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Starfruit Leaves as Glucose Absorption Inhibitor in Mice's Small Intestinal Epithelial Cells

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Abstract

Background: Starfruit **(***Averrhoa carambola*) leaves contain flavone derivatives that exhibit anti-hyperglycemic effects. This study aims to determine the effect of starfruit leaves in reducing glucose absorption in intestinal epithelial cells of mice. **Methods:** This study was done by performing perfusion on the small intestines of mice. The mice that were used in this study were divided into four groups. The control group was given glucose solution without infused starfruit leaves whereas, the remaining 3 groups were given 3 mmol (540 mg/dL) glucose solution with infused starfruit leaves of varying concentrations; 200, 400, and 600 mg/kg. Samples were collected at 0, 15th, 30th, 45th, and 60th minute. The sample was tested for glucose levels using spectrophotometry. **Results:** Test of significance showed a significant difference between the control group and the test group with $p < 0.05$. **Conclusions:** Starfruit leaves have a reduction effect towards glucose absorption in the small intestines in Wistar strains where the group using 600 mg/kg of infused starfruit leaves have the most significant effect as compared to other groups.

Keywords: absorption, Averrhoa carambola, glucose, infusion, intestine small

Introduction

Studies about the usage of herbal based medicine to cure diseases has been done, including to decrease the blood glucose level.[1] Previously known that the mechanism of glucose absorption can occur in a few ways; from glucose diffusion and protein transport. There are two type in glucose's protein transport mechanism; Sodium-Glucose Transport Proteins (SGLT1) and Glucose Transporter 2 (GLUT2).[2-4] The mechanism of decreasing blood glucose could occur in a few ways. The fibers that are in herbal substances could inhibit glucose absorption.[5-11] Fibers are complicated to dissolve to smaller molecule because human's intestine is lack of enzyme that can dissolve the fibers. Hence, the monosaccharides that bond with the fiber are difficult to absorb in human's intestine.[11] Aside from that, there are certain active substances that have glucose inhibition activity in the intestinal epithelial cells.[12,13]

Starfruit leaves has flavone derivatives in the form of apigenin-6-C-β-$_L$-fucopyranoside (Figure 1) and apigenin-6-C-(2-O-α-L-rhamnopyranosyl)-β-1-fucopyranoside (Figure 2) that has a potential of anti-hyperglycemic effect.[1,14,15] Even though it is known that starfruit leaves exhibits an anti-hyperglycemic effect, the molecular mechanism of starfruit leaves in lowering the blood glucose level is still exactly unknown. However, on previous study conclude that starfruit's leaves infusion

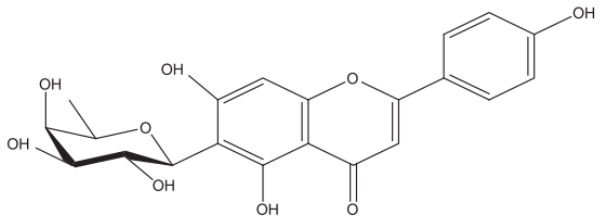

Figure 1. Apigenin-6-C-β-$_L$-fucopyranoside[1]

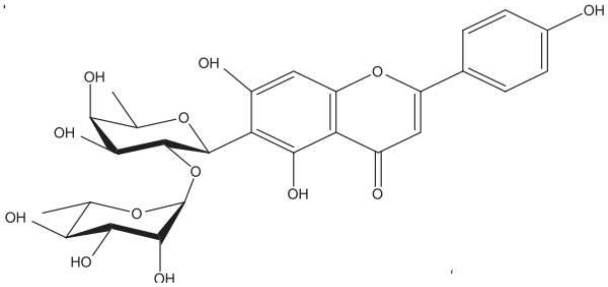

Figure 2. Apigenin-6-C-(2-O-α-L-rhamnopyranosyl)-β-1-fucopyranoside[1]

had effect on activity of glucose transportation on small intestine; apigenin-6-C-β-$_L$-fucopyranoside and apigenin-6-C-(2-O-α-L-rhamnopyranosyl)-β-1-fucopyranoside activate Phosphoinositide 3-Kinase inhibitor (PI3K inhibitor), Protein Kinase C inhibitor (PKC inhibitor), and Mitogen-activated Protein Kinase inhibitor (MEK inhibitor) to involve the glucose absorption activity.[1] Hence, this study has a purpose to observe the effect of infused starfruit leaves on glucose transport in the intestinal epithelial cells of mice.

