# Effect of Administration of Kawista Juice (*Limonia acidissima*) on Blood Glucose Levels of Wistar Strain (*Rattus norvegicus*) Hyperglycemia

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**Abstract.** Hyperglycemia is a condition of glucose levels in the blood that exceed normal limits, this occurs due to a lack of insulin so that glucose levels are high. Kawista contains antioxidants that can lower blood glucose levels. The purpose of this study was to determine the effect of giving kawista juice (limonia acidissima) on random blood glucose (RBG) levels in the hyperglycemic wistar (rattus norvegicus) strain. This research is true-experimental with pre and post test controlled group design. The research method was the induction of hyperglycemia in white rats by giving Streptozotocin (STZ) 55 mg/kgBW for 1 day then the white rats were checked for RBG levels before intervention. White rats were intervened with kawista fruit juice to see the effect of giving on RBG levels. Mice were divided into K+ groups (without intervention); P1 (dose 7.6 ml/day); P2 (dose 15.2 ml/day); P3 (22.8 ml/day dose). Shapiro Wilk statistical test, paired t test, one-way ANOVA and LSD test. In this study, kawista juice with a dose of 15.2 ml/day can reduce RBG levels but has not reached normal levels.

**Keywords:** Kawista Juice, Blood Glucose Levels, Hyperglycemia

## 1 INTRODUCTION

The diet of the world's population globally has changed with the times. This is due to the advancement of food management technology and the increasing level of well-being. This change sometimes has a bad impact, namely the increased tendency to consume sweet foods.

Hyperglycemia is a condition of glucose levels in the blood that exceed normal limits, this occurs due to lack of insulin so that glucose levels are high. If it occurs continuously and lasts for a long time, it will result in diabetes mellitus[1]. Hyperglycemia in diabetes is associated with long-term damage, dysfunction and the presence of organ failure (eyes, kidneys, nerves, heart and blood vessels). In impaired


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insulin secretion and insulin work often occur simultaneously and it is often not clear which abnormality is the main cause of hyperglycemia[2].

Blood glucose commonly called glucose is a sugar that is in the blood. This sugar comes from the food consumed and is the body's main source of energy. Blood will carry this glucose to all body cells to be used as energy[3].

Kawista is included in the annual horticultural crops in Rembang, Central Java[4]. Kawista fruit contains fruit acids, vitamins such as vitamin C, and minerals. The dried flesh of the fruit contains 15% citric acid, potassium, calcium and iron salts. Seeds and fruits contain oil and protein; oil consists of palmitic, oleic, linoleic and linolenic acids in addition to traces of palmitoleic and stearic acids; β-sitosterol, β-Amirin, lupeol and stigmasterol from unsaponifiable material from seed oil. In addition, kawista fruit also contains flavonoids, glycosides, saponins, tannins, some coumarins, and tyramine derivatives that function as antioxidants[5].

Giving antioxidants is an effort to inhibit intracellular free radicals from producing to prevent the emergence of vascular complications related to diabetes[6]. These beneficial antioxidants can reduce oxidative damage in people with diabetes or hyperglycemia. Based on the CDC (Centers for Disesase Control and Prevention) levels of vitamin A, low Vitamin E in diabetics, and it is also said that giving high doses of vitamin C that is 2 grams / day can improve the health of diabetics[7].

That kawista juice can be an alternative drink to control blood glucose levels because it has a glycemic index and glycemic load which is included in the low category. This kawista juice is recommended as a functional drink to control blood sugar levels. In the study, it was also explained the glycemic index of kawista fruit juice (<55) which is included in the low category, due to the content and owned by kawista fruit[8].

Experimental animal models are classified into two, namely, spontaneous experimental animals or experimental experimental animals. Spontaneous animal models use experimental animals that have phenotypic similarities with humans. While experimental animals use experimental animals that are often used compared to genetic models because they are cheaper, have a lot of availability and easier induction methods[9].

