The Effect of Mangosteen Rind Glucose Transport in Small Intestine Cell Membrane of Wistar Rats

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Abstract

Seven percent of the people in the United States have hyperglycemia, which a third of them are unaware with the condition. To prevent this from occurring, mangosteen rind can be taken as daily supplements. Its fruit hull has been used as traditional medicine in Southeast Asia countries. The objective of this research is to study the effect of mangosteen rind (Garcinia mangostana) on glucose transport across small intestine cell membrane of wistar rats. Perfusion method of mangosteen rind was used to carry out this experiment. Nine rats were used after overnight fasting. They were divided into three groups where each group received 20%, 40% and 60% of mangosteen rind. Each rat received simple glucose solution as the control and glucose with mangosteen rind infusion as the treatment. The sample was taken every 15 minutes for 1 hour from the small intestine and was measured using a spectrophotometer. The results were not statistically significant for all three different concentration of mangosteen rind. The mangosteen rind has inhibitory effect but it is not statistically significant on glucose absorption across small intestine cell membrane. Further investigation is needed to be done by using higher concentration of mangosteenrind infusion.
Keywords: *Mangosteen rind, Garcinia mangostana), antihyperglycemic, glucose transport, small intestine*

Efek Kulit Manggis terhadap Transpor Glukosa Melalui Membran Sel Epitel Usus Halus Tikus Wistar

Abstrak

Banyak orang tidak sadar bahwa mereka menderita hiperglikemia. 7% dari orang-orang di Amerika Serikat, hampir sepertiga dari mereka saat ini tidak menyadari bahwa mereka memiliki hiperglikemia. Untuk mencegah hal ini, kulit buah manggis dapat dijadikan sebagai suplemen harian karena memiliki banyak manfaat. Kulit buahnya telah digunakan sebagai obat tradisional di negara Asia Tenggara karena anti-inflamasi, antiulkus dan antiseptik. Tujuan dari penelitian ini adalah untuk mempelajari pengaruh dari kulit buah manggis (Garcinia mangostana) pada transportasi glukosa yang melintasi membran sel usus kecil pada model tikus wistar. Metode perfusi dari kulit buah manggis digunakan dalam percobaan ini. Sembilan tikus digunakan dipuasakan selama 8-10 jam. Tikus tersebut dikelompokkan menjadi tiga kelompok dan masing-masing kelompok menerima 20%, 40% dan 60% dari kulit buah manggis. Setiap tikus menerima larutan glukosa sederhana sebagai kontrol dan glukosa dengan kulit buah manggis sebagai eksperimen. Sampel diambil dari usus kecil lalu diukur dengan spektrofotometer. Ditemukan hasil yang tidak signifikan secara statistik pada semua kelompok dengan tiga konsentrasi berbeda kulit manggis. Kulit buah manggis memiliki efek penghambatan tetapi tidak signifikan secara statistik pada penyerapan glukosa melalui membran sel usus kecil.
Kata Kunci*: Kulit manggis, Garcinia mangostana, antihiperglikemik, transpor glukosa, usus kecil*

Introduction

The main role of dietary carbohydrate is to provide energy. Carbohydrate in the diet is classified into monosaccharides and disaccharides, which are also known as simple sugars that can be found in fruits, honey, milk and artificial sweeteners. Another form of carbohydrate is polysaccharides, known as complex carbohydrate found in plants in the form of starch. The common sources of starch are potatoes, grains, wheat, and beans. Another form of carbohydrate is dietary fiber or non-digestible carbohydrate which are present in plants.[1]

Carbohydrate is absorbed in the small intestine in the form of glucose. Glucose could not enter directly into the epithelial cell membrane but by sodium independent facilitated diffusion. Glucose binds to GLUT-2 transporter, found in the small intestine and act to glucose across the cell membrane. The movement of glucose follows the concentration gradient, which does not require energy. Insulin increases the glucose uptake by increasing the number of GLUT-2 in the cell membrane.[1] When there is a decrease in glucose utilization, increase in glucose production and reduced insulin secretion, it will eventually lead to hyperglycemia.[2] Hyperglycemia is the excess of glucose in the blood.[3] If hyperglycemia is not treated, it may lead to complications.

