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Sparing Muscle Glycogen in Rats with Brown Sugarcane Supplementation

Sparing Muscle Glycogen *Otot pada Tikus dengan Suplementasi Gula Tebu Merah*

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ABSTRACT

Background: Carbohydrates supplementation before exercise is known to delay fatigue in athletes, especially for endurance type of sports. Brown sugarcane (Saccharum officinarum) mostly contains sucrose. The breakdown of sucrose into glucose and fructose is used by the body as an energy-providing substrate, especially when exercising for a long duration – endurance. Consumption of brown sugarcane before exercise is expected to keep blood glucose in normal condition and preventing from muscle glycogen catabolism.
Objectives: This research aimed to investigate the effect of carbohydrate supplementation with brown sugarcane and glucose on blood glucose and muscle glycogen levels.
Methods: 36 male Sprague Dawley rats at 8 weeks old were involved in this study. There were 4 groups of intervention, brown sugarcane + swimming (BS), glucose + swimming (G), water + swimming (W), and brown sugarcane without swimming (S). The dose of intervention was 0,3 g sucrose/100 g body weight of rats. The supplementation was given 10 minutes before doing the swimming activity. A statistical test with SPSS software was used to analyze the results. One-way ANOVA and t-test were used to analyze before and after supplementation.
Results: The results showed that the rats who were given sugar cane supplementation before swimming had a smaller increase in blood glucose than the other groups. The increasing of blood glucose in each group were BS = 7.95 mg/dl; G = 21.19 mg/dl; W = 35.64 mg/dl; S = 4.57 mg/dl; p=0.000. Muscle glycogen levels in the rats given sugar cane supplementation group were higher than in the other groups (p=0.000).
Conclusions: Carbohydrate supplementation with brown sugarcane before endurance type of exercise was able to maintain blood glucose on normal condition and prevent muscle glycogen catabolism in experimental animals. Research on the development of sports spesific products based on brown sugarcane can be carried out to see its effects directly on humans.

ABSTRAK

Latar belakang: *Pemberian suplementasi karbohidrat sebelum olahraga diketahui dapat menunda kelelahan. Gula merah tebu (Saccharum officinarum), diketahui memiliki kandungan utama sukrosa. Pemecahan sukrosa menjadi glukosa dan fruktosa digunakan tubuh untuk substrat penyedia energi, utamanya saat melakukan olahraga dengan durasi waktu yang lama (endurance). Pemberian gula merah tebu sebelum olahraga diharapkan dapat menjaga glukosa darah tetap normal dan mencegah pemecahan glikogen dalam tubuh.*
Tujuan: *Penelitian ini bertujuan untuk mengetahui pengaruh suplementasi karbohidrat dari gula merah tebu dan glukosa terhadap kadar glukosa darah dan glikogen otot.*
Metode: *Penelitian ini melibatkan 36 ekor tikus Sprague Dawley jantan yang berusia 8 minggu. Terdapat 4 kelompok intervensi yaitu tikus yang diberikan gula merah tebu (BS), glukosa (G), dan air aquades (W) sebelum olahraga renang. Kelompok tidak aktif/sedentary yaitu diberikan gula merah tebu namun tidak diberikan intervensi renang (S). Dosis intervensi yang diberikan yaitu 0,3 g gula sukrosa/100 g berat badan tikus. Suplementasi diberikan 10 menit sebelum*

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aktivitas berenang. Analisis statistik menggunakan software SPSS digunakan dalam penelitian ini. Uji menggunakan One way ANOVA dan t-test dilakukan untuk mengetahui perbedaan sebelum dan sesudah suplementasi.
Hasil: *Hasil penelitian menunjukkan bahwa tikus yang diberikan suplementasi gula merah tebu sebelum olahraga renang memiliki kenaikan glukosa darah yang lebih kecil daripada kelompok lainnya. Kenaikan gula darah pada masing-masing helompok BS = 7,95 mg/dl; G = 21,19 mg/dl; W = 35,64 mg/dl; S = 4,57 mg/dl; p=0,000. Kadar glikogen otot pada kelompok tikus yang diberikan suplementasi gula merah tebu lebih tinggi daripada kelompok lainnya (p=0,000).*
Kesimpulan: *Penelitian ini menunjukkan bahwa suplementasi karbohidrat dengan gula merah tebu sebelum olahraga tipe endurance mampu menjaga kadar glukosa darah dalam kategori normal dan mencegah pemecahan glikogen otot pada hewan coba. Penelitian lanjutan untuk pengembangan makanan atau minumana olahraga dengan gula merah tebu dapat dilakukan untuk melihat efeknya pada manusia.*

