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The beetroot (*Beta vulgaris*) powder improves blood pressure and glucose level Wistar rats after high intensity exercise

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Abstract

High-intensity exercise can increase oxygen consumption. Beetroot contains phytochemicals that are biologically active substances able to improve post-exercise conditions. Beetroot can be processed into powder. This study aimed to determine the effect of beetroot powder on the improvement of blood pressure and blood glucose level in Wistar rats that received high-intensity exercise. The thirty Wistar rats were randomly divided into 5 groups (n = 6), they controlled negative (G1); control positive (G2); received exercise and 0.36 g/200 g b.wt/day of beetroot powder (G3); received exercise and 0.72 g/200 g b.wt/day of beetroot powder (G4) and received exercise and 1.08 g/200 g b.wt/day of beetroot powder (G5). The treatment was given once a day after exercise for 28 days. The blood pressure was measured using the S-2 sphygmomanometer while blood glucose levels were measured using the GOD-PAP method by taking retroorbital vein blood. The data were analyzed using paired sample t-test and one-way analysis of variance (ANOVA). The results obtained were differences in blood pressure and blood glucose levels before and after the administration of beetroot powder as much as 0.36 g/200 g b.wt/day; 0.72 g/200 g b.wt/day and 1.08 g/200 g b.wt/day in Wistar rats when receiving high-intensity exercise ($p<0.005$). G4 has the most decrease in systolic blood pressure. G5 showed the lowest increase in blood glucose levels compared to all other groups. Beetroot powder was effective in improving blood pressure and blood glucose levels in Wistar rats who received high-intensity exercise.

1. Introduction

High-intensity aerobic exercise with light recovery or no exercise is a form of high-intensity interval training (HIIT). The accumulated high-intensity physical activity has a minimum duration of 75 mins per week with a maximum heart rate of 85 - 95% (Taylor *et al.*, 2019). The energy contribution in high-intensity exercises comes mainly from glycolytic metabolism and a small contribution from high energy phosphagens and oxidative phosphorylation (Domínguez *et al.*, 2018). Exercise with a short- or long-term pattern can significantly reduce blood pressure in various populations (Costa *et al.*, 2016).

Body metabolism caused by high-intensity exercise can increase oxygen consumption. The maintenance of normal blood pressure is influenced by the balance between cardiac output and peripheral vascular resistance. Exercise, diet, obesity, and genetic changes are factors that affect blood pressure. Systolic blood pressure indicates the highest pressure while diastolic blood pressure shows the smallest pressure in the blood vessels at a given moment. Research conducted by Webb showed that 500 mL of beet juice can lower systolic and diastolic blood pressure. This decrease is caused by the presence of inorganic nitrate content in beets which allows for a pleiotropic effect (Siervo *et al.*, 2013).

Muscle glycogen is the main raw material used during exercise. Muscle glycogen stores will decrease during exercise and will replenish during the recovery period. When muscle glycogen decreases during exercise, muscle capacity will also decrease, leading to fatigue (Betteridge *et al.*, 2016). Foods that contain high antioxidants are beginning to be considered as an alternative to support athletes' performance. The high

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antioxidant content is found in many fruits and vegetables. Exercise can trigger glycogen depletion through the activation of autophagy. Activation of autophagy can stimulate catabolism and activation of protective endogenous mechanisms. Protein quality control, stimulation of endogenous antioxidant defence and even gluconeogenesis are the reactions of protective endogenous mechanisms (Cherkas *et al.*, 2020).

Beetroot is a vegetable that is quite popular among athletes. It is a great source of antioxidants and micronutrients including potassium, betaine, vitamins and nitrates. The colour of beetroot comes from the purple and yellow pigments known as betalains. These betalains have the potential for antioxidant capacity (Ormsbee *et al.,* 2013).

Beetroot is an annual or biennial plant from the family *Beta vulgaris* subsp. *Vulgaris conditiva* that was originated from the Middle East then spread to America, Europe and Asia. Beetroot contains biologically active substances, vitamins, and minerals that are used as supplements to support health and are finally applied as functional food ingredients (Baiáo *et al.*, 2020). The role of beetroot as an additional material to support athlete performance is widely used as an ergogenic (Clifford *et al.*, 2015). This study used beetroot in powdered form because they have low moisture content, are light in weight, have a longer shelf life and are easy to consume. This study was aimed to determine the effect of beetroot powder on Wistar rats that were exposed to high-intensity exercises.