Methods

This study was done in November 2015 in the Biochemistry Laboratory, Faculty of Medicine, Universitas Padjadjaran. The starfruit leaves were obtained from the Sekolah Ilmu Teknologi Hayati, Bandung Institute of Technology. The leaves were dried and infused using leaves that weighed 20, 40, and 60 g to produce sequential infusions with concentrations of 200, 400 and 600 mg/kg of the mice weight. Each of the infused starfruit leaves was then mixed with 3mmol glucose solution with a ratio of 1:24 for the mice group that was given an infusion. Meanwhile, the control group was given distilled water (aqua dest) and 3 mmol glucose solution with the same ratio. For all subject enrolled in this study was approved by research ethics committee from Faculty of Medicine Universitas Padjadjaran with number 558/UN6.C1.3.2/KEPK/PN/2015.

The mice used in this study were healthy, male mice with Wistar strains aged 3-4 months with a weight of 200-300 g. The mice were adapted for a week in the Biochemistry Laboratory, Faculty of Medicine, Universitas Padjadjaran. During adaptation, the shelter are cleaned and mice are gave proper food and water. Mice that died before the study or during the data collection were not included in the sample group.

In the study, 24 mice were divided into four groups. Researcher used Federer's formula to get the smallest sample population. The mice were fasted for 8 hours then given an anesthetic which was intramuscular ketamine 0.4 mL to anesthetized the mice during experiment. Then, cannulas were inserted 10 cm distally from the pylorus for the first cannula and 25 cm from the first cannula for the second cannula. The intestines of the mice were then cleaned using 0.96% NaCl solution three times.

The tested solution was flowed through the small intestine of the mice using a perfusion device for 60 minutes. Sample collection was done every 15 minutes from the 0 to the 60th minute. Samples were examined using the Trinder enzymatic method. The data analysis was done using the Shapiro-Wilk method to check the data distribution and the one-way Analysis of Variance (one-way ANOVA) method to calculate the significance of data.

In this experiment, the researcher need to take notice the ethical aspect of animal experiment. In reduction aspect, researcher has used Federer's formula to acquire the smallest population samples on each group. In refinement aspect, researcher has provided proper mice's shelter and provision of food and water on adaptation phase. On data collection phase, researcher has used ketamine as anesthetic agent to relieve the mice from pain. And in replacement aspect, researcher has decided to use mice as population sample in order to use the lower animal levels.

Results

The comparison in glucose levels for the control group, 200, 400, and 600 mg/kg of the mice weight was presented in Figure 3. Based on the Shapiro-Wilk data normality test, it was found that the data distribution of each group had a significance value of < 0.05 with an interpretation of normal data distribution. The value of glucose levels for the control group, 200, 400, and 600 mg/kg mice weight were chronologically presented in Table 1.

Table 1. Glucose Level in Infusion

		Glucose Level in Infusion (mg/dL)			
		15'	30'	45'	60'
Control	1	25.397	17.063	13.492	12.302
	2	24.603	23.413	20.238	15.079
	3	40.079	38.889	29.762	20.635
	4	28.571	25.397	21.825	17.857
	5	34.921	31.349	26.190	19.841
	6	29.762	19.841	17.857	15.476
Mean		30.556	25.991	21.561	16.865
200 mg/kg mice weight	1	112.698	111.508	108.730	107.143
	2	113.095	111.111	107.937	106.746
	3	112.698	110.317	108.333	105.556
	4	112.698	109.921	95.635	90.873
	5	135.714	111.508	95.635	88.095
	6	124.603	121.429	120.635	104.762
Mean		118.585	112.632	106.151	100.529
400 mg/kg mice weight	1	142.857	141.270	139.286	119.841
	2	129.365	127.381	118.651	140.476
	3	146.825	119.048	114.286	109.921
	4	142.460	132.937	122.619	102.381
	5	137.302	130.556	129.365	123.810
	6	138.492	120.635	124.603	123.016
Mean		139.550	128.637	124.801	119.907
600 mg/kg mice weight	1	122.619	121.429	120.238	117.460
	2	117.063	115.873	113.492	105.159
	3	121.825	118.651	112.302	110.317
	4	154.365	146.825	142.857	134.524
	5	157.540	150.794	149.206	144.444
	6	146.825	139.683	135.317	132.143
Mean		136.706	132.209	128.902	124.008