In experimental animal models, hyperglycemia can be caused by administration of streptozotocin (STZ) which can cause damage to pancreatic beta cells[10]. STZ has a long half-life and is not easily oxidized. STZ works by forming highly reactive free radicals that will then cause damage to cell membranes, proteins and DNA (deoxyribonucleic acid) so that it will cause disruption of insulin production by pancreatic beta cells[11].

Based on the description above, a study was conducted on the effect of giving kawista juice (limonia acidissima) on blood glucose levels in white rats wistar strain (ratus norvegicus) induced hyperglycemia.

## 2 METHOD

This research is a real laboratory experimental (true-experimental) with Pre and post test controlled group design. The population in this study was white rats (Hundred

Norvegicuss) males aged 2-3 months with a body weight of 160-250 grams placed in the Experimental Animal Laboratory of the University of Muhammadiyah Semarang. The sample of this study was white rats (Rattus norvegicus) male wistar strain obtained from the Experimental Animal Laboratory of the University of Muhammadiyah Semarang and has met the requirements for inclusion and exclusion criteria.

In this study, the total sample to be used was 16 mice. This study had 1 control group and 3 treatment groups. Based on the data above, the total number of samples was 4 groups with the need for 4 male white rats for each treatment. The calculation of this sample size uses the Feesting formula from Charn et al (2013) which obtained 4 samples for each treatment group. The sampling technique in this study used the simple random sampling method.

Experimental animals are kept in cages in groups with a total of 4 cages, each cage is filled with 2 rats and marked odd / even in each cage to make it easier if given treatment. Experimental animals are kept with temperatures around 18-26ºC if the temperature is higher than the range, the animals will experience stress and have a high chance of dehydration[12]. Animal cages try to be cleaned every morning.

Hyperglycemia conditioning is performed by STZ injection. Streptozotocin was dissolved into a 0.01M citrate buffer solution with a PH of 4.5 and prepared when it would be treated on rats[13]. Streptozotocin was injected at a dose of 55 mg / kg body weight in white rats.

Measurement of blood glucose levels is done by GOD-PAP examination. Blood collection of white rats was done through the orbital sinuses of white rats as much as ±3 ml inserted into the microtube. Blood will be centrifuge at a speed of 7000 rpm for ± 5 minutes. The serum formed from the treatment will be taken as much as 10 µl and added 1000 µl reagent will then be incubated for ± 10 minutes at a temperature of 20-25ºC. Blanks, standards and samples will be read using a spectrophotometer with a wave of 546 nm, 100f, program c / st. RBG levels were analyzed using the GOD-PAP method (RBG levels of rats, normal <135 mg / dl).

Making kawista fruit juice is done by washing then opening the skin of the kawista fruit then taken fruit flesh and weighing using digital scales. After that blended added water with a ratio of 1 : 2 + 100 ml, then after smooth filtration is carried out the filtering process.

Giving kawista fruit juice is given through the sonde for the stomach and is done twice a day, namely in the morning and afternoon for 2 weeks after the pre-test blood glucose level examination with the provision of Dose 1 7.6 ml / day; Dose 2 15.2 ml / day ; Dose 3 22.8 ml/day. Then after intervening for 18 days, the RBG test was carried out again using GOD-PAP.

The data obtained were tested for normality with the Shapiro-wilk test due to n<50. Then after that, continued the Paired Sample T-test and one-way Anova test and LSD test (post hoc).

# 3 RESULTS AND DISCUSSION

Research on the effect of giving kawista juice (limonia acidissima) on blood glucose levels in hyperglycemia-induced wistar (ratus norvegicus) white rats has been conducted. A total of 16 mice were taken blood samples to analyze RBG levels. Pre and post data have been tested for normality using Shapiro-wilk test and homogeneity test, the results of data are normally distributed and homogeneous.

Based on table 1, it can be seen that the paired test analysis of t-test samples, rat body weight experienced significant weight loss in the P3 group with a delta of -29.5±8.81 g (p 0.007). While in the K + group the data was not significant but experienced weight gain which can be seen from delta data of 3.2±13.04 g (0.653). Then the results of the one-way ANOVA test obtained a p value of 0.118 (p >0.05) which concluded there was no significant difference in the average body weight of pre and post intervention mice.

