AMJ. 2019;6(2):80–5

Annona Muricata L Leaf Infusion Effect on Glucose Absorption in the Intestinal Cells Membrane of Wistar Rats Model

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Abstract

Background: Diabetes mellitus (DM) is a chronic disease that disrupts the homeostasis of glucose. DM is the second of most causes of deaths in urban areas, as well as the sixth in rural areas. Therefore, herbal medicine is constantly developed. Soursop leaves have been long known to have good effects in lowering glucose levels in our blood. This study explored the soursop leaves infusion on glucose absorption in the intestinal cell membrane of the *Wistar* Rats Model.

Methods: This was an exploratory study performed in October 2012 on five male rats *Wistar*, aged 3-4 months, weighing about 150-300 grams. Using the in situ perfusion method, each rat was given the following; 3 X 10-3 M glucose solution for one hour; 3 X 10-3 M glucose solution with 1 mL of infusion of soursop leaves (*Annona muricata L*) for one hour; 3 X 10-3 M glucose solution for the last hour. The glucose absorption levels were measured using spectrophotometry method. The glucose level between treatments was analyzed using a paired sample t-test.

Results: The concentration of glucose absorption was decreased, the first treatment was 24.42+14.29 mg/dL, the second treatment was 18.63+10.37 mg/dL, and the third treatment was 18.24+8.02mg/dL), however, the decrease of glucose absorption was statistically not significant.

Conclusions: There is a slightly decreased of the glucose absorption in the intestinal cell membranes of the *Wistar* Rats Model after infusion of soursop leaves. Further study is needed to explore the role of soursop leaves infusion in glucose absorption.

Keywords: Diabetes mellitus, glucose absorption, soursop leaves, *Wistar* Rats

Introduction

All parts of the *Annona muricata (A. muricata)* tree are claimed to be used in herbal treatment, including the bark, leaves, root, and fruit-seeds. It commonly called "Soursop". The fruit is one of many fruits that extensively used to prepare candies, syrups, ice cream, and beverages and also provide benefits for our health. It has been long used as a herbal medicine for different purposes, like pain relief for arthritic pain, neuralgia, as an astringent for diarrhea, to cool fever, malaria, anticancer and it is also eaten to elevate a mother's milk after childbirth. The bark, leaves, and roots are considered had anticonvulsant activity, antispasmodic, hypoglycemic, and hypotensive.[1–4]

Several studies have been conducted to reveal the functions of soursop leaves. Many bioactive compounds and phytochemicals have been found in *A. muricata* leaves, but the majority is *annonaceous acetogenins*, that play a role as antidiabetic. The leaf aqueous extracts proved beneficial as antioxidant, hypolipidemic and protection of β-cells that believes beneficial in diabetic.[1,5]

The soursop leaves (*Annona muricata*) affect to lower the glucose level in our blood by inhibiting α-glucosidase that can delay enhancement of blood glucose level. However, as of today, there have been no data in situ using the perfusion method of soursop leaves on glucose absorption in the small intestines. With its capability to lower glucose level in the blood, it is very likely there will be any effects found in this research.[1,3,6]

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Methods

This experimental research was an exploratory study in situ perfusion method.[7] Five adult male Wistar rats were included, aged 3-4months, weighing 150–300 grams. The research was conducted at Biochemistry Laboratory, Faculty of Medicine, Universitas Padjadjaran, and approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran.

About 200 g soursop leaves were mixed and heatedat 90^0C with 200 mL of aquadest for 15 minutes. After the adaptation period for 7 days, the rats were fasted for about 20 hours and were anesthetized with ketamine. Rat abdominal was dissected and two cannulas inserted in the intestine; 10 cm from the pylorus and 25 cm from the first cannula. The intestines were cleaned by a 0.9% NaCl solution and by blowing the cannulas three times. The cannulas were connected to the vessel filled with a solution. In the first hour, 25 mL glucose solution was given, continued in the next hour with 25 mL glucose solution and with additional the soursop leaves infusion, then

Table 1 The Concentration of Glucose Absorption at Baseline Before Soursop Treatment

Rats no.	Time (minutes)	Glucose Concentration (mg/dL)	Glucose Absorption (mg/dL)	Average Glucose Absorption (mg/dL)
	0	106.47		
	15	100.00	6.46	
1	30	89.55	16.91	18.53
	45	81.59	24.87	
	60	80.59	25.87	
	0	126.66		
	15	99.48	27.17	
2	30	88.20	38.46	39.49
	45	80.51	46.15	
	60	80.51	46.15	
	0	121.46		
	15	94.14	27.31	
3	30	84.39	37.07	38.17
	45	78.04	43.41	
	60	76.58	44.87	
	0	102.01		
	15	99.49	2.51	
4	30	96.48	5.52	5.78
	45	94.97	7.03	
	60	93.97	8.04	
	0	57.14		
	15	39.90	17.23	
5	30	36.28	20.86	20.13
	45	36.05	21.08	
	60	35.82	21.31	
Average				24.42