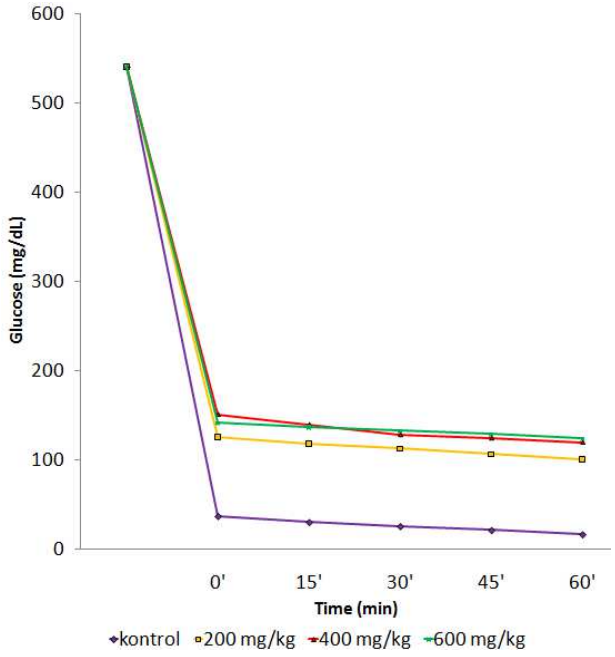

Figure 3. Glucose Level in Infusion

Discussion

Based on previous studies, it is known that starfruit leaves have flavone derivatives that exhibit an effect in lowering glucose levels.[1,15,16] The flavone derivatives found in starfruit leaves consisted of apigenin-6-C-β-$_L$-fucopyranoside and apigenin-6-C-(2-O-α-L-rhamno-pyranosyl)-β-1-fucopyranoside[15,16] Based on early studies about starfruit leaves; it was found that the starfruit leaf had an effect of reducing blood glucose level and increasing the entry of glucose into the soleus muscle of mice.[1,14,17] The decreasing of blood glucose level and increasing of glucose entry into muscles are caused by the starfruit leaves' effect towards glucose transportation and insulin release from β cells of the pancreas.[1]

Based on Figure 3, there was a gradual decrease in blood glucose level from minute 0 to minute 60 in every group that showed that there was glucose absorption through the intestinal epithelial cells of mice in each group. In previous studies, there were results from the administration of starfruit leaves extract that intervened to decrease the glucose level based on measurements of mice blood samples.[1] The lowest blood glucose level decrease by infusion was found in the test group with 600 mg/kg infused starfruit leaves. A previous study also found that the administration of 400 mg/kg starfruit leaves extract had the most potent effect in lowering the blood glucose level in mice.[1]

There is a significant difference between the administration of infused starfruit leaves and control solution on the decrease of glucose absorption. Based on that, the infused starfruit leaves inhibits the glucose absorption in the intestinal epithelial cells of mice. The glucose rate of 600 mg/kg infused starfruit leaves during every 15 minutes had a higher value compared to the control group, 200 and 400 mg/kg infused starfruit leaves. This is caused by the bigger values of active substances in 600 mg/kg infused starfruit leaves that led to a bigger intervention in glucose absorption compared to other groups. The limitation of this study is that the researcher does not have definite knowledge about the molecular mechanism of infused starfruit leaves on glucose transport in the intestinal epithelial cells of mice.

Conclusions

The conclusion of this study is that the administration of infused starfruit leaves decreases glucose absorption in the intestinal epithelial cells of mice with the highest rate of glucose absorption occurring in the first 15 minutes. The 600 mg/kg infused starfruit leaves had the highest inhibitory effect on glucose absorption compared to the control group and other infusions.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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