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HSP70 (heat shock protein 70) expression and antioxidant as a protective againts oxidative stress triggered by sub-maximal physical activity

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Abstract. Sub-maximal physical activity will interfere with the ROS and antioxidants balance, so oxidative stress occurs. Oxidative stress can induce increased expression of HSP70 in cells to produce a cytoprotective effect. Red dragon fruit in biological systems can neutralize free radicals, because it contains flavonoid compounds and polyphenols. There were 20 participants, male, athletes, non-smokers and 2 weeks before and during the study, no consumption of supplements and antioxidants. Participants were randomly divided into 2 teams. Team A had sub-maximal activity and no red dragon fruit given. Team B had activity sub-maximal and was given red dragon fruit juice. Study found that HSP70 expression was lower in the team B than in team A. There was significancy difference between team A and team B in expression of HSP70. We concluded that, HSP70 expression and antioxidants can be the protective function against oxidative stress triggered by sub-maximal physical activity.

1. Introduction

Human muscles can adapt to broad capacities in response to stress caused by physiological and mechanical changes in physiology. Physical activity accompanied by mechanical and metabolic disorders can be a stressor. When skeletal muscle experiences physiological stress, it adapts to changes in the cellular mechanism. An important protein produced by cells in response to the stress conditions is HSP (Heat Shock Protein) [1].

The mechanism for the creation of free radicals during submaximal physical activity starts with muscle contractions during explosive power movements. At the time of the explosive power movement, ischemic to the muscle will occur, Alternating muscle relaxation followed. This muscle motion hampers the blood flow due to muscle contraction. Then, as blood vessels relax easily flow, ischemia-reperfusion occurs, which causes the release of an electron from the respiration chain to form free radicals or known as ROS (Reactive Oxidative Species) [2,3].

Submaximal physical activity can interfere with the balance between ROS and antioxidants, thereby producing oxidative stress. Oxidative stress often has many parameters in biological systems including an increased production of free radicals and other oxidants and reduced enzyme antioxidants Like catalase, superoxide dismutase (SOD), and glutathione peroxide (GPx). An imbalance of redox reactions in cells can also cause oxidative damage to cell components, such as fat, protein, and DNA [4]. Another indicator of cell damage is increased expression of the HSP70 protein due to oxidizing stress. Consequently, oxidative stress can increase HSP70 expression through the formation of reactive oxygen species (ROS).

HSP70 expression is a highly essential protein molecule for cell recovery and homeostasis maintenance. HSP70 is a protein that is strongly induced after stress, such as oxidative stress [5]. Oxidative stress can induce an increase in HSP70 expression in cells to provide a cytoprotective effect. When cells are ischemic, HSP70 expression helps prevent cell death by binding proteins which play a role in cell necrosis inhibition [6]. Self-care behavior and the relationship with exercise can be influence metabolic control [7].

Oxidative damage due to physical exercise can be prevented by increasing the antioxidant content of food [8]. Therefore, the body must have optimal antioxidant defenses to protect against free radicals. Many athletes believe that consuming antioxidants can reduce muscle damage and increasing defenses and fatigue so that it will improve their performance [9].

Red dragon fruit (*Hylocereus polyrhizus*), which belongs to the cactus category, is a nowadays very common and cultivated fruit, especially in Indonesia. Red dragon fruit is considered natural antioxidant. Numerous studies demonstrated the possible antioxidant of red fruit extract.

Red dragon fruit is known to have the ability to serve as a natural antioxidant. Many studies have shown that the extract from red dragon fruit had antioxidant possible [10-12]. The ability of red dragon fruit as an antioxidant in biological systems will neutralize free radicals because it contains flavonoid compounds and polyphenols [13]. This research aims to find out the protective role of HSP70 and antioxidants against oxidative stress, based on the description above.

2. Methods

2.1. Ethical clearance

Universitas Sumatera Utara (no.59/KEP/USU/2020) of the Committee on Ethics for the Implementation of Scientific Research in the Faculty of Medicine approved this work ethically.

2.2. Subjects

The participants were 20 persons, male, who were qualified as athletes, non-smokers and no consumption of vitamins and antioxidants 2 weeks before and throughout the research. The subjects were split into two teams, randomly. Team A had activity sub-maximal and no red dragon fruit given. Team B had activity sub-maximal and obtained juice from red dragon fruit. Blood is obtained for evaluation of HSP70 expression before and after diagnosis.