Kata kunci: *Gula merah tebu, Glukosa, Glikogen, Renang, Karbohidrat*

INTRODUCTION

Optimization of performance for athletes needs a comprehensive approach. An athlete's body will respond to exercise training differently based on duration, intensity, type of exercise, as well as quantity and quality of nutrition[1]. An absence of nutrition before exercise begins will lead the athlete to fatigue condition, especially for athletes who compete in endurance exercises. If it happens over a long time, overtraining syndrome could occur to the athlete and it will reduce the exercise performance[2]. A good plan to feed the athlete before exercise with the right nutrients is necessary to prevent fatigue during a long duration of exercise.

The International Society of Sports Nutrition (ISSN) states that there are a couple of nutrients important for athletes before exercise, but mostly it is about carbohydrate feeding[2]. Simple carbohydrates such as sucrose will give a positive impact to maintain blood glucose during endurance exercises[3,4]. The development of sports products containing simple carbohydrates is needed to give a wide choice for athletes.

A simple carbohydrate generally found in sports food is granulated sugar; if there is >5% of the net weight in food or >2.5% in drinks it is categorized as high in sugar. If we looking for another substitution for white sugar as simple carbohydrates, brown sugarcane could be a good option. Brown sugarcane is widely known as a traditional sugar and has different local names such as *Panela* (Latin America), *jaggery* (India), *kokuto* (Japan), *hakura* (Sri Lanka), *rapadura* (Brazil), *gur* (Pakistan), *gula merah* (Indonesia and Malaysia)[5,6]. Some researches show the benefits of brown sugarcane over granulated sugar[7,8]. Over granulated sugars, brown sugarcane is rich in nutraceutical properties because it contains not just sugars but also vitamin, mineral, and even antioxidant properties[7] Yukiko et al. reported that phenolic compounds detected in brown sugarcane contribute to lowering stress hormone (serum corticosterone) in rats given several conditions of exercise[9]. Sakhtibalan et al. also reported that brown sugarcane contains iron that has benefits in enhancing hemoglobin levels in female students with iron deficiency anemia[10]. It was explained that sugars in brown sugarcane gave benefits to insulin production under the condition of type 2 diabetes mellitus in rats[11].

Brown sugarcane is known to have more complex sugar contents by interacting with other macronutrients and micronutrients[7,12]. Micronutrients such as vitamins, minerals, and bioactive components are able to influence glucose absorption. Research shows that bioactive component, polyphenol can inhibit amylase and glycosidase enzymes which can lead to decreasing glucose absorption. Another mechanism that polyphenols can inhibit is protein transport of glucose since they use the same protein transport in its absorption[7,12]. It also has sucrose as simple carbohydrate content which makes brown sugarcane suitable to be an option as sugar for sports food development[13]. For this reason, we hypothesize that supplementation of brown sugarcane before exercise will give a positive impact on blood glucose and also in sparing glycogen. The novelty of this research is the new study to examine the effect of brown sugarcane on blood glucose and glycogen content under exercise conditions as it isn't reported before. We used experimental research in animal study because analysis of muscle glycogen is hard to execute.

METHODS

Animal Treatment and Preparation

Thirty-six male Sprague-Dawley rats (SD rats) with body weights of approximately 177 g and aged 8 months were used in the study. The rats were purchased from The Center Study of Food and Nutrition, Universitas Gadjah Mada, Indonesia. All the rats were housed in a temperature-controlled room. The study was approved by the ethics commission of animal study from the Faculty of Medicine, Diponegoro University, Indonesia with the registered number 130/EC/H/FK-UNDIP/XII/2020.