2. Materials and methods

The research was conducted in August - October 2020 at the House of Experimental Rats CNFS, Universitas Gadjah Mada, Yogyakarta, Indonesia. Research ethics eligibility letter number 067/KEPK/EC/2020 from the Committee on the Ethics of Health, Universitas Negeri Semarang, Indonesia. Facilities for maintaining, providing training and handling experimental animals during the study using the Guidelines for Care and Use of Laboratory Animals of CNFS Universitas Gadjah Mada, Yogyakarta, Indonesia. This study used 30 male Wistar rats provided by the CNFS House of Experimental Rats, Universitas Gadjah Mada, Yogyakarta, Indonesia, 4 weeks old, 180 to 240 g of body weight and adapted for seven days prior to the study. Wistar rats were placed in individual cages made of stainless steel with a temperature of 25±1°C, normal humidity, and had a cycle of dark and light of 12:12 hours each. Each Wistar rat was given 20 g of standard chow with ad libitum water. Daily cage cleaning was performed to reduce stress levels in Wistar rats and to maintain their health.

2.1 Preparation of beetroot powder

The beetroot was obtained from the organic beetroot farming in the villages of Pakis and Ngablak, Magelang Regency, Central Java. The making of beetroot powder was in accordance with Rahayu's research (Rahayu *et al.*, 2020). The process of making beetroot powder started with sorting then washing, cutting and drying. The beetroot sorting process aims to reduce physical and microbiological hazards such as soil, gravel and caterpillars. The process of washing beetroot using running water was then followed by the cutting process using a copper bowl and drying under sunlight for one day. The beetroot was cut to 1 mm in size to speed up the drying process. The next step was crushing the beetroot using a juice extractor before placing the beetroot crush into a spray dryer to produce the powdered form (Figure 1). The determination of the vitamin C and flavonoid levels of beetroot powder was carried out at the Laboratory of the Food and Agriculture Center for the Food Technology Study Program, Faculty of Agricultural Technology, UNIKA Soegijapranata Semarang, Indonesia.

Figure 1. Schematic of beetroot powder

2.2 Preparation of high-intensity exercise and experimental design

Wistar was chosen as an experimental animal because it has metabolic activities similar to humans. The high-intensity exercise performed by Wistar rats was swimming for 30 mins for 5 days/week. The swimming pool size was 50×30 cm with a depth of 25 cm and a water temperature of 28°C. Each Wistar rat received 20 g/day standard chow and beetroot powder in the morning after swimming with a dose of Group 3 (G3) was 0.36 g/200 g b.wt /day; Group 4 (G4) as much as 0.72 g/200 g b.wt/day and Group 5 (G5) as much as 1.08 g/200 g b.wt/day. The beetroot powder given to each group was previously diluted in 3 mL of water and given through a gastric sonde for 28 days. The dosage of beetroot powder in this study was based on the flavonoid content (Carreiro *et al.*, 2016) and the portion of vegetables and fruit on DASH Diet (Clements *et al*., 2014; Alissa and Ferns, 2017).

The Wistar rats were randomly divided into five groups consisting of 6 rats each. G1: negative control rats (did not exercise and did not receive any treatment); G2: positive control group (exercised rats, but did not receive any treatment); G3: Exercised rats that received beetroot powder 0.36 g/200 g b.wt./day; G4: exercised rats that received beetroot powder 0.72 g/200 g b.wt/day; G5: exercised rats that received beetroot powder 1.08 g/200 g b.wt/day.

2.3 Parameter analysed

Blood pressure and blood glucose levels of the Wistar rats were measured before and after the intervention. Blood pressure was measured using the S-2 sphygmomanometer made in Germany using the tail-cuff method. Wistar rats' blood pressure was measured 5 mins after the rats were placed on the device. Blood glucose levels were measured using the GOD-PAP method. The blood samples were from the retro-orbital vein and centrifuged at 3000 rpm for 15 mins to obtain serum. Blood glucose levels were measured using a spectrophotometer. The Wistar rats were sacrificed through cervical decapitation and then burned in the incinerator at the end of the study.

2.4 Statistical analysis

Statistical data for each group used means±standard deviation (SD). Paired sample t-test was used to determine the differences before and after the intervention in each group. The differences between groups were assessed by one-way analysis of variance (ANOVA). If one-way ANOVA yielded significant results, Tukey was performed for intergroup comparisons using the least significant difference test. The p-values were considered statistically significant when they were <0.05.

