

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

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Submission date: 19-Oct-2022 03:03PM (UTC+0700)

Submission ID: 1929489084

File name: 13._FAJAR_APOLLO_SINAGA_ICOSTA.rtf (1.16M)

Word count: 4011

Character count: 22968

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

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Submitted :..... Reviewed :..... Accepted:.....

ABSTRACT

Lipid peroxidation, which is marked by increased malondialdehyde levels and decreased antioxidant levels, may be increased by strenuous physical activity. This can contribute to the athlete's decreased 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, but its effectiveness in reducing lipid peroxidation caused by physical activity needs to be investigated. The goal of this study was to determine the impact of beetroot juice on the malondialdehyde concentration and antioxidant status during strenuous physical activity. The research used was experimental (pre-test-post-test) using a control group. A total of 30 students who met the criteria were divided 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 that, strenuous physical activity was carried out using a beep test, and the MDA, TAC, and VO₂max concentrations were checked again. The findings indicated a decrease in MDA concentration as well as an increase in TAC and VO₂max to the training group that received 250ml beet juice (p <0.05). The study concluded that the administration of beetroot juice during exercise could reduce malondialdehyde concentration and increase total antioxidant capacity and VO₂max in athletes.

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

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INTRODUCTION (11pt)

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, resulting in an increase in mitochondrial electron leakage that will become reactive oxygen compounds (Nemes et al. 2018). Generally, 2-5 percent 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 radical development exceeds antioxidants for cellular protection, where physical activity is one of the contributing factors (Kawamura and Muraoka 2018; Marius-Daniel, Stelian, and Dragomir 2010). In conditions of oxidative stress, free radicals can cause lipid peroxidation to the cell membrane resulting in cell damage (Ayala, Muñoz, and Argüelles 2014; Evans 2000). During strenuous physical exercise especially 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 amount of free radicals formed (Liu et al. 2019).

The findings show that when undergoing heavy and prolonged physical exercise, it will cause a decrease in endogenous antioxidant concentration (Thirumalai et al. 2011). In the meantime, (Bulduk 2011) recorded that volleyball athletes running a 20-metre shuttle run can cause lipid peroxidation, characterized by a rise in malondialdehyde concentration and a decrease in catalase and glutathione peroxidase antioxidant concentrations.

Due to maximum and sub-maximal physical activity, decreased antioxidant levels and increased CK and lipid peroxidation can be prevented by improving diet, especially by increasing the content of antioxidants (Kim et al. 2005; Pingitore et al. 2015; Simioni et al. 2018). Accordingly, (Gomez-Cabrera, Domenech, and Viña 2008) said that oxidative damage due to physical activity can be prevented by consuming foods with high antioxidant content. According to (Yulia 2015) the efficacy of antioxidants will be more effective when consuming antioxidant-rich vegetables or fruits of various types rather than using a single antioxidant such as vitamin E. This may be due to the presence of other components and their interactions in vegetables and fruit those that play a positive role.

It is known, beetroot (*Beta vulgaris* L) contains various types of natural antioxidants. One of them is betalain, which is a substance that has strong antioxidants that can to be an effective scavenger 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 flavonoid compounds in beet root include biliroside, rhamnetin, kaempferol, astragalol, rhamnocitrin (Liliana and Oana-Viorela 2020; Mirmiran et al. 2020), cochliophilin, betavulgarin, betagarin, and dihydroisorhamnetin (Kujala et al. 2002). Phenolic acid compounds in red beetroots include trans-feruloyltyramine, N-trans-feruloylhomovanillylamine (Kujala et al. 2002), caffeic acid, gallic acid, ferulic acid, chlorogenic acid, quercetin, p-coumaric acid, kampferol, sinigic acid, ellagic acid, myricetin, ellagic acid and vanillic acid (Chhikara et al. 2019; Koubaier et al. 2014). A number of studies have reported that giving beets in the form of juice can have anti-anemia effects (Putri and Tjiptaningrum 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), antidiabetic (S et al. 2020).

