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Effect of Beetroot Juice (*Beta Vulgaris L*) During Training on Malondialdehyde Level in Athletes

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Abstract: Severe physical activity can cause lipid peroxidation marked by increased levels of malondialdehyde (MDA) which can reduce athlete's performance and health. Lipid peroxidation can be prevented or reduced by administering antioxidants. Beets (*Beta Vulgaris L*) contain various types of natural antioxidants but have not been investigated for their efficacy to reduce lipid peroxidation that is triggered by physical activity. The purpose of this study was to determine the effect of beetroot juice during exercise on lipid peroxidation during maximum physical activity. This type of research is an experimental study with a randomized control group pretest-posttest design research design. The study was conducted at the Unimed Stadium and the USU Faculty of Medicine Integrated Laboratory. The sample was 30 students of Sports Science who met the criteria. Pretest is done by checking MDA levels. Furthermore the sample was divided into 2 groups (P1 = 15, P2 = 15). During the training program group P1 was given 300 ml beet juice while P2 was given as control. Then all samples performed maximum physical activity using a bleep test, and MDA levels were re-examined. Then all samples performed maximum physical activity using a bleep test, and MDA levels were re-examined. The results showed a decrease in MDA levels in the training group that was given 300ml beet juice compared to the control group ($p < 0.05$). The conclusion of the study was that the administration of beet juice during training could reduce MDA levels when athletes performed maximum physical activity.

Keywords: beet juice, malondialdehyde, antioxidant, maximum physical activity

1. Introduction

Heavy physical activity can increase oxygen consumption 100-200 times due to an increase in metabolism in the body [1]. Increased use of oxygen mainly by contracting muscles causes an increase in electron leakage from the mitochondria which will become reactive oxygen compounds [2]. Generally 2-5% of oxygen used in metabolic processes in the body will become superoxid ions so that during heavy physical activity an increase in free radical production occurs [3]. When free radical production exceeds cellular defense antioxidants, oxidative stress can occur, where one of the contributing factors is physical activity [4,5]. Under conditions of oxidative stress, free radicals will oxidize lipids and damage the organization of cell membranes [6,7]. Malondialdehyde (MDA) is one of the results of lipid peroxidation induced by free radicals during maximal physical exercise or endurance training with high intensity [8,9,10], so that MDA is a general indicator used to determine the amount of free radicals and indirectly assess the body's oxidant capacity [8]. Bulduk et al reported that volleyball athletes who run a 20 meter shuttel run can cause lipid peroxidation which is marked by an increase in MDA levels and a decrease in CAT and GPx antioxidant levels [11]. The results of research conducted by Eroglu on judo athletes who perform sub-maximal physical activity also concluded that an increase in MDA and a decrease in the concentration of Cu and Zn ions which is an indicator of oxidative stress and lipid peroxidation [12]. The results of a study conducted by Moflehi et al reported that increasing the intensity of exercise can cause increased levels of MDA and creatine kinase levels [13]. It is known, CK is an indicator of damage to muscle cells [14,15]. The decrease in antioxidant levels and the increase in creatine kinase and lipid peroxidation due to maximum and sub-maximal physical activity can be prevented by optimizing nutrition,

especially by increasing antioxidant content [3,16]. Accordingly, Gomez said oxidative damage due to physical activity might be prevented by optimizing nutrition, especially by increasing the antioxidant content of food [17]. According to Silalahi, antioxidant properties 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 fruits that play a positive role [18]. Some natural plants have been tested for antioxidant activity to prevent lipid peroxidation due to physical activity including ginseng extract [19], grape seed extract [20], green tea leaf extract [21], plant extracts of *ixora parviflora* [22], lemon verbena [23], licopene from tomatoes [24], *rhodiolo rosea* [25], apple juice [26] and red fruit oil [27,28].

One alternative natural ingredient that has antioxidant content is red beet (*Beta vulgaris L*). Red beet is known to have betalain content which is a compound that has a very high antioxidant that is able to neutralize free radicals [29]. Other compounds in red beets (*Beta vulgaris L*) that function as antioxidants are betain [30], vitamin C, carotenoids, phenolic acids such as and flavonoids [31,32,33]. Phenolic acid compounds in red beets include ferulic acid, caffeic acid, p-coumaric acid, syringic acid, and vanillic acid [34]. The specialty of red beet (*Beta vulgaris L*) which has various types of antioxidants makes researchers feel interested and need to examine the antioxidant effect of red beet juice on MDA levels in maximum physical activity.

2. Methods

2.1 Material

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Research materials include Red Beet (*Beta vulgaris* L) obtained from MMTC Medan Market, 1% EDTA solution, Aquadest, MDA Kit purchased from Shanghai Korain Biotech Co., Ltd (Shanghai, China)

2.2 Tool

Research tools include: Spectrophotometer (Shimadzu), Glassware, Spuit, Juicer.

2.3 Types of Research

This type of research is experimental research with pre-test post-test design, control group design. The location of the study was conducted at the Unimed Stadium, the UNIMED FIK Physiology Laboratory and the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra, Medan.