**Table 1.** Average body weight of pre-intervention and post-intervention mice

| Group | Average BB Mouse (g) ± SD | | | $p^1$ | $p^2$ |
|---|---|---|---|---|---|
| | **Pre-intervention** | **Post-intervention** | ***delta*** | | |
| K+ | 195.5±22.7 | 198.7±14.9 | 3.2±6.5 | 0,653 | 0,118 |
| P1 | 201.0±31.4 | 170.7±10.1 | -30.2±11.3 | 0,076 | |
| P2 | 195.2±33.6 | 159.2±7.8 | -36.0±18.3 | 0,145 | |
| P3 | 176.7±5.1 | 147.2±8.5 | -29.5±4.4 | 0,007* | |

Description:

a) [1]Paired sample t-test; [2]One-way ANOVA; *significant ($p<0.05$); b) Pre-intervention body weight was the body weight of mice before STZ injection and kawista administration; c)K+: Positive control group of normal feed and drinking; d) P1 : Treatment 1 dose 7.6 ml / day; e) P2 : Treatment 2 doses 15.2 ml / day; f) P3 : Treatment 3 doses 22.8 ml / day

The decrease in the average body weight of mice in the hyperglycemia treatment group occurred due to STZ induction which triggered hyperglycemia. This is in accordance with the research of Zafar et al (2010), Nurliyani et al (2015) and Muntafiah (2017) which states that there is a relationship between hyperglycemia and rat weight loss in diabetic models. This weight loss is due to protein degradation that occurs because there is not enough glucose and lipids, then protein will be used as the main source of energy in the body. Structural proteins can contribute to weight loss[14,15,16].

Weight gain experienced by rats due to excessive thirst conditions (Polydipsy). This was observed during the study period of rats in the control group always spent the drink provided. Polydipsia is caused because the kidneys produce more urine to remove excessive blood glucose from the body. The body will lose a lot of fluid, until finally the brain sends a signal to drink more to replace the fluid loss[17].

Based on table 2, it is known that after the intervention of the positive control group (K+) that did not experience a significant decrease was characterized by a p-value of

0.055 (P<0.05). In the K+ group there was a decrease in RBG levels as in table 2, delta K+ was -8,500±5.56 the decrease in RBG levels is thought to be due to stress factors experienced by white rats during the study period. Physical activity, stress, anxiety and excessive depression can trigger the body to reject insulin so that RBG levels can rise or fall. Signs of symptoms of decreased RBG levels can also be characterized by a state of dehydration[18]. During the study period white rats of group K+ were dehydrated, this was observed during the study period. After drinking is done, drinking water will soon be exhausted by mice.

**Table 2.** Average random blood glucose levels of pre-intervention and post-intervention mice

| Group | Average RBG(mg/dl)±SD levels | | | $p^1$ | $p^2$ |
|---|---|---|---|---|---|
| | Pre-intervention | Post-intervention | *delta* (mg/dl) ± SD | | |
| K+ | 261.25±27.10 | 252.75±22.47 | -8,500±5.56 | 0,055 | 0,001* |
| P1 | 254.25±24.54 | 235.00±24.42 | -19.20±6.29 | 0,009* | |
| P2 | 247.00±28.15 | 216.50±25.31 | -30.50±4.79 | 0,001* | |
| P3 | 225.75±20.74 | 203.00±17.56 | -22.75±4.99 | 0,003* | |
| Total | 247.06±26.46 | 226.81±28.05 | | | |

Description: [1]Paired sample t-test; [2]One-way ANOVA; * p-value <0.05 significant data
a) K+ : Positive control group of normal feed and drinking; b) P1 : Treatment 1 dose 7.6 ml / day; c) P2 : Treatment 2 dose 15.2 ml / day; d) P3 : Treatment 3 doses 22.8 ml/day

The most significant decrease in the treatment group was found in treatment group 2 (P2) because the p-value was 0.001 (p<0.05) with delta or mean difference was -30.50±4.79 mg / dl. Giving kawista juice had an effect on reducing rat RBG levels with the most decrease found in the 2nd treatment of (-30.50±4.79 mg / dl). To prove the effect of treatment from the control group and treatment, further tests will be carried out using the LSD test in table 3.