Researchers are interested in the specialty of red root beet that has different types of antioxidants and 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

Red 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 study used 30 sports science students. The study criteria were male, aged 20-22 years, had a good VO₂max level, were not smokers, had a good Body Mass Index (BMI), two weeks before and during the study, vitamins and antioxidants were not used, were prepared to be he subject. Study and ethical permission has been obtained from the Faculty of Medicine Ethical Commission, University of North Sumatra No:277 / KEP / USU/2020

Research Implementation

All subjects were subjected to hematological examination to measure MDA and TAC levels after the athlete had maximal physical activity by performing a beep test (pre test). Furthermore, the athletes were divided into 2 groups, namely the experimental group (given beet juice, n = 15) and the control (as a placebo, n = 15). Subjects for four weeks consumed 250 mL of beet root juice (BRJ) 1 hour before training. Then all athletes perform full maximum physical activity after one month by doing a beep test. After performing the beep test, another hematology examination was performed to measure the MDA and TAC levels (post test).

MDA and TAC determination

Blood (5 mL) was obtained with a needle from the antecubital vein before and after treatment and put in test tubes containing an anticoagulant material. To test the MDA and TAC, blood was taken to the laboratory. The enzyme-linked immunosorbent assay (ELISA) colorimetric approach was used for serum MDA and TAC analysis. Inspection procedures by following the procedures set out in each MDA and TAC kit.

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Data Analysis

All research data are reported as means and standard deviation. The research data were analyzed using IBM statistics for windows 25. Statistical analysis using paired t test was used to determine the average difference between pre-test and post-test data. Statistical analysis using independent samples t test was used to determine the difference between the experimental group and the control group. The statistical significance was set at $P < 0.05$ prior to analyses

5 RESULT AND DISCUSSION

Impact of Beetroot Juice (*Beta Vulgaris L*) on Malondialdehyde Levels

The pretest malondialdehyde (MDA) amount in the treatment group was 1.98 ± 0.37 nmol/ml based on the results of the research, 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 results of the MDA measurement 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-test 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 ($p = 0,000$)

MDA levels in the group that was not given beetroot juice were higher than the group given beetroot juice in the analysis. High levels of MDA in the group that were not given beetroot

juice shows that during maximum physical activity will produce free radicals that oxidize cell membranes (Figure 1).

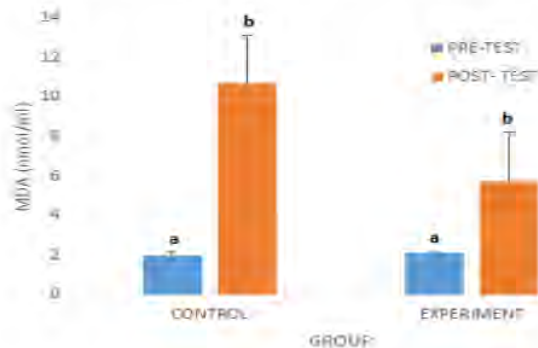


Figure 1. Impact of supplementation with BRJ on the level of malondialdehyde. In each group, the data is mean±SD for n=15. Different letters suggest a major difference between paired t-test samples at $p < 0.05$.

Several reports support the findings of research results including reporting that acute aerobic physical activity contributes to the occurrence of 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), during maximum physical activity oxygen consumption throughout the body increases to 20 times, 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. The findings of this study 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 by Bulduk, et al., (2011) 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 $41 \pm 0.30 \text{ nmol/ml}$ to $2.06 \pm 0.08 \text{ nmol/ml}$, while non-athletes with VO_2max levels of $26.7 \pm 3.67 \text{ ml/kg/min}$, 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; Sinfali and Angelino 2013). Some findings of in vitro research indicate that betalain pigments can protect cellular components from oxidative injury (Kanner, Harel, and Granit 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 authors also report that betanine, the most abundant betalaine (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 capacity to disperse very reactive radicals that attack cell membranes (Kanner, Harel, and Granit 2001). In addition, beets also 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)

Based on the research results obtained pre-test TAC levels in the treatment group were 1.53 ± 0.06 U/ml, while the control group 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 results of the measurement of the TAC post-test of the treatment group were 3.39 ± 0.08 U/ml, while the control group obtained TAC levels of 2.46 ± 0.11 U/ml. The results of statistical tests using the t-test showed differences in pre-test-post-test TAC levels in the experimental group or in the control group ($p = 0.000$). The results of statistical tests using the Independent Sample t-test showed a significant difference in the levels of posttest TAC between the treatment and control groups ($p = 0.000$).

