which caused a hyperglycemic effect until the final stage of treatment.

The mice groups of K_3 and K_4 experienced a decrease in blood glucose levels which showed a hypoglycemic effect. K_3 of mice group experienced a decrease in blood glucose levels about 3.346% and the K_4 group experienced a decrease in blood glucose levels about 6.536%. The difference in decrease of blood glucose levels due to differences in metabolic conditions between diabetic and non-diabetic groups. According to Hutapea, et al. (2016) the non-diabetic population had normal functioning insulin, so that blood glucose levels only increased in the first 1 hour and again fell due to insulin work in the second hour after the soda was induced [14].

K_4 of mice group which was a diabetes group experienced a decrease in diabetes sugar levels greater than K_3. This was due to the influence of pirdot leaf extract and juice of red watermelon

albedo found in carbonated coconut drink. Plant extracts contain not only one type of active component and have different pharmacological effects. The active ingredient can react with organ-receiving cells, thus forming a transduction signal that was able to control blood glucose [15].

Pirdot leaf water extract is able to inhibit the activity of α-glucosidase as well as acarbose which is commonly used as an antidiabetic drug. This can be seen from the IC_{50} value where pirdot leaf water extract has an IC_{50} value about 75.56 μg/mL while the IC_{50} value of acarbose about 150.63 μg/mL [11]. Pirdot leaf extract contains flavonoids which can inhibit the action of α-amylase and α-glucosidase, so that the amount of post-prandial monosaccharides and hyperglycemia will be absorbed [16].

Watermelon albedo juice can also reduce blood sugar which was almost comparable to the administration of glibenclamide. This was due to the presence of citrulline content [12]. Citrulline acts as a trigger agent for the productivity of nitric oxide. Nitric oxide is a compound that is directly related to controlling insulin secretion.

K_5 of group mice which was a normal group of mice given commercial soda for 14 days, experienced an increase of blood sugar levels about 12.750%, and K_6 which was a group of diabetic mice given commercial soda for 14 days, experienced an increase of blood sugar levels about 19.787%. An increase of blood sugar levels due to the high sugar content in commercial soda.

Carbonated drinks are known to have a large sugar content, which is 40-50 g [1]. Increased blood sugar levels of the commercial soda treatment on K_5 was lower than K_6. This was due to the non-diabetic population having normal insulin levels in their body, so that it can metabolize high sugar content [14].

Normal adults produce insulin about 20-60 units per day, if excessive glucose can not be metabolized, resulting in increased blood glucose [17]. K_6 of mice group which was a diabetes, where according to Rias and Sutikno, (2017) the sugar metabolism of the diabetes mellitus group into cells is inhibited due to insulin resistance, so that blood glucose remains high [18].

Decreased blood glucose levels in the K_4 of mice group after induced carbonated coconut drink for 14 days were in fact unable to normalize blood glucose levels. Blood glucose levels in this group are still in a state of diabetes (Table 1). This was because carbonated coconut drink has a variety of active substances consisting of coconut water, pirdot leaf extract, and juice of albedo red watermelon.

Active substances in plant extracts cause of the substance to work is not specific. This was due to the activity of natural ingredients which are multicomponent, where the effects of the various components of these substances can be synergistic, additive, or antagonistic [19]. Based on this, further research is needed with a longer testing period to see a decrease in optimal blood sugar levels to be within normal condition.

The dose of carbonated coconut drink induced in diabetes mice was 0.6 mL/day for 14 days. Based on observations, the dose is not sufficient to normalize the blood sugar levels of diabetes mice. This was thought to be due to the ceiling effect, which is the effect arising from drug compounds at certain dosage levels has exceeded its optimum limit. Ceiling effect arises due to saturation of the bond between the drug and the receptor, so the receptor is not able to react with the drug [20]. So, further studies are needed regarding the optimal dose of carbonated coconut drink to reduce blood sugar levels in mice within normal condition.

3.2. Body weight

Administration of aquades, carbonated coconut drink, and commercial soda for 14 days had a highly significant effect ($P < 0.01$) on the percentage change of body weight of mice. Measurement of body weight of mice (Table2) was carried out on day-0, which was the diabetic mice group was induced by STZ 50 mg/kg, then on day-8 which was the beginning of the treatment because the STZ induced diabetes group had blood glucose levels above 200 mg/dL, then the blood glucose level of mice was measured on the day-22 which was where each group of mice had been given treatment for 14 days. The effect of giving aquades, carbonated coconut drink, and commercial soda on the percentage change of body weight of mice for 22 days is presented in Table 2.