Table 2 The Concentration of Glucose Absorption during Soursop Treatment

Rats no.	Time (minutes)	Measured of Glucose Concentration (mg/dL)	Concentration of Glucose Absorption (mg/dL)	Average Concentration of Glucose Absorption (mg/dL)
	0	105.97		
	15	79.60	26.37	
1	30	78.61	27.36	26.87
	45	78.61	27.36	
	60	79.60	26.37	
	0	110.26		
	15	87.18	23.08	
2	30	81.54	28.72	27.31
	45	82.05	28.21	
	60	81.03	29.23	
	0	102.93		
	15	92.69	10.24	
3	30	80.98	21.95	20.37
	45	78.54	24.39	
	60	78.05	24.88	
	0	103.52		
	15	104.02	-0.50	
4	30	103.02	0.50	1.88
	45	102.01	1.51	
	60	97.49	6.03	
	0	53.06		
	15	36.28	16.78	
5	30	36.28	16.78	16.72
	45	36.51	16.55	
	60	36.28	16.78	
Average				18.63

followed by the last one hour of which 25 mL glucose solution was again given. The sample was taken every 15 minutes, consisting of the residual concentration of glucose absorption (Peridochrom Glucose/GOD-PAP reagent kit). The concentration of glucose absorption was measured by a spectrophotometer with a wavelength of 505 nm, and calculated using the formula. [7–9]

The analysis was performed using paired sample t-test, to compare the concentration of glucose absorption between treatments.

Results

The concentration of glucose absorption before and during the treatment of soursop leaves infusion was shown in Table 1 and Table 2 to explore the effect of soursop leaves infusion. The average level of glucose absorption before treatment was 24.42 mg/dL (Table 1). The average level of glucose

Table 3 The Concentrations of Glucose Absorption After Soursop Treatment

Rats no.	Time (minutes)	Glucose Concentration (mg/dL)	Glucose Absorption (mg/dL)	Average Concentration of Glucose Absorption (mg/dL)
	0	82.58		
	15	67.66	14.92	
1	30	65.92	16.66	16.60
	45	65.67	16.91	
	60	64.67	17.91	
	0	108.71		
	15	98.71	10.00	
2	30	91.79	16.92	15.13
	45	92.30	16.41	
	60	91.53	17.17	
	0	119.02		
	15	109.51	9.51	
3	30	98.53	20.48	20.61
	45	98.53	20.48	
	60	87.07	31.95	
	0	103.26		
	15	102.26	1.00	
4	30	94.72	8.54	8.54
	45	92.96	10.30	
	60	88.94	14.32	
	0	78.00		
	15	64.05	13.94	
5	30	50.11	27.89	30.30
	45	42.85	35.14	
	60	33.78	44.21	
Average				18.24

absorption when used soursop leaves infusion in the second treatment was 18.63 mg/dL (Table 2), whereas the average level of glucose absorption after soursop treatment was 18.24 mg/dL (Table 3). Furthermore, there was a tendency of the lower level of glucose absorption when soursop leaves infusion was given, however, the decrease was statistically not significant. The effect of soursop leaves infusion was further irreversible in one hour after the treatment (Table 4).

Discussion

Soursop leaves infusion has a tendency to inhibit glucose absorption in the Rat's intestine cell membrane. Our study has shown that the average concentration of glucose absorption is decreased even though the decrease is not statistically significant. The decrease in concentration of glucose absorption may be due to effect of bioactive substances of soursop leaves such as phenol, flavonoid, alkaloid, and triterpenoid. These components inhibit the

Table 4 The Average of Glucose Absorption Level Before, During and After Soursop Treatment

Rats no.	Before (mg/dL)	During (mg/dL)	After (mg/dL)
1	18.53	26.87	16.60
2	39.49	27.31	15.13
3	38.17	20.37	20.61
4	5.78	1.88	8.54
5	20.13	16.72	30.30
Mean ± sd.	24.42+14.29	18.63+10.37	18.24+8.02
p-value		0.26[a]	0.39[b]

Note: p-values of<0.05 were considered statistically significant, [a]Paired sample t-test between first and second treatment (pre and during Soursop treatment), [b]Paired sample t-test between first and third treatment (pre and after Soursop treatment)

activities of α-glucosidase, an enzyme that plays a role a key of breaking down starch or carbohydrates. The enzyme α-glucosidase is a hydrolase enzyme that catalyzes the reaction of the non-reductive terminal hydrolysis of substrates to produce α-glucose. Inhibition of α-glucosidase activities may inhibit glucose absorption.[6,10–12] However, soursop leaves contain a chemical substance glycoside, which is a molecule consisting of sugar group (glycone) and non-sugar group (aglycone or genin).With glucose content in the glycoside, it is most likely that soursop leaves affect the overall glucose levels, to disguise from the effectiveness of other compounds that help glucose absorption.[6]

The inhibitory effect of soursop leaves infusion is irreversible despite also not significant, indicating that after administration of soursop leaves infusion, glucose absorption levels do not return to normal.

In conclusion, soursop leaves (Annona muricata) has slightly an inhibitory effect on glucose absorption irreversibly. More studies are in need to explore further the role of soursop in the antihyperglycemic treatment.

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