The food and water were provided *ad libitum*. The light of our animal room was maintained with a 12:12 h light-dark cycle. After arrival at our animal room, the rats were acclimated for three days before the experiment began.

Preparation of Brown Sugarcane Solution

Brown sugarcane was obtained from producers in the city of Kudus, Central Java, Indonesia. Brown sugarcane craftsmen used sugar cane varieties BR-579 or *Bululawang* (BL) as the basic material for making brown sugarcane. The decree of the Minister of Defense Republic of Indonesia number 322/kpst/SR120/5/2004 states that BL sugarcane variety was officially released in 2004. BL is a sugarcane variety that always grows with the emergence of new shoots. The weight potential of the superior BL varieties of sugarcane will be very high when the shoots continue to

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grow when harvested. Brown sugarcane comes in solid form and then was crushed into granular before being diluted with water.

Solutions of brown sugarcane (30%) (w/v), glucose (30%) (w/v), and water were prepared. The total volume for each rat was 1 ml/ 100 g body weight[14]. The solution was given orally using a sonde. Ten minutes after ingestion of the drink, three groups were administrated swimming exercise and the other one was resting.

Exercise Protocol

Thirty-nine males SD rats were divided into four groups: 1) swimming exercise with brown sugarcane supplement (BS), 2) swimming exercise with glucose supplement (G), 3) swimming exercise with water supplement (W), 4) sedentary rats with brown sugarcane supplement (S). The use of the four groups has the following objectives: glucose supplementation to examine the effect between single type sugar and complex sugar (from brown sugarcane); water supplementation to illustrate exercise conditions without the supply of carbohydrates, and finally, sedentary condition to describe a normal condition when there wasn't any exercise involved. After three days of being acclimated, SD rats began conditioning with swimming exercises for five straight days. The conditioning exercise began at 7 in the morning, with the duration of swimming 5-10 minutes. The water temperature was monitored at 30 - 34ºC with the depth of water about ± 50 cm.

As the conditioning exercise finished, SD rats were administrated into the real experiment. The activity began at 7 in the morning, all the SD rats had blood taken to analyze glucose. After that, the SD rats were given supplementation depending on their group, with doses of 30% (w/v) for 1 ml/100 g body weight[14]. Ten minutes after being supplemented, the rats were doing swimming exercises for 10 minutes with an additional load of 6% body weight. The water temperature was monitored at 30-34ºC with the depth of water about ± 50 cm.

As the SD rats finished their swimming exercise, blood was taken again to analyze glucose. Shortly after the rats finished swimming and blood was drawn, the rats were sacrificed by overdose of ketamine. The soleus muscle tissue was taken and then analyzed for muscle glycogen content.

Analysis of Blood Glucose

The blood glucose variable was taken two times, pre-and post-intervention. The method used for blood glucose analysis was glucose-oxidase by spectro-photometry. The principle of this method is the oxidation of glucose by oxygen with the enzyme glucose oxidase as a catalyst, then it will form gluconic acid and hydrogen peroxide. Hydrogen peroxide will react with 4-aminoantipyrin and phenol with the peroxidase catalyst enzyme catalyst to form quinamine and water. Quinonimine is an indicator that shows blood glucose levels and will be analyzed using spectrophotometry.

Analysis of Glycogen

The muscle glycogen variable was taken at post-exercise. Since the method to collect glycogen samples must be through the dissection of muscle tissue, then the variable can be taken after the rat is sacrificed. Muscle glycogen analysis used the sandwich method - ELISA. Rat soleus muscle tissue was taken and separated from the blood because it could interfere with the ELISA test. An amount of 1 g of muscle tissue would be taken for muscle glycogen analysis. The ELISA kit was obtained from Wuhan Fine Biotech Co., Ltd. The principle of this analytical method is that biotin will capture antibodies that are markers of muscle glycogen. Standards, samples, and biotin detection antibodies will be put into the well and then rinsed using a buffer solution. HRP - Streptavidin is added and if no conjugation is formed, it will be lost during the rinsing process using a buffer solution. TMB substrate is used to visually see the color changes that occur due to enzymatic reactions by HRP. TMB will be catalyzed by HRP and produce a blue color. After adding the acid solution as a stop solution, the blue color will turn yellow. The yellow density will then be seen using a microplate reader using a wavelength of 450 nm.