Table 2. Results of measurements body weight of mice for 22 days

Group	Body weight (g)			Percentage change of body weight (%)
	Day-0 x±SD	Day-8 x±SD	After treatments Day-22 x±SD	
K_1	27.4 ± 1.517	29.6 ± 1.673	34.4 ± 2.074	$16.196 \pm 0.312^{e,E}$
K_2	27.0 ± 1.000	31.2 ± 0.837	23.6 ± 1.140	$-24.386 \pm 0.159^{b,B}$
K_3	27.0 ± 1.414	28.6 ± 1.517	29.8 ± 1.789	$4.171 \pm 0.194^{d,D}$
K_4	27.0 ± 1.581	31.8 ± 1.304	32.6 ± 1.140	$2.564 \pm 0.358^{d,D}$
K_5	26.4 ± 1.949	28.6 ± 1.342	26.6 ± 0.894	$-6.884 \pm 0.385^{c,C}$
K_6	26.4 ± 1.140	31.4 ± 1.342	20.8 ± 0.837	$-33.750 \pm 0.040^{a,A}$

The (+) sign represents an increase; The (-) sign indicates a decline

The body weight of mice in the K_1, K_3 and K_4 groups increased, while the K_2, K_5, and K_6 groups experienced weight loss. The observations (Table 2) show that K_1 was a normal group of mice treated with aquades which has increased body weight about 16.196%. The increase in body weight of mice is influenced by the feed given ad libitum.

K_2 was a group of diabetic mice treated with aquades experienced weight loss that about 24.386%. According to Albu, et al., (2010) inhibited insulin production of diabetes group, resulting in high blood glucose levels [21]. If the body is not fulfilled by the amount of energy from sugar, the body will produce components of other substances to be broken down into energy such as fat and protein, thus causing weight loss.

K_3 was a group of normal mice that gained weight about 4.171%. This was due to the presence of sugar in carbonated coconut drink. That was because seeing the behavior of mice after giving carbonated coconut drink, their appetite increased. Wolfenshon and Lloyd (2013) describe that weight gain of mice is caused by mice experiencing polydipsi and polyphagia. This is seen from the amount of feed that always runs out before the next administration period.

K_4 was a group of diabetic mice that gained weight about 2.564%. This is presumably because the bioactive component of carbonated coconut drink derived from pirdot leaf extract and juice of red watermelon albedo, that help regenerate β-cells in the pancreas and provides therapy for hyperglycemia mice. Weight gain in the diabetes group was due to regeneration of the β-cell population and to influence insulin receptors in the β-cells due to STZ. This reduces the buildup of

blood glucose, thereby improving the absorption of other nutrients and thus contributing to weight gain [23].

Commercial soda with a dose of 0.6 mL per day for 14 days caused a decrease in body weight of mice, both of K_5 group (6.884%) and K_6 (33.750%). This was influenced by the high amount of sugar contained in carbonated drinks. Carbonated drinks contain a lot of sugar, but in liquid form. As a result, if consumed continuously will produce an equivalent effect on satiety such as ad libitum food supply. Liquid food causes accumulation of substances in the stomach and feeling full. Satiety reduces the energy supply in food [24].

K_6 was a group of diabetic mice that experienced a very drastic weight loss about 33,750%. This is due to the induced streptozotocin effect that causes muscle atrophy. As a results weight loss and structural protein loss, due to the absence of carbohydrates as an energy source [25].

The commercial soda treatment on K_6 group caused an increase in blood glucose levels, so the appetite was reduced. The result was a decrease of body weight, because commercial soda contains sugar about 40-50 g. The sugar content has exceeded 4 times the recommended sugar consumption per day which is 6-12 g. This situation worsened the condition of the diabetes. Glucostatic theory explains that an increase in glucose levels in the body causes a decrease in appetite, so a person will not increase his energy supply [26].

4. CONCLUSION

The addition of pirdot leaf extract and juice of red albedo watermelon on carbonated coconut drinks has an effect on decreasing blood glucose levels, both in diabetic and non-diabetic mice. This is caused by the presence of antioxidant from coconut water, flavonoids from pirdot leaf extracts, and citrulline from juice of red watermelon albedo. These bioactive components provide therapeutic effects, especially for the diabetes. As a result, insulin productivity increases and triggers weight gain.

The treatment of commercial soda, both in diabetic and non-diabetic mice gave an influence on increased of blood glucose levels. This was due to sugar content in commercial soda causing severe damage to the pancreas. As a result, blood glucose levels get higher and absorption of nutrients is inhibited resulting in weight loss.

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