2.4 Research Subject

The study used 30 sports science students with criteria of having a good VO₂max level, male sex, age 20-22 years, having a good BMI, not smoking, not taking supplements and antioxidants 2 weeks before and during the study, willing to be the subject research and has received ethical clearance from the ethics committee of the Faculty of Medicine, University of North Sumatra.

2.5 Research Implementation

All subjects underwent hematological examination to measure MDA levels after the athlete had maximal physical activity by performing a bleep test (pretest). Furthermore, athletes are divided into 2 groups (P1 = 15, P2 = 15). During the training program with moderate intensity for one month, the treatment group (P1) was given 300 ml of beetroot juice (*Beta vulgaris* L) while P2 as a control. Then after one month, all athletes perform maximum physical activity by doing a bleep test. Furthermore, a hematologic examination was performed again to measure MDA levels (posttest).

2.6 Malondialdehyde Examination

MDA examination is carried out using a spectrophotometer using the enzyme-linked immunosorbent assay (ELISA) method. Inspection procedures by following the procedures set out in each kit

2.7 Data analysis

Data analysis with normality test, homogeneity and t test.

3. Result and Discussion

Based on the results of the research, the pretest malondialdehyde (MDA) level in the treatment group was 1.98 ± 0.37 nmol / ml, while the control group was 2.11 ± 0.24 nmol / ml. The normality and homogeneity test shows that the data is normally distributed and homogeneous ($p > 0.05$). The results of the analysis using the independent t test

showed that there were no differences in pretest MDA levels between the treatment group and the control group. The results of measurement of MDA levels posttest treatment group was 5.75 ± 0.51 nmol/ml, while the control group obtained MDA levels 10.69 ± 0.97 nmol/ml. Normality and homogeneity test showed that MDA data posttest treatment group and control group were normally distributed and homogeneous ($p > 0.05$). The results of the analysis using the t dependent test obtained differences in the levels of pretest-posttest MDA treatment groups and control groups ($p = 0.000$). Statistical test results with the independent t test obtained a significant difference in posttest MDA levels between the treatment and control groups ($p = 0.000$).



Figure 1: Effect of administration of Beet Juice (*Beta Vulgaris* L) during exercise on malondialdehyde levels on maximal physical activity. Data are mean \pm SD, $n = 15$, $p < 0.05$.

Malondialdehyde is a dialdehyde compound with the molecular formula $C_3H_4O_2$, which can be produced from oxidation of unsaturated fatty acids by free radicals. Therefore, high MDA concentrations indicate an oxidation process in the cell membrane [35]. In the study obtained levels of MDA in the group that was not given beet juice was greater than the group given beet juice. High levels of MDA in the group that were not given Beet Juice showed that during maximum physical activity would produce free radicals which oxidize cell membranes (Figure 1). The results of this study are supported by several study results, that acute aerobic physical activity contributes to oxidative stress especially when exercising with 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 [36]. 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 [37]. Under conditions of oxidative stress, free radicals will cause lipid peroxidation of cell membranes and damage the organization of cell membranes [7]. Increased levels of MDA triggered by physical activity have been reported by many researchers including Moflehi, who examined the effects of aerobic exercise with different intensities (low intensity; moderate and high) on increasing levels of MDA and CK in people who are not athletes [13]. The results of this study indicate that the higher the intensity of exercise the greater the MDA level. 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±1.32µmol/L). Meanwhile, a study conducted by Bulduk, et al., (2011) reported that volleyball athletes who performed a 20 meter shuttle run test with a VO₂max level of 41.78±4.91ml/kg/min turned out to increase MDA levels from 1.41±0.30 nmol/ml to 2.06±0.08 nmol/ml, while non-athletes with VO₂max levels of 26.91±3.67 ml/kg/min, MDA levels increased from 0.94±0.24 ml/kg/min to 1.10±0.21 ml/kg/min.

Giving Beet Juice during exercise in this study can reduce MDA levels when athletes perform maximum physical activity when compared with 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 [31,32,38]. Some in vitro research results show betalain pigments can protect cellular components from oxidative injury [39,40]. For example, in the study by Kanner et al two betalain metabolites (betanin and betanidin) were shown to reduce linoleic damage caused by cytochrome C oxidase and lipid membrane oxidation induced by methyoglobin-H₂O₂-activated and free iron (AA-Fe). The authors also report that betanin, the most abundant betalain found in beets (300-600 mg / kg), is the most effective inhibitor of lipid peroxidation. High antioxidant activity of Betanin is thought to originate from its extraordinary electron donation capacity and ability to diffuse radicals that very reactive that targets cell membranes [39]. In addition, beets also contain NO₃ compounds (nitrates) which have been shown to suppress the formation of free radicals such as superoxide and hydrogen peroxide [41,42].

4. Conclusion

The conclusion of the study is that the administration of beet juice during training can reduce MDA levels when athletes carry out maximum physical activity.

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