Statistical Analysis

Data analysis was carried out using SPSS. First, a normality test was carried out to determine the distribution of data. The normality test used the Shapiro-Wilk test. A one-way ANOVA test was conducted to see differences in muscle glycogen levels and blood glucose levels after swimming between groups. Muscle glycogen analysis was used to compare between groups. Blood glucose analysis was performed to see blood glucose levels after swimming exercises between groups. The follow-up analysis used was the Bonferroni post hoc test when the data had the same variant or using Tamhane's post hoc test when the data variants were not the same. The t-test was used to see changes in blood glucose levels, and to compare before and after the intervention in each group.

RESULTS AND DISCUSSION

Effect of Brown Sugarcane Supplementation on Blood Glucose of SD Rats

Body weights and blood glucose of experimental animals before being given supplementation and swimming exercise did not differ much between groups (Table 1). These results indicate that the experimental animals have homogeneous data at baseline. Blood glucose levels were carried out before and after the rats were given intervention.

Blood glucose levels increased significantly after supplementation in each group when tested using paired t-test analysis (Figure 1). Another analysis of post-intervention blood glucose showed different levels between groups (Figure 1). The group that was given brown sugarcane supplementation without sports activity showed the lowest blood glucose value at the end of the study.

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Table 1. Body weights, blood glucose, muscle glycogen concentration, and swimming duration of rats

	Brown Sugarcane	Glucose	Water	Sedentary
Body weights (g)	189.22 ± 3.83*	190.00 ± 5.12*	190.02 ± 3.41*	191.44 ± 5.5*
Blood glucose pre (mg/dl)	71.58 ± 2.06[1,2]	70.94 ± 1.78[1,2]	69.4 ± 2.72[1,2]	71.71 ± 1.78[1,2]
Blood glucose post (mg/dl)	79.53 ± 2.41[1,3]	92.13 ± 1.65[1,3]	105.04 ± 2.63[1,3]	76.28 ± 1.78[1,3]
Muscle glycogen (mg/g)	10.97 ± 0.94[4]	7.36 ± 0.94[4]	5.55 ± 0.93[4]	14.2 ± 1.84[4]

Values are expressed as mean ± standard deviation; *) the results of One Way Anova analysis with p > 0.05; [1]) the results paired t-test to see differences in each group before and after supplementation, p = 0.000; [2]) the results of One Way Anova analysis with p > 0.05; [3,4]) the results of One Way Anova analysis with p = 0.000

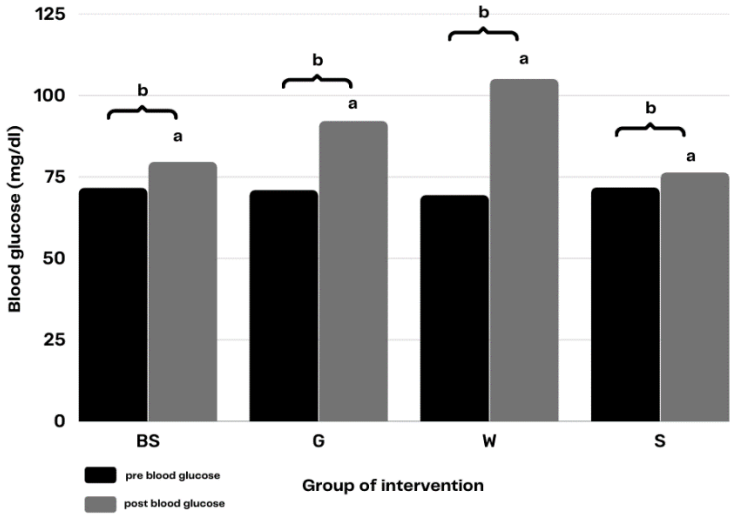

Figure 1. Effect of carbohydrate supplementation on rats blood glucose; [a]) the results of One Way ANOVA with significance p=0.000; [b]) the results of paired t-test before and after supplementation on each group intervention with p=0.000; all data are distributed normally (statistic analysis using Kolmogorov Smirnov)