Nowadays, people are too busy working and never taking a good care of their health. Hence, they may unknowingly suffer from hyperglycemia due to its asymptomatic effect. Seven percent of the people in the United States, nearly a third of them are presently unaware of having hyperglycemia.[4] To prevent this from occurring, mangosteen rind can be taken as daily supplements due to its many benefits. Its fruit hull has been used as traditional medicine in Southeast Asia because of its anti-inflammatory, antiulcer and antiseptic purposes. The mangosteen fruit, *Garcinia mangostana,* is one of the most attractive tropical fruits. Mangosteen tree is widespread in Southeast Asia and are now highly popular because of its biological activity. This study will show awareness of the effects of mangosteen rind on hyperglycemia, as this will be very useful for people with their busy lifestyle to help maintaining their health. Thus, mangosteen rind can be used as preventive medicine in treating hyperglycemia. The objective of this research is to determine the effect of mangosteen rind on the glucose transport across small intestine cell membrane of wistar rats.

Methods

The experiment was conducted in October-November 2015 at the Biochemistry Laboratory, Faculty of Medicine Universitas Padjadjaran. The experiment has been approved by the Ethical Clearance Committee of the Faculty of Medicine Universitas Padjadjaran. The study is a laboratory experiment using homogenous subject. Research materials used for this experiment were mangosteen rind infusion, distilled water, glucose solution 3mM, sodium chloride solution 0.9%, anesthetic solution of ketamine, alcohol 70%, artificial perfusion equipment, reagent set for the examination from ST Reagensia, eppendorf micropipette size 50 µL, 100 µL, 0.5mL, 1 mL equipped with disposable tips, test tubes, minor surgical tool, disposable syringe 3cc, spectrophotometer, centrifuge equipment, cannula, stopwatch, infusion pot, thermometer, sample bottles, and gloves. Tools used for the experiment were rat cage, food container, water bottle, and table lamp.

The mangosteen fruit was purchased from the local market in Bandung and approved by Department of Biology, Faculty of Mathematics and Science Universitas Padjadjaran. The mangosteen rind was boiled at 40-45^{o}C until all the moisture from the rind is evaporated. The mangosteen rind was grinded into fine powder and left to dry. The powder was weighed (20, 40 and 60 grams) using a digital scale, then put into the beaker and aquades was added until it reached 100mL. The solution was removed into the infusion pot and left to boil at 90^{o}C for 15 minutes. A sieve was used to filter out the powder from the infusion pot.[5]

Federer's formula was used to obtain the health of male wistar rats aged 3–4 months old weighing 250-300 grams used in this study. The rats were placed in the Pharmacological Department of Medical Faculty of Universitas Padjadjaran for adaptation and access to food and water. Before the experiment, the rats were fasted overnight

The rat was anesthetized with 0.5ml of ketamine. The abdomen was dissected and a cannula was inserted into the intestine firmly at a distance of 10 cm from the pylorus and 25cm after. Two solutions were given to the rat, which were 25 ml glucose solution for 60 minutes and 24 ml glucose solution with added 1ml mangosteen rind infusion with the conentration of 20% powder for 60 minutes. One mililiter of sample was taken every 15 minutes from the intestine. These steps were repeated for 40% and 60% of mangosteen rind infusion.[6]

Trichloroaceticacid (TCA) 8% was used for every sample collected at every 15 minutes in 60-minute period, then centrifuged for 10 minutes at 3000 rpm. The supernatants were mixed with glucose reagents and left for 20 minutes at room temperature. A spectrophotometer was used to measure the samples at a wave length of 5nm.[6] Paired t-test was used to analyze the data and saphiro-wilk was used to analyze the normality of the test. The data was analyzed using the SPSS software. The results were considered significant when $p \leq 0.05$.

Results

Table 1 and figure 1 below showed the average of absorbed glucose for all 3 rats in every 15 minutes for 60 minutes without mangosteen rind and with mangosteen rind. The average absorbed glucose for 5 minutes without mangosteen rind was 65.862mg/dL and with mangosteen rind was 63.334mg/dL. This shows that there is a slight inhibition in 5 minutes. For 15 minutes the average absorbed glucose without mangosteen rind was 64.253mg/dL and with mangosteen rind was 65.287. This shows that there is no inhibition in 15 minutes. For 30 minutes the average absorbed glucose without mangosteen rind was 72.987 mg/dL and with mangosteen rind was 71.264 mg/dL; there is a slight inhibition in 30 minutes. For 45 minutes the average absorbed glucose without mangosteen rind was 76.092 mg/dL and with mangosteen rind was 81.034 mg/dL meaning there is no inhibition in 45 minutes. For 60 minutes the average absorbed glucose without mangosteen rind was 81.149 mg/dL and with mangosteen rind was 82.873 mg/dL, which shows that there is also no inhibition in 60 minutes.