3. Results and discussion

Beetroot contains phytochemicals such as flavonoids, polyphenols, vitamins, inorganic nitrates (NO_3) and minerals such as potassium, sodium, phosphorus, calcium, magnesium, copper, iron, zinc, and manganese. Previous studies showed that vitamin C content and total flavonoid content in beetroot juice were 10.75-20.36 mg/100 g and 2.02-2.36 mg/100 g, respectively (Mirmiran *et al.*, 2020). Meanwhile, the beetroot powder used in this study contains 60.29 mg/100 g of vitamin C and 254.12 mg/100 g of total flavonoids. Beetroot is classified as one of the plants that have the highest antioxidant activity due to the betalains content that is consistently stable even when processed into powder form, dye extract or other derivative forms (Khan, 2016). Betalains belong to the soluble fibres group and nitrogen-containing pigments synthesized from tyrosine. Betalains are divided into two groups: red -violet (betacyanins) and yellow (betaxanthins). At room temperature, betacyanins are more stable than betaxanthins because of their glycosylated structure (Azeredo, 2009). Betalains content in beetroot allows them to have a stable colour. This colour stability depends on pH and temperature. In this study, the powder form does not affect the betalains content in the beetroot. The use of powder form is also based on the research that revealed that betaxanthin has a higher susceptibility to temperatures than betacyanin (Ceclu and Nistor, 2020).

Figure 2 shows the gain of body weight in each group of Wistar rats. Those given beetroot powder at1.08 g/200 g BW/day had the highest body weight gain after the intervention compared to all groups. Inorganic nitrate (NO_3-) levels in beetroot powder can increase NO levels in the digestive system. NO_2- nitrate is produced by NO_3 - which is assisted by the NO_3- reductase enzyme. Swimming exercise with a duration of 30 mins for 5×/ week did not cause weight loss of the Wistar rats during the study. The carbohydrate content in the chow and beetroot powder did not allow weight loss to occur in each treatment group. The main source of fuel for energy production is carbohydrates which occur through the high energy phosphagen system and glycolysis. The high energy phosphagen system and glycolysis gradually provide up to 50% energy (Domínguez *et al.*, 2017).

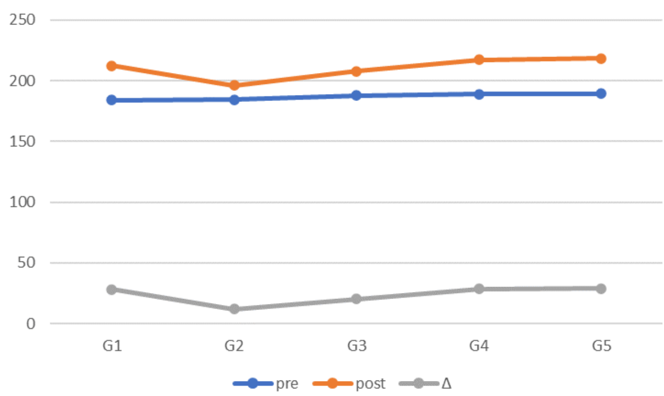

Figure 2. Body weight in Wistar rats during the study

Table 1. Mean level of parameters in Wistar rats

Parameters tested	G1[a]	G2[b]	G3[c]	G4[d]	G5[e]
Systolic blood pressure					
pre	130.17±16.63	157.50±10.29	152.17±7.70	152.33±3.98	149.00±6.78
post	125.83±8.47	135.00±6.48	119.83±7.28	118.00±4.43	116.67±5.32
Δ	4.33±9.87	22.50±14.75	32.33±13.13	34.33±6.06	32.33±7.87
p	0.002[b,c,d,e]	0.001*[a]	0.001*[a]	0.001*[a]	0.014*[a]
Diastolic blood pressure					
pre	89.83±16.10	121.83±6.21	125.17±4.88	122.67±6.77	120.83±2.64
post	92.33±21.05	116.00±20.56	87.00±4.43	85.50±2.17	83.00±3.22
Δ	2.50±7.18	5.83±17.79	38.17±5.38	37.17±6.31	37.83±3.31
p	0.001[c,d,e]	0.001[c,d,e]	0.001*[a,b]	0.001*[a,b]	0.014*[a,b]
Blood glucose levels					
pre	69.38±1.48	68.32±0.99	69.79±1.62	70.26±2.09	69.67±1.74
post	70.97±1.51	112.88±3.78	94.32±1.72	88.89±1.32	81.20±3.38
Δ	1.59±1.20	44.57±3.76	24.54±2.39	18.63±2.81	11.53±2.43
p	0.001*[b,c,d,e]	0.001*[a,c,d,e]	0.001*[a,b,d,e]	0.001*[a,b,c,e]	0.001*[a,b,c,d]