Fasting rat blood glucose levels have the same normal value as humans, which is <100 mg/dl. A study conducted by Thannoun showed fasting blood glucose values in mice of ± 63.54 mg/dl[15]. The results of fasting blood glucose in this study are similar to the literature. As rats exercise, blood glucose levels are influenced by the presence or absence of carbohydrate intake. Several studies have shown that mice given glucose-type carbohydrate supplementation before exercise, have peak blood glucose levels at 45-60 minutes[15,16]. Another method in the same study showed rats given carbohydrates supplementation peaked at 15 minutes after supplementation[16].

The results of blood glucose analysis after the intervention showed that the group that was not given carbohydrate supplementation had the highest blood glucose levels. This is because the body responds to glucose needs in muscles by breaking down glucose from substrates other than carbohydrates, otherwise known as gluconeogenesis and glycogenolysis[17]. The results of the study on sugar cane supplementation were able to increase blood glucose but not as big as other groups of intervention. This slow rise in blood glucose can indicate that brown sugarcane has a lower glycemic index than glucose. When viewed further related to the development of sports drinks, drinks containing a low glycemic index are suitable to be given before exercise takes place. This can be a reference if the development of sports drinks with brown sugarcane will be carried out, then it can be given before exercising.

A couple of factors could be interfering with the result of blood glucose after supplementation. The level of processing of food ingredients, the content of fructose or lactose, and components of anti-nutritional substances such as phytate or lectins are known to affect the rate of absorption and absorption of foods containing carbohydrates. Also, brown sugarcane has a more complex sugar content, because sucrose binds to other macro and micronutrients so that sugar absorption is slower[7]. Antioxidant content on brown sugarcane also can interfere with the process of glucose absorption. Umeno et al. explained that polyphenol in brown sugarcane suppresses the activities of amylase and glycosidase enzymes which can decrease glucose absorption in the gut[18]. Another research reported that polyphenol content in brown sugarcane altered glucose absorption because it uses the same protein transport as glucose for absorption[11].

The results comparison between brown sugarcane and control (no sugars) supplementation showed that blood glucose after the intervention had a higher level in control group. It can be explained because the source of glucose supply in the control

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group comes from gluconeogenesis induced by the glucagon hormone. Glucagon hormone is sensitive to glucose availability when it comes to exercise[19]. In the control group when there is no supply of exogen carbohydrates, it may enhance glucagon secretion and lead to gluconeogenesis to maintain blood glucose.

Effect of Brown Sugarcane Supplementation on Glycogen

Muscle glycogen levels were analyzed using soleus muscle in experimental animals. Muscle tissue retrieval was carried out after the experimental animal was sacrificed so that the data obtained are the data after the intervention. Muscle glycogen analysis used the Sandwich ELISA method. The results of statistical analysis showed that there were significant differences between groups for muscle glycogen levels (p = 0.000) which can be seen in Figure 2.

