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The Effect of Carbonated Drinks on Blood Glucose Levels in Mice (*Mus musculus*)

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ABSTRACT

The habit of people who live in cities and villages likes instant smells, for example, consuming soft drinks. One of the soft drinks popular in the community is carbonated drinks. This study aims to determine the effect of carbonated drinks on blood glucose levels of mice. This study used 21 male mice Balb C strain aged 2-3 months, which was divided into 3 groups, namely K1 given aquadest dose of 0.86 ml / 20 g body weight. K2 is given a carbonated drink dose of 0.43 ml / 20 g body weight. K3 was given a carbonated drink dose of 0.86 ml / 20 g body weight. All treatments were given for 14 days. The average increase in blood glucose levels of mice in KI, K2, and K3 were 16.71 mg / dl, 11.63 mg / dl, and 67.59, respectively (P<0.05). The results of the study showed that there was an increase in blood glucose levels in all treatments. However, the highest increase in blood glucose levels is in K3 (67.59 mg / dl). From the results of the study, it was concluded that carbonated drinks can affect blood glucose levels in mice, but this increase is still within normal limits, thus providing information and increasing knowledge about the effect of soft drinks on blood glucose levels.

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INTRODUCTION

Improving living standards in developed countries and big cities has impacted changes in people's lifestyles and consumption patterns. The occurrence of dietary changes in big cities impacts various health problems. Based on research conducted by Fitriati et al. (2017), nowadays, one kind of diet widely loved by children and adults is consuming carbonated soft drinks (soft drinks). Carbonated drinks, commonly called soft drinks, have become a lifestyle. When viewed by place of residence, urban residents consume more carbonated drinks than rural residents. Residents in urban areas consume carbonated beverages at 0.027 per 250 ml/day, and in rural areas by 0.012 per 250 ml/day (Bidang Statisti Sosial, 2015). Children and teenagers are the target consumers of carbonated beverages (Brannan, 2005). The consumption of carbonated drinks by children is at a problematic level. According to research conducted on children aged 8-14 in Australia, 41.8% of respondents consume carbonated drinks weekly, and 13.3% consume daily (Pettigrew et al., 2015). A study on children aged 8-13 in the United States noted that 30% of respondents consumed carbonated drinks

daily (Grimm et al., 2004). The consumption of this drink continues to increase, not only in developed countries but also in developing countries such as Indonesia (Cinteza, 2011; Shaw et al., 2018).

Carbonated drinks are a kind of drink that dissolves carbon dioxide into drinking water (Novidahlia et al., 2014). In carbonated drinks, carbon dioxide produces a characteristic sour taste in the mouth and will cause a fresh taste when carbonated drinks are drunk (Fitriati et al., 2017). The content of the carbonated drink itself consists of carbonated water, sugar, acidity regulators, flavorings, and preservatives. Carbonated drinks can be harmful to health if consumed in large quantities. These carbonated drinks are not suitable for health, primarily because of the presence of preservatives, sugar-sweetened, and sugar content in carbonated drinks that can increase glucose levels in the blood (Kregiel, 2015).

In the human body, glucose can be stored as glycogen in the muscles and liver and stored in the bloodstream as blood glucose (Irawan, 2007; Montung et al., 2015). Blood glucose level is the amount of glucose in the blood (Adrien jems, 2012). The regulation of blood sugar levels is closely related to the hormone insulin. The hormone insulin is a hormone produced by cells β in the pancreas. The hormone insulin will be secreted by the pancreas when there is an increase in the concentration of glucose in the blood (Mark et al., 2000).

The hormone insulin is a hormone that functions in lowering blood glucose levels. Insulin lowers blood glucose levels by moving glucose into cells with the help of glucose transporters (Haviz, 2012). When the amount of insulin hormone produced is not proportional to the amount of glucose, the hormone insulin will not be able to move all the glucose into the cells of the body, as a result of which excess glucose will remain in the blood, and this will lead to high levels of glucose in the blood (Rahmawati et al., 2014).

The lack of the hormone insulin in the blood will lead to hyperglycemia. Hyperglycemia is a condition in which an excessive amount of glucose and exceeds normal limits in the blood. Hyperglycemia is one of the leading causes of the appearance of diabetes (Hasanah, 2013).

Diabetes mellitus is a disease that occurs in almost all countries, and the number of people with diabetes mellitus continues to increase (Shaw et al., 2010). Diabetes mellitus is a metabolic disorder characterized by increased blood sugar levels caused by impaired insulin secretion (Bolla et al., 2012; Punthakee et al., 2018).

Carbonated drinks harmfully affect the health of the body. Consuming carbonated beverages can cause damage to liver function (Milei et al., 2011). Daily intake of carbonated drinks can reduce bone density and increase the risk of osteoporosis (Chen et al., 2020). The results of the study by Basu et al. (2013) stated that there is a relationship between the consumption of carbonated drinks with obesity and diabetes.

