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RESEARCH ARTICLE

URL of this article: http://heanoti.com/index.php/hn/article/view/hn20205

Effects of Purple Sweet Potatoes on Oxidative Stress Biomarkers in Rats Subjected to Exhaustive Exercise

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ABSTRACT

Oxidative stress is a condition caused by the imbalance between the production of free radicals or ROS and the antioxidants; the level of free radicals is higher than the antioxidants. Exhaustive exercise is one of the causes of this oxidative stress. However, it can be reduced by antioxidants found in purple sweet potatoes (PSP) (*Ipomoea batatas* L). This study aims to evaluate the effect of two weeks supplementation of PSP extract on rats induced-oxidative stress. Twenty rats were distibuted into four groups consisted five rats each. Rats in the treatments groups were administered PSP extract for two weeks then were forced to swim until exhausted. Bloods from all samples were drawn to see the changes in the levels of malondialdehyde ans superoxide dismutase after two weeks of treatment. The data was then analyzed using descriptive and inferential statistic. The results showed that mean level of MDA and SOD differ significantly ($p<0.05$) between groups after the administration of PSP extract as well as after exhaustive exercise. The extracts and exhaustive exercise concurrently showed a significant effect on oxidative stress biomarkers ($p<0.05$). It concluded that PSP extract can protect cells against free radical produced by exhaustive exercise.

Keywords: Purple sweet potatoes, Physical activity, Oxidative stress, MDA, SOD

INTRODUCTION

Regular exercise and physical activity have been known to have beneficial effects in prevention of chronic disease such as obesity, diabetes mellitus, cardiovascular disease, and cancer. However, the beneficial effects of physical activity or exercise are lost with exhaustion and lack of training[(1),(2)]. Exhaustive exercise induces a complex stress response which further leads to enhance production of ROS[(3)]. ROS in appropriate level actually play an important role in modulation of various cellular functions such as signalling process[(4)]. However, overproduction of ROS in response to exhaustive exercise can lead to oxidative stress, which further causes lipid peroxidation and damages to proteins, lipids, and DNA[(5)]. The oxidative damages caused by ROS associated with the type, frequency, intensity, and duration of exercise. Excessive production of ROS also contributes to disruptions in many biological process, such as metabolic, physiological, psychological, and immunological systems[(6)].

It is well established that exhaustive exercise increases the formation of ROS both in animal and human studies. Studies investigated the effects of acute exercise on oxidative stress showed the increased of ROS following lipid peroxidation and decreased antioxidant defense[(7)]. Other studies showed that the increase of malondialdehyde and hydroperoxide have been found in rats induced swimming test until exhausted[(8)].

Naturally, body has a defense mechanism against ROS by endogenous antioxidant system which consists of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT). Superoxide Dismutase (SOD) is the primary enzymatic antioxidant defense system in cell[(8)]. This enzyme plays an important role as first-line protection against the harmful effects of ROS generated by various sources. However, when production of ROS is excessive, the function of endogenous antioxidant will be limited. Therefore, the supplementation of exogenous antioxidant from diet becomes important to protect cells against the deleterious effect of ROS[(5)]. Epidemiological studies showed that high intake of vegetables and fruits rich in antioxidant has many potential health benefits which are associated with reduction the risk of lifestyle related disease such as cancer, diabetes, CVD, and hypertension[(9)].

One of dietary antioxidant that is often used is purple sweet potato (PSP). Purple sweet potato (*Ipomoea batatas* L.) contains high amount of polyphenol and flavonoids such as anthocyanins. Anthocyanins are chemical substances with strong antioxidant properties that exhibit many health-promoting effects such as

antiinflammatory, antidiabetes[10], anti-aging and antihypertension[11]. The antioxidant effect of PSP is approximately 2.5 times higher than those in berries. Studies reported that supplementation of PSP extract can lower MDA level in rats and mice after being exposed to exhausted physical training[12] and lower the level of cell damages in rats

This study was undertaken to evaluate the effect of purple sweet potato (*Ipomoea batatas* L.) extract on the biomarkers of oxidative stress in male Wistar rats subjected to exhaustive exercise.

METHODS

Twenty healthy male Wistar rats weighing 150-200 grams were randomly divided into four groups, consist of one control (K1) and three treatment groups (K2, P1, and P2). They were all maintained on a 12-h on and 12-h off lighting cycle in room temperature area and fed with standart rat chow and tap water ad libitum. The experiments began after rats being adapted for seven days.

Rats in treatment groups were subjected to swimming test until exhausted twice a week for two weeks. They were then given purple sweet potatoes extract with different dosage (P1=0.5 mL and P2=1 mL). At the end of treatment, all rats were euthanized and 5 mL intracardiac blood samples were collected to measure malondialdehyde (MDA) level as well as superoxide dismutase (SOD). MDA level were detected by thiobarbituric acid method (TBARS) and SOD level were detected by xanthine oxidase method. All procedures were approved by Ethic Committee of Faculty of Public Health, Airlangga University.

The numeric data were analyzed using: 1) descriptive in form of mean and standard deviation[13] and 2) inferential statistic. Manova test was performed to analyze the effect of purple sweet potato extract to rats. Tukey test was used for further analysis to determine which groups showed the differences.