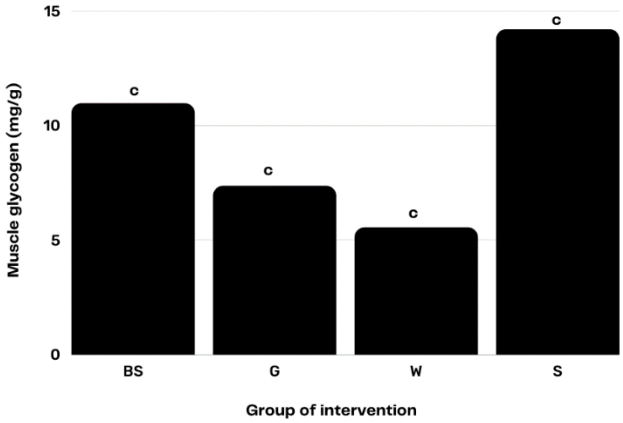

Figure 2. Effect of carbohydrate supplementation on rats soleus muscle glycogen; the data is normally distributed (statistic analysis using Kolmogorov Smirnov); [c]) the results of One Way ANOVA analysis with p = 0.000

Generally, glycogen is found in muscle and liver tissue. The main function of glycogen is to provide energy reserves when the body needs a substrate to produce energy, which means it is important to consider when doing endurance type of sports. The normal value of glycogen stores in muscle in rats is 9 mg/g[14]. A study conducted by Lin et al. stated that normal muscle glycogen levels were 13 mg/g[20]. Muscle glycogen levels will decrease with exercise. Research conducted by Morifuji stated that the muscle glycogen levels of rats after swimming were 6 - 6.5 mg/g[14].

The value of rats' muscle glycogen if compared with literatures was still parallel. This study showed that supplementation of carbohydrates with brown sugarcane before exercise successfully prevents glycogen catabolism. This condition is also called sparing glycogen at endurance exercise. It can be shown from the group of rats with brown sugarcane supplementation had higher content compared to glucose and no sugar supplementation (under the same condition and swimming intervention). Sugar from brown sugarcane supplementation enters the blood circulation and is used as a source for muscle contraction during exercise, and it prevents the breakdown of muscle glycogen.

The results of the analysis of rats' blood glucose and muscle glycogen show that brown sugarcane give a positive impact. At the circular level, this could happen because of several processes. Figure 3 contains the summary of how brown sugarcane will give an effect if the experimental study is applied to humans. First, sucrose from brown sugarcane is the main type of simple carbohydrate that will play a role.

In the gut, sucrose will degrade to form glucose and fructose with the catalyzing of sucrose enzyme[21]. Researches show that supplementation carbohydrate with sucrose is more effective than with glucose or fructose alone because the combination of two monosaccharides gives the best results[22–24]. From the intestinal lumen, glucose will move to the enterocyte through protein transport SGLT1 and fructose through GLUT5[25,26]. Inside the enterocyte cell, glucose will directly distribute to the bloodstream through GLUT2 while for fructose two possible processes will happen. Fructose will change to glucose form or directly transport to the bloodstream as fructose. Both of the molecules will pass through GLUT2[27]. As the exercise happens, the body will give more blood supply to muscles to support its contraction. Glucose in the bloodstream will move to muscle cells through GLUT4. Exercise will give a positive impact on GLUT4 translocation from inside the cell to move on the surface of the cell[25]. This action will increase the uptake of glucose to muscle cells.

Fat of glucose molecules on the muscle cell could happen in four different pathways[25]. Hexosamine pathway, pentose phosphate pathway, glycogen synthesis, and glycolysis can happen to glucose molecules, but when it comes to the exercise condition then glycolysis will dominate it[25,28–30]. The glucose molecule from sucrose degradation will activate the substrate to produce ATP. ATP will be used to maintain muscle contraction during exercise. There are three main sources for the formation of ATP during endurance exercise; blood glucose, fatty acids, and muscle glycogen[28]. It should be noted that fatty acids are obtained from the breakdown of fat tissue, whereas athletes or people who like to exercise tend to have non-dominant fat tissue, so the source of ATP mainly comes from blood glucose and muscle glycogen. As the

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duration of exercise happens in the endurance type, then the glucose molecule is more needed. The storage of muscle glycogen doesn't use catabolism, since the glucose supply comes from brown sugarcane absorption. This condition will lead to glycogen sparing and prevent fatigue if the duration of exercise takes longer.