The results of the study of Soniya et al. (2023), which tested the effect of giving carbonated drinks on increasing blood sugar levels of white rats (Rattus norvegicus) male strain Sprague Dawley with four treatment groups, namely control (K) was only given aqueous dose 6 ml / 200 gr/day, treatment 1 (P1) given carbonated drinks dose 3 ml / 200 gr/day, treatment 2 (P2) given carbonated soft drinks dose 6 ml / 200 gr/day and treatment 3 (P3) given soft drinks carbonated dose 12 ml / 200 gr/day for 30 days. The results showed that there was an increase in the average blood glucose

level of rats in the P1 treatment (90.76 ± 5.750 mg/dl), P2 (106.50 ± 7.868 mg/dl), and P3 (138.00 ± 16.745 mg/dl).

Carbonated drinks can cause negative health effects. Carbonated drinks consumed in excess can cause hepatocyte cell damage to the liver (Murti et al., 2016), kidney tubule damage (K, 2016), and obesity (Putri et al., 2017; Faisal & Anayanti, 2021). So far, research on the influence of carbonated beverages can increase blood glucose levels (Soniya et al., 2023). Soniya et al. (2023) showed that blood glucose levels in the blood of white male rats of the Sprague Dawley strain might increase after consumed of carbonated drinks at a dose of 3 ml/200 g body weight, 6 ml/200 gr body weight, and 12 ml/200 g body weight.

From the background above, it is clear that carbonated drinks adversely affect health. The many effects of carbonated drinks that are harmful to health and the lack of research on the effects of carbonated drinks on blood glucose levels caused researchers to feel interested in conducting this study to determine the effect of carbonated drinks on the blood glucose levels of mice (*Mus musculus*). This research is aimed to provide information and improve knowledge about the effects of carbonated drinks on health. Furthermore, research on the effect of carbonated drinks on blood glucose levels can be a reference for reducing the consumption of carbonated beverages.

METHOD

This research was conducted as an experimental laboratory study. The design of this research used a complete randomized design (CRD). This technique was dependent on the experimental units' relatively homogeneous conditions. The laboratory experiment in this study was by experimenting on mice to determine the blood glucose level of mice after giving carbonated drinks for 14 days. Ngatidjan (2006) refers to testing the period and states that in a subacute toxicity test, the testing time is carried out at least two weeks. A subacute toxicity test is a test that aims to obtain data on drug or chemical poisoning that is used intentionally or unintentionally into the body repeatedly over a long period. Purposive *sampling* is a technique used the sample selection. The Mice used in this experiment are male mice of the Balb/ C strain, aged 2-3 months, with a body weight ranging from 20-26 g. Firstly, all laboratory animals were acclimatized for seven days to adapt to the new environment before the experiment began.

The laboratory animals are divided into three groups, each group consisting of seven heads. Every group has seven repetitions. The determination of dose refers to converting doses between species based on body surface area (Priyanto, 2015). The first group is the group that was treated with distilled water (aqueous) at a dose of 0.86 ml / 20 g body weight. The second group was treated with carbonated drinks at a dose of 0.43 ml/20 g body weight plus 0.43 ml/20 g body weight. The third group was treated with carbonated drinks at a dose of 0.86 ml / 20 g body weight. From the initial day of treatment (day 1), an initial blood sample was taken, and the final blood sampling was carried out 14 days after treatment (day 15). A blood sample is taken from the orbital sinuses. The blood sample obtained from the initial and final treatment was centrifuged at 4000 rpm to utilize the blood serum. The blood serum was then put into a test tube and diluted with 1000 µl of DiaSys (Diagnostic Systems) brand Glucose GOD FS. The homogeneous sample obtained is homogenized using a vortex. The spectrophotometer with a wavelength of 500 nm was used to read the absorbance of the sample. The

variance analysis (ANOVA) was used to analyze. The data were obtained at a level of 5% and continued with the Tukey's Honest Significant Difference test using the SPSS software version 25.

RESULTS AND DISCUSSION

Based on the results of studies that have been carried out, the average initial blood glucose level (before treatment) and the average final blood glucose level (after treatment) can be seen in Table 1. In contrast, the average value of the difference in the increase in blood glucose levels can be seen in Table 2.

Table 1. Average blood glucose levels for each treatment group on the day before and after treatment

Group	Average blood glucose level (mg/dl) after treated carbonated drinks for 14 days	
	Before Treatment	After Treatment
Aqueous 0.86 ml/20 g body weight	65.85	82.55
Carbonated drink 0.43 ml/20 g BB plus aqueous 0.43 ml/20 g body weight	67.30	78.93
Carbonated drink 0.86 ml/20 g body weight	67.45	135.04

Table 2. The average value of the difference in blood glucose levels

Group	The average difference in blood glucose levels before and after treated carbonated drinks	
Aqueous 0.86 ml/20 g body weight	$16.70^{a} \pm 7.63$	82.55
Carbonated drink 0.43 ml/20 g BB plus aqueous 0.43 ml/20 g body weight	$11.63^{a} \pm 6.50$	78.93
Carbonated drink 0.86 ml/20 g body weight	$67.59^{b} \pm 9.63$	135.04

Description: *The number after the symbol ± indicates the standard deviation (SD) value.
*Numbers followed by different letters indicate a noticeable difference in Tukey test with a grade of 5% (P<0.05).

