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Impact of supplementation with beetroot juice (*Beta vulgaris* L) on levels of malondialdehyde and antioxidant status in athletes

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ABSTRACT

Strenuous physical activity can increase lipid peroxidation, which is characterized by increased levels of malondialdehyde (MDA) and decreased levels of antioxidants. It can drop the athlete's performance and health. It is possible to avoid or reduce lipid peroxidation through supplementation with antioxidants. Beetroot (*Beta vulgaris* L) produces different forms of natural antioxidants; however, its effectiveness still needs to be investigated. This research aimed to determine the impact of beetroot juice on the malondialdehyde concentration and antioxidant status during strenuous physical activity. The research used experimental (pre-test-post-test) using a control group. The researchers divided a total of 30 students who met the criteria into two groups (Experiment; n = 15; control; n = 15). The experimental group drank 250 mL of the juice 1 hour before training for four weeks. After the strenuous physical activity was carried out with a beep test, the researchers also checked the concentrations of MDA, TAC, and VO₂max. The findings indicated a decrease in MDA concentration and an increase in TAC and VO₂max to the training group that received 250 ml beet juice (p <0.05). The research concluded that beetroot juice administration during exercise could reduce malondialdehyde concentration and increase total antioxidant capacity and VO₂max in athletes.

Keywords: beetroot juice, malondialdehyde, antioxidants, strenuous physical activity, exercise

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INTRODUCTION

Exercise causes various physiological changes of varying magnitude and direction, depending on the exercise characteristics and the subject's health and training level. Oxygen flux can be increased by 100-200 times during strenuous exercise because of an improvement in metabolism in the body (Joyner and Casey, 2015). Enhanced use of oxygen predominantly by muscle contraction, increasing mitochondrial electron leakage that will become reactive oxygen compounds (Nemes et al., 2018). Generally, 2-5% of the oxygen in the body used in metabolic processes can become superoxide ions so that free radical development increases during intense physical activity (Simioni et al., 2018). Oxidative stress will occur when free revolutionary development exceed antioxidants for cellular protection, where physical activity is one of the contributing factors (Kamura and Muraoka 2018; Marius-Daniel et al., 2010). In oxidative stress conditions, free radicals can cause lipid peroxidation to the cell membrane resulting in cell damage (Ayala et al., 2014; Evans 2000). During strenuous physical exercise or high-intensity resistance training, malondialdehyde will form as a result of lipid peroxidation (Bao et al., 2016; Liu et al., 2019; Xu et al., 2017). The increase in MDA concentration during strenuous physical exercise has been used as an indicator measuring the number of free radicals formed (Liu et al., 2019).

The findings show that it will cause a decrease in endogenous antioxidant concentration (Thirumalai et al., 2011). Meanwhile, recorded that volleyball athletes running a distance of 20 meters could cause lipid peroxidation characterized by an increased concentration of malondialdehyde and decreased antioxidant catalase concentration glutathione peroxidase.

Due to maximum and sub-maximum physical activity, decreased antioxidant levels and increased CK and lipid peroxidation can be prevented by improving diet, primarily by increasing antioxidants' content (Kim et al., 2005; Pingitore et al., 2015; Simioni et al., 2018). Accordingly, (Gomez-Cabrera et al., 2008) said that the oxidative damage that occurs after strenuous physical activity is preventable by consuming high antioxidant content. Vegetables and fruits with antioxidant-rich are more effective than single antioxidants such as vitamin E. The components and interactions of antioxidant-rich vegetables and fruits may play a positive role.

Beetroot (*Beta vulgaris* L) contains various types of natural antioxidants. One of them is betalain as a substance with healthy antioxidants, which can be an effective antidote to free radicals (Clifford et al. 2015). Other compounds that act as powerful antioxidants in beetroot include betaine (Liliana and Oana-Viorela, 2020; Zhao et al., 2018), phenolic acids, vitamin C, carotenoids, triterpenes, coumarins, and flavonoids. The beetroot's flavonoid compounds include tiliroside, rhamnetin, kaempferol, astragaln, rhamnocitrin (Lilia and Oana-Viorela, 2020; Mimiran et al., 2020), cochliphilin, betavulgarin, tagarin, and dihydroisorhamnetin (Kujala et al., 2002). Phenolic acid compounds include N-trans-feruloyltyramine, N-trans-feruloylhomovanillylamine (Kujala et al., 2002), caffeic acid, gallic acid, ferulic acid, chlorogenic acid, quercetin, p-coumaric acid, kampferol, syringic acid, ellagic acid, myricetin, ellagic acid and vanillic acid (Chhikara et al., 2019; Koubaier et al., 2014). Several studies have reported that giving beets in the form of juice can have anti-anaemia effects (Putri and Tjijptaningrum, 2016), blood pressure and lipid-lowering (Hobbs et al., 2012), anti- inflammation (Clifford et al., 2015), anticancer (Lechner and Stoner, 2019), antioxidative (Kozłowska et al., 2020), anti-diabetic (Sanjeev et al., 2020).