In table 3, it shows that significant differences exist in the K+ and P2 groups p-value 0.043 (<0.05) and K+ and P3 p-value 0.009 (<0.05). The effect of feeding kawista fruit juice on other groups has no significant value. The effect of giving kawista fruit juice from the results of the LSD test can be concluded that at treatment dose 2 (P2) the most influential p-value is 0.043 (<0.05) compared to treatment dose 3 p-value 0.009 (<0.05). It is concluded from the paired t-test results of treatment group 2 (P2), p-value 0.001 (p<0.05) and results from LSD that P2 has significant data.

In the P1 group, there was no effect of administration (p 0.289) or the data were not significant. The cause is not yet known. However, there is a possibility due to the result of giving relatively fewer doses than other treatment groups so that the insulin response in the body has not been able to function normally. So that it can trigger no significant effect of giving kawista fruit juice to rats.

**Table 3.** LSD Test for rat RBG levels

| Post hoc *LSD* Test | RBG mean difference (mg/dl) | *p* |
|---|---|---|

| | | |
|---|---|---|
| K+ vs P1 | 17,750 | 0,289 |
| K+ vs P2 | 36,250* | 0,043 |
| K+ vs P3 | 49,750* | 0,009 |
| P1 vs P2 | 18,500 | 0,270 |
| P1 vs P3 | 32,000 | 0,069 |
| P2 vs P3 | 13,500 | 0,416 |

Description: LSD test (*different means $p<0.05$) with 95% confidence; a) K+ : Positive control group of normal feed and drinking; b) P1 : Treatment 1 dose 7.6 ml / day; c) P2 : Treatment 2 dose 15.2 ml / day; d) P3 : Treatment 3 doses 22.8 ml/day

Kawista juice contains flavonoids and vitamin C which are included in the antioxidant group. In some studies it is mentioned that antioxidants can protect pancreatic beta cells from toxic effects produced during hyperglycemia, so that insulin levels in the body are maintained and blood glucose levels remain normal[19]. Flavonoids have the ability to regenerate and stimulate the release of pancreatic beta cells[20]. Flavonoids against beta cell damage as insulin producers and can increase insulin sensitivity[21]. Vitamin C is an antioxidant that inhibits the negative impact of excess oxidants due to hyperglycemia so that it can inhibit oxidative damage to a cell[22]. From the results of research Atiqoh (2006) stated that flavonoids can reduce blood glucose levels by reducing damage to pancreatic cells in the islets of Langerhans by producing and stimulating insulin and its release into the blood[23].

In Rustiah, et al (2018) research on antioxidant tests from kawista fruit extract (Limonia acidissima) using a UV-VIS spectrophotometer, research results were obtained that the antioxidant test of kawista fruit extract has a low IC50 value of 1275 ppm because it is above 150 ppm[24]. The levels of this antioxidant are relatively weak. Based on the results of statistical testing, there is a significant decrease in blood glucose levels. However, if reviewed again, in table 2, the average data of the total sample RBG levels of post-intervention rats was 226.81±28.05 mg / dl (normal <135 mg / dl). At the P2 dose as the difference in decreasing RBG levels in the most pre and post intervention with the average decrease in the P2 group was 216.50±25.31 mg/dl (normal <135 mg / dl), this level was still above normal. Giving kawista fruit juice in this study can only reduce RBG levels when given intervention but has not been able to reduce RBG levels to normal category (<135 mg / dl). The condition of decreasing RBG levels is not normal is suspected from mice that still experience a state of stress during the study period. Physical stress can trigger the release of hormones that can cause high blood glucose levels[25].

## 4 CONCLUSION

This research has shown that kawista fruit juice given as an intervention has not been able to reduce blood glucose levels to normal again. However, there is still an effect of giving to be able to reduce RBG levels but not to normal RBG levels.

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