Sampling was done 28 days after start of treatment.
GI: Control negative; G2: control positive; G3: exercise, treated with beetroot powder of 0.36 g/200 g b.wt/day; G4: exercise, treated with beetroot powder of 0.72 g/200 g b.wt/day; G5: exercise, treated with beetroot powder of 1.08 g/ 200 g b.wt/day.
Values represent the mean±SD for observation mode on six rats in each group
Statistical analysis: p*; paired t test, significant difference ($p<0.05$); One-way analysis for variance (ANOVA), where significant, post hoc testing (least significant difference) was done for intergroup comparisons.
[a]Statistically significant difference ($p<0.05$) when compared with G1 values
[b]Statistically significant difference ($p<0.05$) when compared with G2 values
[c]Statistically significant difference ($p<0.05$) when compared with G3 values
[d]Statistically significant difference ($p<0.05$) when compared with G4 values
[e]Statistically significant difference ($p<0.05$) when compared with G5 values

3.1 Blood pressure in Wistar rats

Blood pressure measurements were taken twice, before exercise and 15 mins after exercise. Table 1 shows that there is a significant difference in systolic blood pressure before and after the intervention in the high-intensity training group which was given the intervention of 0.36 g of beetroot powder/200 g b.wt / day (G3); 0.72 g/200 g b.wt /day (G4) and 1.08 g /200 g b.wt /day (G5). The Tukey test showed that there was a difference in systolic blood pressure in high-intensity exercise and beetroot powder groups compared to the group that was not given exercise and intervention (G1). The highest decrease in systolic blood pressure showed in the group that was provided with 0.72 g of beetroot /200 g b.wt/day (34.33±6.06 mmHg), although it was not significantly different compared to the positive control group (G2).

In this study, a difference in diastolic blood pressure before and after the intervention in all groups that were treated with beetroot powder was found. The difference in diastolic blood pressure was also observed in the control and the intervention groups. The highest decrease in diastolic pressure is in the G3 which was treated with 0.36 g of beetroot powder/200 g b.wt/day. This study showed that giving 0.36 g of beetroot powder and the equivalent of 0.22 mg flavonoid had a reduction effect on diastolic blood pressure.

This study is in line with previous research which reported that the consumption of 140 mL of beetroot juice (12.9 mmol nitrate content) did not affect the total VO2 or work performance after being given a specific rating of perceived exertion (RPE) 13 exercise but could reduce resting systolic blood pressure (Rienks *et al.*, 2015). Increasing exercise intensity will increase blood pressure, heart rate, and lung function. Blood pressure reduction, either systolic or diastolic, indicates a change in body metabolism as an adaptation to high-intensity exercise and the effect of beetroot powder administration. The possible mechanism of beetroot powder in reducing blood pressure is because beetroot powder may trigger the epinephrine hormone release. The hormone causes the dilation of the blood vessel so that blood volume decreases and triggers the extracellular release of blood plasm, which leads to blood pressure reduction (Manansang *et al.*, 2018).

In addition, beetroot powder reduces blood pressure through the bioconversion of nitrates to nitric oxide. The digestive process can convert nitrate to nitrite to be stored or circulated in the blood. When oxygen availability is low, nitrite can be converted to nitric oxide

(NO) (Ferguson *et al.*, 2013). NO is a gaseous signalling molecule that modulates physiological functions and metabolic control, such as regulation of bloodstream, neurotransmission, immune function, glucose and calcium homeostasis, muscle contractility and mitochondrial respiration. NO is produced through the oxidation of the amino acid L-arginine in the catalyzed reaction by NO synthase (NOS) with nitrite (NO_2-) and nitrate (NO_3-) resulting in NO as the oxidation product. Under suitable physiological conditions, NO can be generated through the NO_2- reduction. This reduction process becomes important, especially when oxygen availability is low and NOS function is impaired (Amaral *et al.*, 2019).