Table 1. Absorbed Glucose with 20% Mangosteen Rind

Sample collected for every 15 minutes	Average absorbed glucose (mg/dL) or all 3 rats		Inhibition (%)
	Control	Mangosteen rind	
5	65.862	63.334	3.838
15	64.253	65.287	-1.609
30	72.987	71.264	2.361
45	76.092	81.034	-6.495
60	81.149	82.873	-2.124

The negative sign means that there is no inhibition in 15, 45 and 60 minutes

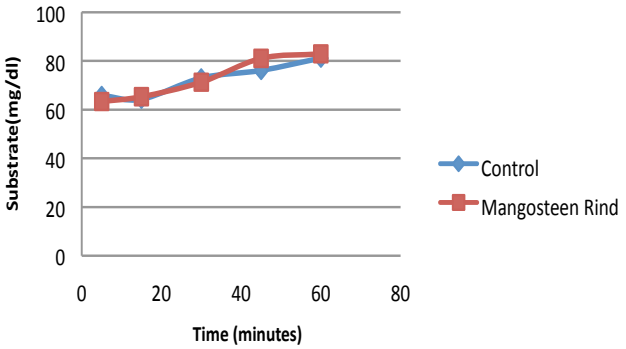

Figure 1. Absorbed Glucose with 20% between Control and Treatment Group

Table 2 and figure 2 showed the average absorbed glucose for all 3 rats in every 15 minutes for 60 minutes without mangosteen rind and with mangosteen rind. The average absorbed glucose for 5 minutes without mangosteen rind was 44.87mg/dL and with mangosteen rind was 52.681mg/dL. This shows that there is a no inhibition in 5 minutes. For 15 minutes the average absorbed glucose without mangosteen rind was 79.371mg/dL and with mangosteen rind was 75.408mg/dL; there is a slight inhibition in 15 minutes. For 30 minutes the average absorbed glucose without mangosteen rind was 80.536 mg/dL and with mangosteen rind was 84.266mg/dL meaning there is no inhibition in 30 minutes. For 45 minutes the average absorbed glucose without mangosteen rind was 89.394mg/dL and with mangosteen rind was 89.161mg/dL. This shows that there is a slight inhibition in 45 minutes. For 60 minutes the average absorbed glucose without mangosteen rind was 93.939mg/dL and with mangosteen rind was 87.879mg/dL; shows that there is inhibition in 60 minutes.

Table 2. Absorbed Glucose with 40% Mangosteen Rind

Sample collected for every 15 minutes	Average absorbed glucose (mg/dL) in all 3 rats		Inhibition (%)
	Control	Mangosteen rind	
5	44.87	52.681	-17.408
15	79.371	75.408	4.993
30	80.536	84.266	-4.64
45	89.394	89.161	0.261
60	93.939	87.879	6.451

The negative sign in the percentage of inhibition shows that there is no inhibition in 5 and 30 minutes

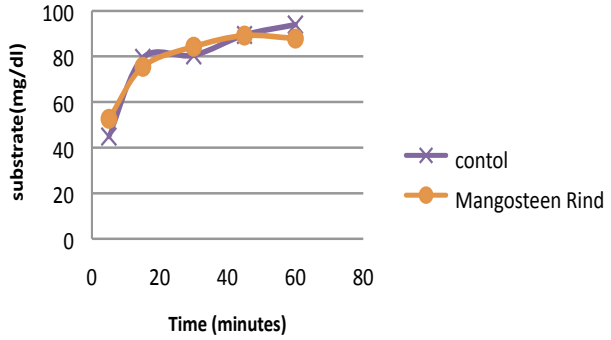

Figure 2. Absorbed Glucose with 40% between Control and Treatment Group

Table 3 and figure 3 showed that the average absorbed glucose for all 3 rats in every 15 minutes for 60 minutes without mangosteen rind and with mangosteen rind. The average absorbed glucose for 5 minutes without mangosteen rind was 77.208mg/dL and with mangosteen rind was 63.818mg/dL; there is a inhibition in 5 minutes. For 15 minutes the average absorbed glucose without mangosteen rind was 78.781mg/dL and with mangosteen rind was 75.499mg/dL. This shows that there is a slight inhibition in 15 minutes. For 30 minutes the average absorbed glucose without mangosteen rind was 89.316mg/dL and with mangosteen rind was 79.202mg/dL meaning there is inhibition in 30 minutes. For 45 minutes the average absorbed glucose without mangosteen rind was 92.593mg/dL and with mangosteen rind was 77.635mg/dL. This shows that there is inhibition in 45 minutes. For 60 minutes the average absorbed glucose without mangosteen rind was 95.156mg/dL and with mangosteen rind was 82.194mg/dL. This shows that there is inhibition in 60 minutes.