From table 1 and table 2, the results of measuring blood glucose levels showed a difference in glucose levels in blood between the three treatments. Table 2 shows the highest blood glucose levels in the third group (P<0.05). The statistical analysis showed that adding carbonated drinks at a dose of 0.86 ml significantly differed from that of carbonated drinks at a dose of 0.43 ml and aqueous. This result suggests that increased blood glucose levels occur significantly at high doses compared to low-carbonated drinks.

The blood glucose levels increment is due to carbonated drinks having content that improves blood glucose levels, which we call sugar. Generally, the ingredient of the carbonated drink itself consists of carbonated water, sugar, acidity regulators, flavors, and preservatives. Carbonated soft drinks generally have a sugar between 10-12% (Ashurst, 1998). Regarding the Regulation of the Minister of Health of the Republic of Indonesia Number 30 of 2013 Article 4 paragraph (2), the amount of sugar in food or beverages, the recommended sugar consumption limit is no more than 50 g per person per day. In line with this study, Erlianawati reported that giving carbonated drinks at a dose of 1.8 ml for 14 days can increase the blood glucose levels of mice (Handayani, 2021).

Sugar from carbonated drinks is part of the group of carbohydrates with the type of disaccharide sucrose. Sucrose consists of two monosaccharide molecules, one glucose molecule, and one fructose molecule (Cakrawati, D., & Mustika, 2014). The sucrose will first be converted into monosaccharides in the small intestine with the help of sucrose enzymes before being absorbed by the body (Cakrawati, D., & Mustika, 2014; Wulandari, 2016).

Glucose is one of the essential fuels used to produce the energy required by the body's cells. When the glucose in the blood increases, this increment will be detected by the beta cells of the pancreatic islets and causes the pancreas to release the hormone insulin into the blood. Furthermore, insulin will stimulate adipose cells and muscle cells to take glucose from the blood, then insert it into the cells (Hasanah, 2013; Pahlawan, P.P., & Oktaria, D, 2016). When adequate, glucose will be stored as glycogen reserve in the liver (Hasanah, 2013). Inside the small intestine, glucose will be absorbed into the bloodstream leading to the liver through the hepatic portal vein. Glycogen will be stored in the liver and converted back into glucose when blood glucose levels decrease (Hasanah, 2013). When blood sugar levels decrease, the pancreas will produce the hormone glucagon to stimulate the liver to free glucose from glycogen so that normal blood sugar levels return (Pahlawan, P.P., & Oktaria, D, 2016).

Nugrahani stated that normal mice's blood glucose levels ranged from 62.8 mg/dl - 176 mg/dl. In comparison, the blood glucose level of diabetic mice is ≥ 200 mg/dl (Nugrahani, 2012). Diabetes mellitus occurs when there are problems in insulin secretion, one of which is the exhaustion of beta cells in secreting insulin. This condition happens when the amount of incoming glucose intake is not proportional to insulin production. If beta cells experience fatigue in producing insulin, it will cause beta cell function to decrease. When pancreatic beta cell fatigue occurs, diabetes mellitus will arise, characterized by increased blood glucose levels (Bolla et al., 2012; Punthakee et al., 2018; Rahmawati et al., 2014; Banjarnahor & Wangko, 2013). Based on this, the results of this study reflect that the blood glucose levels of the three groups after giving carbonated drinks are all within normal limits because they are in the range of 78.94-135.04 mg/dl. Although it is declared relatively safe for consumption, if carbonated drinks are carried out continuously for an extended period, it is likely to cause hyperglycemia (high blood sugar). It will lead to the emergence of diabetes. According to (Kregiel, 2015), consuming foods/drinks that contain excess/high sugar harm health, one of which can cause diabetes mellitus.

CONCLUSION

The blood glucose levels in mice can be affected by carbonated drinks. The increase in blood glucose is still within normal limits. Blood glucose levels increased significantly at a dose of carbonated drinks of 0.86 ml/ 20 g body weight. It is necessary to conduct further research on the stratified consumption of carbonated drinks for a longer time to determine their effect on blood glucose levels. In addition, in line with the increase in blood glucose levels after giving carbonated drinks that are still within normal limits, it is necessary to conduct further research by increasing the dose of carbonated drinks to determine the carbonated drinks that can increase blood glucose levels beyond normal limits.

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