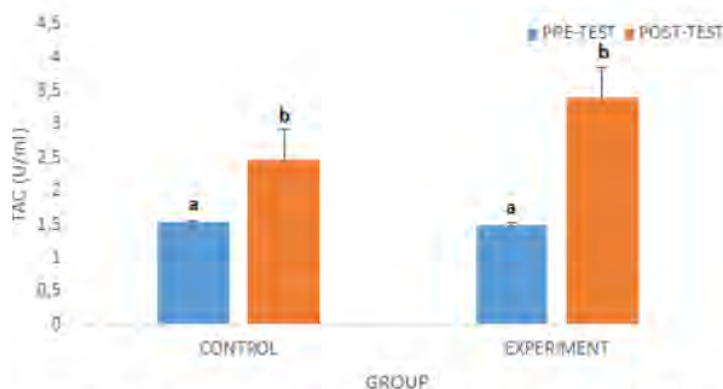


Figure 2. Impact of supplementation with BRJ on the level of total antioxidant capacity. In each group, the data is mean±SD for n=15. Different letters suggest a major difference between paired t-test samples at $p < 0.05$.

Giving beetroot juice in Figure 2 shows that TAC levels will increase when compared to the control group. Increased levels of TAC due to beetroot juice is due to the antioxidant content of beets such as betaine, phenol compounds, flavonoids, vitamin C, carotenoid and betalains. Betalains contained in beets are betacyanin, betaxanthine (Georgiev 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 (CCl₄) administration, confirms the findings of this report. In his research, giving beetroot juice to mice given NDEA and CCl₄ 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 percent and 66 percent, 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, Wang, and Zhang 2009)

has also confirmed the findings of the study. In his study, he investigated the radioprotective role of red beetroot betalate in light-irradiated rats (60) Co gamma (6.0 Gy, at a dose of 1.5 Gy min⁻¹). Rats were randomly divided into five groups, namely the control group and four experimental groups, which obtained one of four red 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 beetains were administered from red beets for the next three days. The study results showed that administration of betalains from red 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 red beetroots and immune system modulation.

Impact of Giving Beet Juice (Beta Vulgaris L) on VO₂max

The pre-test VO₂max levels in the treatment group were 47.59 ± 0.44 ml / kg / min based on the study findings while the control group was 47.75 ± 0.53 ml / kg / min. The findings of the study using separate t-test samples showed that there were no differences between the treatment group and the control group in pre-test VO₂max levels. The results 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 results of the analysis using the Paired Sample t-test showed that there was an increase in VO₂max levels in the pre-test-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).

In Figure 3 shows the administration of beetroots juice during exercise can increase VO₂max when 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 research conducted on 7 adult men (19-38 years) mentioned the consumption of inorganic nitrates (5.1 mmol nitrate/day) in the form of 500 ml beetroot juice for 6 days can reduce pulmonary oxygen uptake (VO₂) in exercise intensity severe so that it can delay fatigue time (Bailey et al. 2010).

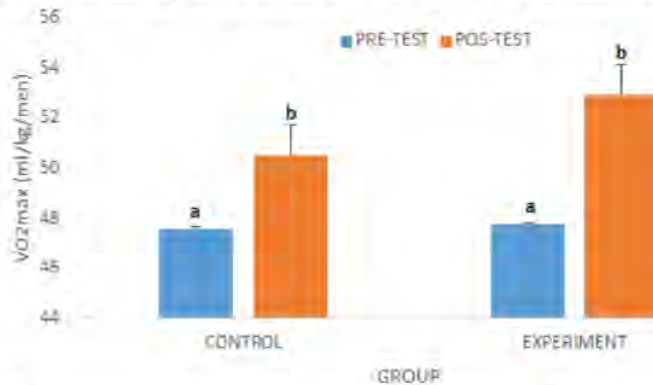


Figure 3. Impact of supplementation with BRJ on VO₂max. In each group, the data is mean±SD for n=15. Different letters suggest a major difference between paired t-test samples at p < 0.05

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 considered to be 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 the recovery of tissue processes (Bloomer 2010).

CONCLUSION

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

ACKNOWLEDGMENT

The authors thank the Fisik Laboratory and the Physiology Laboratory at Universitas Negeri Medan for their invaluable support for this research.

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