Researchers are interested in the speciality of beetroot with different types of antioxidants. They need to analyze the antioxidant impact of supplementation with beetroot juice (BRJ) on malondialdehyde and antioxidant status in maximal physical activity.

MATERIALS AND METHOD

Materials

Beetroot (*Beta vulgaris* L) obtained from the Medan MMTc market, 1% EDTA solution, Aquadest, MDA Kit and TAC purchased from Shanghai, China.

Tool

Spectrophotometer (Shimadzu), glassware, sput, juicer, cones, multistage fitness test audio, compact disk, sheet for performance recording

Methods

Participants

This research used 30 sports science students who were called athletes. The research criteria were male, aged 20-22 years, had an excellent VO₂max level, were not smokers, and had an acceptable Body Mass Index (BMI). Two weeks before and during the study, vitamins and antioxidants were not used, preparing it for the subject. Then obtaining Research and ethical permission from the Faculty of Medicine Ethical Commission, University of North Sumatra No:277 / KEP / USU/2020

Research implementation

The athletes led to haematological examination to measure MDA and TAC levels after strenuous physical activity by performing a beep test (pretest). Furthermore, dividing them into two groups: the experimental group (given beet juice, n = 15) and the control (as a placebo, n = 15). During four weeks, the athletes consumed 250 mL of beetroot juice (BRJ) 1 hour before training. Then, they performed strenuous physical activity after one month by doing a beep test and continuing to another haematology examination to measure the MDA and TAC levels (post-test).

MDA and TAC determination

Researchers drew blood (5 mL) before and after treatment to test for MDA and TAC and brought it to the laboratory. Blood is taken from the antecubital vein and put into a test tube containing an anticoagulant agent. To analyze the MDA and TAC, researchers used ELISA (enzyme-linked immunosorbent assay) colourimetric approach and followed the procedures defined in each MDA and TAC kit.

Data Analysis

Researchers reported all study data as mean and standard deviation. The research data were analyzed using IBM statistics for windows 25. The research data were analyzed using IBM statistics for windows 25. Statistical analysis using a paired t-test used to determine the average difference between pretest and post-test data. Statistical analysis using independent samples t-test used to determine the difference between the experimental and control groups. The statistical significance set at $P < 0.05$ before analyses

RESULT AND DISCUSSION

Impact of beetroot juice (*Beta vulgaris* L) on malondialdehyde levels

The pre-test malondialdehyde (MDA) amount in the treatment group was 1.98 ± 0.37 nmol/ml based on the research results, while the control group was 2.11 ± 0.24 nmol/mL. The results of statistical tests showed that there was no difference in malondialdehyde levels between the experimental group and the control group. The MDA measurement results were 5.75 ± 0.51 nmol/ml in the post-test treatment group, while the control group received MDA levels of 10.69 ± 0.97 nmol/mL. Differences in levels of pre-test-post-treatment MDA treatment groups and control groups ($p=0,000$) were obtained from the results of the analysis using the paired sample t-test. The independent sample t-test statistical test results found a substantial difference in post-test MDA levels between the treatment and control groups ($p = 0,000$)

In the analysis, MDA levels in the group that did not contain beet juice was higher than in the group containing beet juice. High MDA levels in the group that did not contain beet juice indicated that free radicals produced during strenuous physical activity which oxidized the cell membranes (Figure 1).

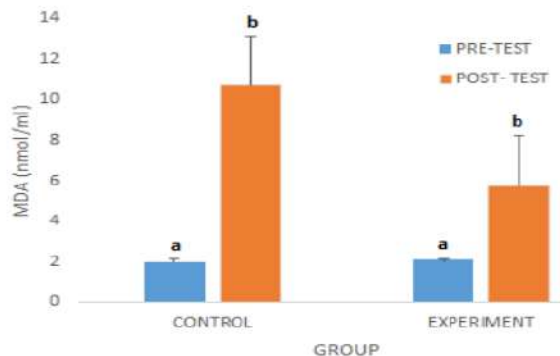


Figure 1. Impact of supplementation with BRJ on the level of malondialdehyde. In each group, the data is mean \pm SD for n=15. Different letters suggest a significant difference between paired t-test samples at $p < 0.05$