Consumption of beetroot powder, which is one of the sources of NO_3-, can increase the plasma concentration of NO_2- and produce NO-like bioactivity (Seremet *et al.*, 2020). The absorption process of NO_3- reaches 25% when it enters the enterosalivary circulation and is concentrated in saliva. Anaerobic bacteria in the oral cavity can reduce NO_3- to NO2-. Then, some NO_2- will turn to NO due to the presence of chloric acid (HCl) in the stomach while the rest will be absorbed to increase plasma circulation (NO_2-). This NO_2- will be reduced to be converted into NO and other reactive nitrogen intermediates when exercise-induced hypoxic tissue (Wylie *et al.*, 2013).

Nitrite compounds have a vasodilating effect on blood vessels and provide resistance to homocysteine. Homocysteine is an intermediate product of amino acids from the methionine to the cysteine cycle. Homocysteine is a marker of chronic inflammation in blood vessels and can lead to congestion of the venous bloodstream (Domínguez *et al.*, 2017). Beetroot also contains potassium and phytochemical compounds, such as flavonoids (Nandani and Sofyaningsih, 2019). The potassium content in beetroot is 325±4.5 mg approximately. Potassium is important in regulating nerve and muscle function and reduces the use of adenosine triphosphate in muscles. Another possible mechanism of lowering blood pressure is the fibre content in beetroot powder. Fibre can bind cholesterol and various proinflammatories that trigger inhibition of the bloodstream. Cholesterol and various proinflammatory bound by fibres are then excreted through faeces that can suppress the increase in blood pressure (de Lade *et al.*, 2018).

3.2 Blood glucose level in Wistar rats

Table 1 shows that blood glucose levels before and after treatment in all groups are significantly different before and after treatment ($p < 0.05$). The post-hoc test showed that each group had a significant difference either in the control group or the intervention group. The highest blood glucose levels increase is occurred in the high-intensity exercise group but not given any intervention (G2). G5 which was given beetroot powder intervention 1.08 g/200 g b.wt /d for 28 days showed the lowest blood glucose levels increase compared to all groups.

This study is in line with research that stated that dietary nitrate can improve insulin sensitivity through stimulation of NO generating pathways. The glucose regulation can be done by increasing NO bioavailability. This event begins with dietary nitrate ingestion on the digestive process (Beals *et al.*, 2017). Glucose expenditure does not increase with exercise when insulin is absent. This indicates that before glycogen is depleted, a stimulus to increase glucose uptake will be made. The increase in the ability of insulin to stimulate glucose utilization and glycogen synthesis is contingent upon the exercise level. Increasing blood glucose levels during high-intensity exercise occurs because catecholamine triggers glycogenolysis in the liver and stimulates the epinephrine and norepinephrine hormones. The process of increasing blood glucose levels during exercise also occurs due to the gluconeogenetic activity of corticosteroids (Sarov and Vlaykova, 2005).

In this study, blood glucose levels have decreased, this process occurs due to intracellular signal transduction modifications as the primary mechanism to decrease blood glucose levels. This mechanism starts with hormonal activation, inhibition of α-amylase and α-glucosidase, and an increase in paraoxonase 1 (PON1). An increase in the stress hormone, such as cortisol, as one of the consequences after exercise also triggers an increase in gluconeogenesis followed by a decrease in glucose concentration. It is related to the secretion of the Adrenocorticotropic hormone (ACTH) and its action on the adrenal cortex level. The decrease in glucose levels due to beetroot powder is also influenced by the polyphenols, betanins and neobetanins content as a result of degradation products, which also affects the nitrite-nitrate pathway or inhibition of hormonal reductase activity (Baiáo *et al.*, 2020). The bioactive components in beetroot can reduce the carbohydrate digestion rate and activate Adenosine Monophosphate Protein Kinase (AMPK), leading to a reduction in intestinal glucose absorption that also reduces the postprandial glucose response (Mirmiran *et al.*, 2020).

4. Conclusion

Beetroot powder is effective in reducing blood glucose levels, systolic and diastolic blood pressure in Wistar rats that performed high-intensity exercise. Beetroot powder is recommended after high-intensity exercise to maintain the stability of blood pressure and blood glucose level.

Conflict of interest

The authors declare no conflict of interest.

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