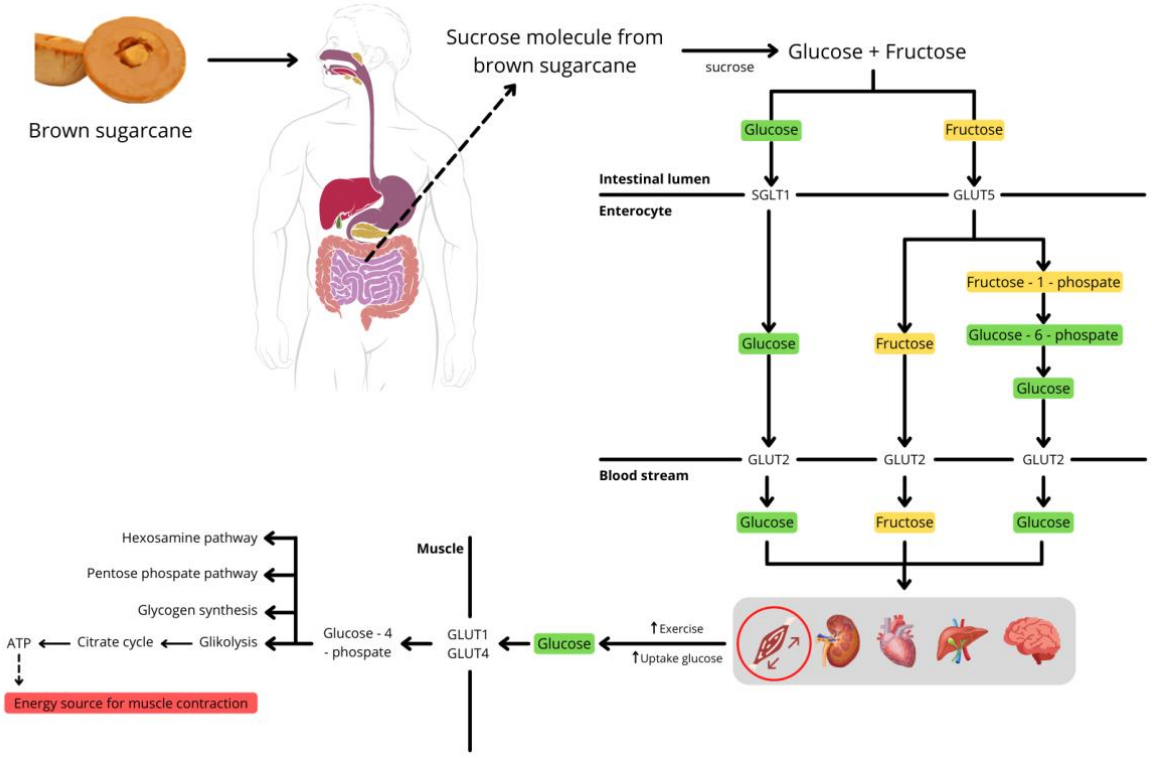

Figure 3. Overview mechanism of the effect of brown sugarcane supplementation on muscle when exercising; SGLT1 – Sodium Dependent Glucose co-Transporters 1; GLUT5 – Glucose Transporters 5; GLUT2 – Glucose Transporters 2; ATP – Adenosine Three Phosphate

This research showed experimental conditions in an animal study, especially for muscle glycogen analysis which still cannot be done in humans because of its invasive method. As there are few differences in glucose metabolism in humans and rats, the results from this study can describe how is the effect if the same condition is conducted in humans. This study also uses brown sugarcane from Indonesia, which can be easily found in the market. The limitation of this study was the supply of glucose for blood from gluconeogenesis cannot yet be explained. In the future, the analysis of muscle glycogen content before intervention can be included to examine if there is a suitable method other than tissue surgery visible to apply.

CONCLUSIONS

Carbohydrate supplementation with brown sugarcane before endurance type of exercise is able to maintain blood glucose and prevent muscle glycogen catabolism in experimental animals. Research on the development of sports-specific products based on brown sugarcane can be carried out to see its effects directly on humans.

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CONFLICT OF INTEREST

All authors have no conflict of interest in this article.

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