Several reports support the findings of research results, including reporting that acute aerobic physical activity contributes to oxidative stress, especially when exercising at high intensity. Two mechanisms that cause oxidative stress in high-intensity aerobic exercise are increased pro-oxidants through the effect of increasing oxygen consumption 10 to 15 times compared to rest and relatively insufficient antioxidants compared to pro-oxidants (Alessio et al., 2000). Meanwhile, according to (Ji, 1999), oxygen consumption throughout the body increases to 20 times during maximum physical activity, while oxygen consumption in muscle fibers is estimated to increase to 100 times. Free radicals induce lipid peroxidation of cell membranes under oxidative stress conditions and destroy the organization of cell membranes (Evans, 2000). Increased levels of MDA triggered by physical activity have been reported by many researchers, including (Moflehi et al., 2012) who examined the effect of aerobic exercise with different intensities on increasing levels of MDA and CK in people who are not athletes. This study's findings show that the higher the exercise intensity, the greater the amount of MDA. In this study, aerobic exercise for 20 minutes with an intensity of 80% can increase MDA levels compared to controls (9.09 ± 2.08 Vs $2.69 \pm 1.32 \mu\text{mol/L}$). Meanwhile, research conducted reported that volleyball athletes who carried out a 20 meter shuttle run test with VO_2max levels of $41.78 \pm 4.91 \text{ mL/kg/min}$ turned out to increase MDA levels from $1.41 \pm 0.30 \text{ nmol/ml}$ to $2.06 \pm 0.08 \text{ nmol/L}$, while non-athletes with VO_2max levels of $26.91 \pm 3.67 \text{ ml/kg/min}$, MDA levels increased from $0.94 \pm 0.24 \text{ mL/kg/min}$ to $1.10 \pm 0.21 \text{ mL/kg/min}$. In this research, giving beetroot juice during exercise can decrease MDA levels when athletes perform full physical activity compared to MDA levels in the control group. The decrease in MDA levels is due to the antioxidant content found in beets (*Beta vulgaris* L). It is known, beets contain antioxidants including phenolic compounds, flavonoids, vitamin C, carotenoids and betalains. Betalains contained in beets are betacyanin and betaxanthine (Georgiev et al., 2010; Kujala et al., 2002; Niri and Angelino, 2013). Some in vitro research findings indicate that betalaine pigments can protect cellular components from oxidative injury (Kanner et al. 2001; Tesoriere et al. 2008). In the study by Kanner et al. for example, two betalain metabolites (betanine and betanidine) showed a reduction in linoleic damage caused by cytochrome C oxidase and metmyoglobin-activated H_2O_2 and free iron (AA-Fe) mediated lipid membrane oxidation. The

researchers also report that betanin, the most abundant betalain (300-600 mg/kg) found in beets, is the most powerful lipid peroxidation inhibitor. Betanin's high antioxidant activity is thought to derive from its remarkable capacity to donate electrons and its power to disperse very reactive radicals that attack cell membranes (Kanner et al. 2001). Also, beets contain NO_3 compounds (nitrates) which have been shown to suppress the formation of free radicals such as superoxide and hydrogen peroxide (Lundberg et al., 2011; Winkler et al., 2005).

Impact of Beet Juice (*Beta vulgaris* L) on Total Antioxidant Capacity (TAC)

The research results obtained pretest TAC levels in the treatment group were 2.53 ± 0.06 U/ml, while the control group was 1.50 ± 0.13 U/mL. The results of statistical tests showed that there was no difference in TAC levels between the experimental group and the control group. The measurement of the TAC post-test of the treatment group was 3.39 ± 0.08 U/mL, while the control group obtained TAC levels of 2.46 ± 0.1 U/mL. Statistical tests using the t-test showed differences in pre-test-post-test TAC levels in the experimental group or the control group ($p = 0.000$). The results of statistical tests using the independent samples t-test showed a significant difference in post-test TAC levels between the treatment and control groups ($p = 0.000$).

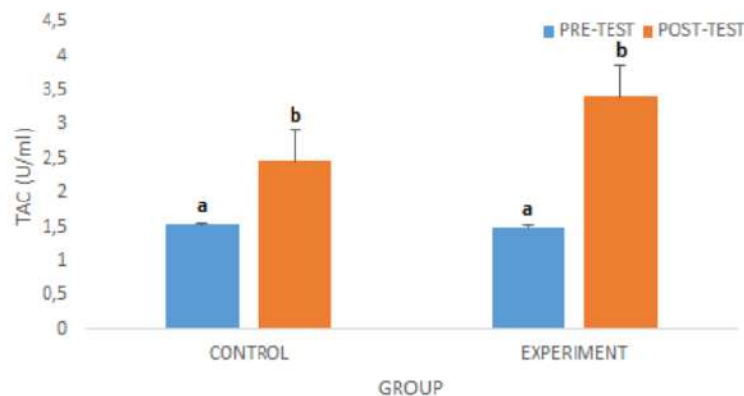


Figure 2. Impact of supplementation with BRJ on the level of total antioxidant capacity. In each group, the data is mean \pm SD for $n=15$. Different letters suggest a significant difference between paired t-test samples at $p < 0.05$