Table 3. Absorbed Glucose with 60% Mangosteen Rind

Sample collected for every 15 minutes	Average absorbed glucose (mg/dL) in all 3 rats		Inhibition (%)
	Control	Mangosteen rind	
5	77.208	63.818	17.343
15	78.781	75.499	4.166
30	89.316	79.202	11.324
45	92.593	77.635	16.155
60	95.156	82.194	13.622

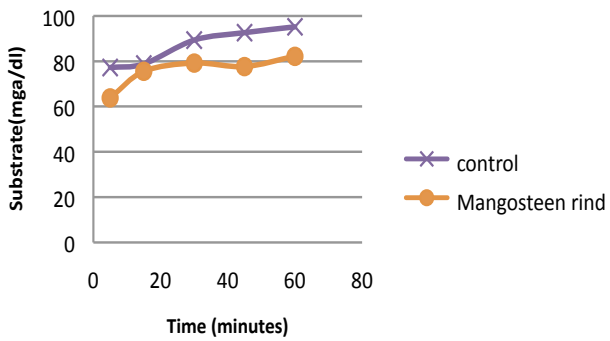

Figure 3. Absorbed Glucose with 60% Mangosteen Rind between Control and Treatment Group

Table 4 showed the absorbed glucose between control and treatment with mangosteen rind with the concentration of 20 grams has a p value of 1.000 which was p>0.05. Hence, there are no changes between the control and the treatment group. Absorbed glucose between the control and treatment with mangosteen rind with the concentration of 40 grams has a p value of 0.368 which was p>0.05, thus, there are no changes between the control and the treatment group. The absorbed glucose between the control and treatment with mangosteen rind with the concentration of 60 grams has a p value of 0.101 which was p>0.05, therefore no changes between the control and the treatment group.

Table 4. Average Absorbed Glucose between Control and Treatment Group

Absorbed glucose		Average	z/t calculation	P Value
20% concentration	Control	72.07	0.000	1.000*
	with MR	72.76		
40% concentration	Control	77.62	-1.153	0.368
	with MR	77.88		
60% concentration	Control	83.93	2.909	0.101
	with MR	78,35		

Discussion

The result from table 1 shows that the highest absorbed glucose is 3.838% in 5 minutes and the lowest is 2.361% in 30 minutes, whereas from table 2 the highest absorbed glucose is 6.451% in 60 minutes and the lowest is 0.261% in 45 minutes. From table 3 the highest absorbed glucose is 17.343% in 5 minutes and the lowest is 4.166% in 15 minutes. Table 4 shows that the result between the control group and the treatment group does not have a difference. Therefore, 20% and 40% are

not statistically significant, however, 60% showed that there was an inhibition, but still not statistically significant. The prenylatedxanthones found in mangosteen rind, which is alpha mangostin has an inhibitory activity on alpha glucosidase in decreasing glucose absorption in the small intestine.[7] However, mangosteen rind infusion did not give a significant effect.

A study by Widjanarko[8] showed that mangosteen peel ethanol extract has a significant effect on lowering the blood glucose level of 250mg/kg BW and 500mg/kg BW in 4 weeks in male wistar rats. This shows that ethanol extract of mangosteen rind has significant effect. From a study done by Atsumi[9] showed that water extract of *Rubus suavissimus* that has been used as an anti-allergic drug had no inhibitory effect on histamine release and prostaglandin E2 synthesis while ethanol extract has inhibitory activity. Another study done by Nagai[10] showed that the constituents of the total phenolic unit were higher in ethanol extract portion than water extract, which shows that the antioxidative activity of ethanol extract is more than water extract.

There are fourteen prenylated xanthones found in mangosteen fruit, which are garcinone C, garcinone D, γ-mangostin, 8-deoxygartanin, gartanin, α-mangostin, garcinone E, β-mangostin, mangostenone A, calabaxanthone, tovophylin B, demethylcalabaxanthone, 11-hydroxy-1-isomangostin, 1,6-dihydroxy-7-methoxy-8-(3-methyl-but-2-enyl)6',6'dimethylpyran ol(2',3',3,2) xanthone. Alpha mangostin has an antihyperglygemic effect by inhibiting alpha glucosidase. This will prevent the catalyzation of hydrolysis of the α-1,4-glucosidic linkage in dissacharides and polysaccharides. Hence, glucose is prevented from being absorbed into the small intestine.[11]

The limitation of the study is that the sample could not be collected at 0 minute because almost half of the solution is being absorbed. From this research, it can be concluded that 20%, 40% and 60% of mangosteen rind infusion does not have significant effect on the transport of glucose across small intestine cell membrane of wistar rat. Further investigation is still needed to be done by using higher concentration of mangosteen rind infusion and mangosteen rind ethanol.

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