2.3. Activity sub-maximal protocol

The subject warms for 3-5 minutes. Perform physical activity by running on treadmill on intensity 80-85% of maximum heart rate, no incline, 11-12 speed stage, 30 minutes length and then cooling down for 3-5 minutes. Treadmill was done for 3 days/week, for 4 weeks. Red dragon fruit drink, 4 weeks a day. The treadmill is done at Physical Laboratory Universitas Negeri Medan.

2.4. HSP70 expression evaluation

Examination of HSP70 expression was carried out with spectrophotometric, the Enzyme-Linked Immunosorbent Assay (ELISA) method Examination and Human Heat Shock Protein 70 (HSP70) reagents ELISA Kit, Catalog Number MBS012990.

3. Results and Discussion

The means of HSP70 expression in team A increased significantly, while team B decreased significantly as shown in Table 1. The expression of HSP70 in team B was lower than in team A as shown in Table 2. Team A and Team B differed significantly in HSP70 speech.

Table 1. Means HSP70 expression on teams

Teams	Pre-test	Post-test	p
	Mean±sd	Mean±sd	
A	16.15±3.20	25.95±7.63	0.02*
B	19.22±5.11	10.42±3.26	0,000*

Note: team A = control; team B = treatment; sd = standar deviation; * = significant

Table 2. Difference in HSP70 expression on team

Teams	Mean	sd	P
A	25.95	7.63	0.000*
B	10.42	3.26	

Note: team A = control; team B = treatment; sd = standar deviation; * = significant

HSP70 expression after submaximal physical exercise increase because it is triggered by oxidative stress, which induced increased production of HSP70 in cells. HSP (Heat Shock Protein) is a protein molecule that plays a role in maintaining the structure and function of cell homeostasis in normal and stressful situations [14]. HSP's anti-apoptotic influence, which repairs damaged cells so that they can return to normal. Thus, an increase in HSP70 levels is compensation for the ischemia state and increased production of ROS due to submaximal physical activity [15]. Research by Nugroho et al. (2020) states that an increase in HSP70 expression triggered by oxidative stress is followed by a decrease in catalase and SOD [16].

The increase in HSP70 expression due to submaximum physical activity is consistent with research by Stranes et al. (2003) [17], who recorded exercise on the treadmill can increase expression of HSP70 in rat muscles. An increase in HSP70 expression functions as a protection against cells that prevents oxidative damage and repairs damaged proteins by balancing the state of ischemia and increasing production of free radicals [18].

HSP70 expression decreased in the submaximum physical activity group given red dragon fruit antioxidants because the antioxidants found in red dragon fruit, such as flavonoids and polyphenols, function to eliminate ROS so that it can suppress HSP70 expression. The findings of this research are consistent with prior Harahap et al . (2019) studies [19], who reported the effect of giving red dragon fruit extract in rats given a heavy physical exercise to decrease HSP70 and cortisol expression.

HSP response is related to training intensity because HSP70 expression is known to be higher after high-intensity exercise compared to the mild and moderate-intensity training [20-21]. Thus, the HSP response is also related to metabolic stress due to increased ROS [22]. Oxidative stress is known to cause an increase in the development of stress protein (HSP), which is an essential component of the cellular protective response and can protect cells from potential oxidative stress damage [23-24].

Red dragon fruit antioxidants are important to prevent protein and other components from being damaged by exercise or ischemia during oxidative stress. HSP70 expression reaction is to help recovery by preventing denatured protein aggregation [25]. Kassaf et al . (2003) research [26] notes that increasing tissue levels of vitamin C can suppress oxidants that are caused by submaximum physical activity. This represents the likelihood of antioxidants and HSP70 defending against oxidative stress [27].

4. Conclusions

Red dragon fruit antioxidants are important to prevent protein and other components from being damaged by exercise or ischemia during oxidative stress. The results showed HSP70 expression decreased with red dragon fruit juice for sub-maximal physical activity. Therefore, it can be concluded of HSP70 expression and antioxidants can be the protective function against oxidative stress triggered by submaximal physical activity.

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