Giving beetroot juice in Figure 2 shows that TAC levels will increase when compared to the control group. Increased TAC levels due to beetroot juice are due to the antioxidant content of beets such as betaine, phenol compounds, flavonoids, vitamin C, carotenoid and betalains. Betalains contained in beets are betacyanin and betaxanthine (Georgi et al., 2010; Kujala et al., 2002; Ninfali and Angelino, 2013). Research conducted by Kujawska et al., on the antioxidant and anti-inflammatory effects of beetroot juice on oxidative stress induced by N-nitrosodiethylamine (NDEA) and carbon tetrachloride (CCl_4) administration confirms the findings of this report. In his research, giving beetroot juice to mice given NDEA and CCl_4 can reduce the process of lipid peroxidation in the liver, which is indicated by a 38% reduction in TBARS levels. In his research, pretreatment with juice induced a partial recovery of 35% and 66%, respectively, of glutathione peroxidase and glutathione reductase activity. Superoxide dismutase activity was approximately 3-fold increased in juice pretreated animals. Both xenobiotics caused an increase in carbonyl plasma protein, which was decreased by 30% in rats pretreated with juice and then injected with NDEA (Kujawska et al., 2009). Analysis performed by Lu et al. (2009) has also confirmed the findings of

the study. In the study, they investigated the radioprotective role of beetroot betalate in light-irradiated rats (60) Co gamma (6.0 Gy, at a dose of 1.5 Gy min⁻¹). They randomly grouped the rats into five, namely the control group and four experimental groups, which obtained one of four beetroot betalain (0, 5, 20 and 80 mg/kg for 30 days) concentrations. Gamma Co (ray) 60 was subsequently exposed to the four groups of experimental mice and betalains were administered from beets for the next three days. The study results showed that administration of betalains from beetroots was radioprotective in mice irradiated by (60) Co in vivo. The underlying mechanism was thought to be mediated by betalain's antioxidant activity from beetroots and immune system modulation.

8 Impact of giving beetroot juice (*Beta vulgaris* L) on VO₂max

The pretest VO₂max levels in the treatment group were 47.59 ± 0.44 mL/kg/min based on the findings, while the control group was 46.75 ± 0.53 mL/kg/min. The study's findings using separate t-test samples showed no differences between the treatment group and the control group in pretest VO₂max levels. The result of the post-test measurement of VO₂max levels for the treatment group were 52.95 ± 0.57 mL/kg/min, while the control group obtained VO₂max levels of 50.53 ± 0.87 mL/kg/min. The analysis results using the Paired Sample t-test showed an increase in VO₂max levels in the pre-post-test treatment group or the control group (p = 0.000). The statistical test results with independent samples t-test showed a substantial difference between the treatment and control groups in post-test VO₂max levels (p=0,000).

6 Figure 3 shows that beetroots juice administration during exercise can increase VO₂max compared to the control group. This increase in VO₂max occurs due to nitrate content and antioxidant content possessed by beetroots. The results showed 500 ml of beetroot juice containing nitrates as much as 5.1-6.2 mmol (Bailey et al., 2009; Lansley et al., 2011). Beet tubers have been shown to affect pulmonary oxygen uptake (VO₂). The results of the study on the consumption of inorganic nitrate (5.1 mmol nitrate/day) in the form of 500 ml beet juice for six days in 7 adult men (19-38 years), can reduce lung oxygen uptake (VO₂) at exercise intensity so that it can delay fatigue time (Bailey et al., 2010).



Figure 3. Impact of supplementation with BRJ on VO₂max. In each group, the mean ± SD is mean ± SD for n=15. Different letters suggest a significant difference between paired t-test samples at p < 0.05

The mechanism of action of nitrate (NO₃⁻) to improve athlete's performance is as follows: Nitrate by bacteria in saliva will be reduced to nitrite (NO₂⁻). Furthermore, in the stomach nitrite will be reduced to nitric oxide (NO) (Lundberg et al., 2011; Shiva et al., 2007). Nitric oxide is an

important signaling molecule with a central role in many physiological processes that can influence sports performance, such as blood flow regulation of tissues, muscle contraction, mitochondrial biogenesis, and absorption of muscle glucose (Stamler and Meissner, 2001). Moreover, improved blood flow from the synthesis of nitric oxide will improve tissue processes recovery (Bloomer, 2010).

CONCLUSION

The research concluded that beetroots juice administration during exercise could decrease MDA levels and increase TAC and VO₂max levels of the athlete.

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