RESULTS

Malondialdehyde Level in Rats

The mean level of serum malondialdehyde (MDA) and superoxide dismutase (SOD) were presented in Table 1.

Table 1. Mean Level of Malondialdehyde and Superoxide Dismutase in Each Groups

Group	Biomarker of Oxidative Stress	
	MDA Level (uM)	Sod Level (U/mL)
K1 (control group)	29.198 ± 1.503	1.220 ± 0.029
K2 (exhaustive exercise only)	38.588 ± 1.716	1.080 ± 0.062
P1 (PSP extract 0.5 mL/day and exhaustive exercise)	31.726 ± 0.791	1.326 ± 0.027
P2 (PSP extract 1 mL/day and exhaustive exercise)	29.728 ± 0.626	1.422 ± 0.046

Values were presented in mean ± SD

The highest level of MDA was found in group K2 which was exposed to exhausted exercise only (38.588 ± 1.716). The MDA level gradually decreased after the supplementation of PSP in group P1 and P2. The lowest level of MDA was found in group P2 which was exposed to exhausted exercise and given PSP extract 1 mL/day. The level of MDA in P2 was almost similar to those in control group (K1).

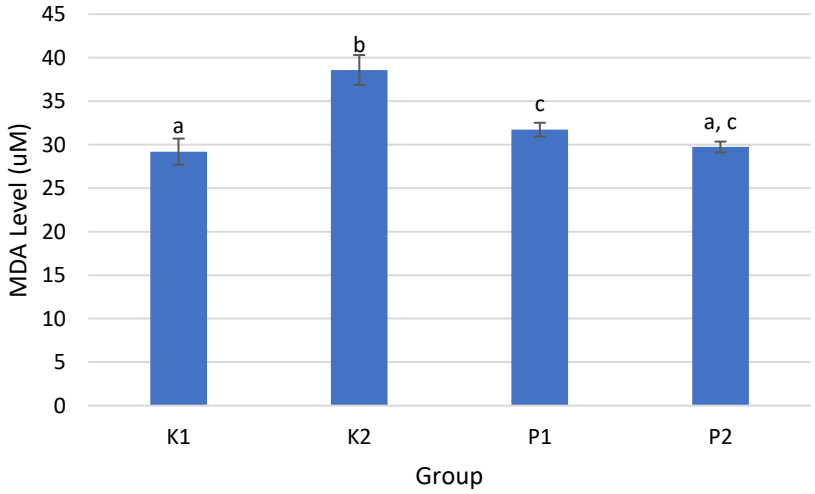

Figure 1. The effects of PSP extract and exhaustive exercise on serum malondialdehyde (MDA) in rats

The data were presented as means ± SD. The significant differents were shown as different alphabet ($p < 0.05$). The PSP extract and exhaustive exercise effects were significant on MDA levels ($p < 0.05$). One way manova test demonstrated that MDA levels were increased in control-exhausted group (K2), but decreased in other exhausted groups after being given PSP extracts with two different dosages. However, post hoc analysis showes that the increases of MDA levels in P2 were not statistically significant with K1 and P1 ($p > 0.05$), while the rest groups showed statistically different.

Superoxide Dismutase Level in Rats

The level of SOD found in K2 was the lowest among all groups (1.080 ± 0.062). The SOD level then increased slowly after being given PSP extract with different dosage, with 1 mL/day dosage showed highest level of SOD among all (1.422 ± 0.046).

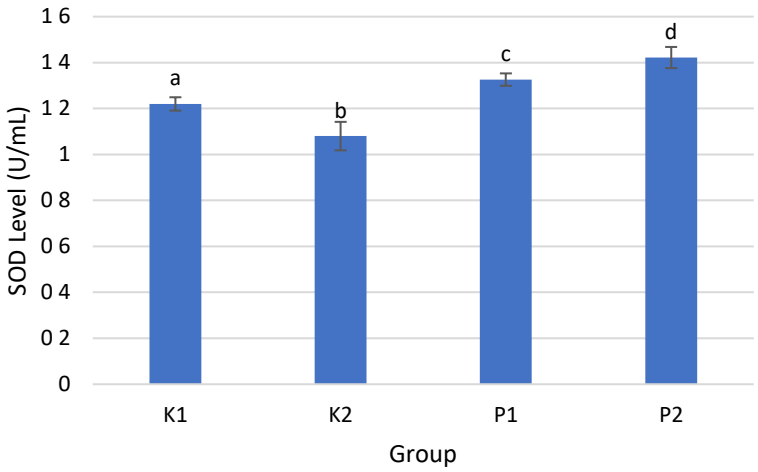

Figure 1. The effects of PSP extract and exhaustive exercise on Superoxide Dismutase (SOD) in rats

The data were presented as means ± SD. The significant differents were shown as different alphabet ($p < 0.05$). The PSP extract and exhaustive exercise effects were significant on SOD levels ($p < 0.05$). SOD level in group K2, P1, and P2 were all significantly higher that in the control group ($p < 0.05$). The exhaustive exercise decreased SOD level in control-exhausted group (K2), while increased in other exhausted group (P1 and P2). Post hoc analysis showed significant differences between groups ($p < 0.05$).

DISCUSSION

In recent years of clinical research, MDA and SOD have often been used as popular matching indicators of oxidative stress. MDA is one of the aldehyde compounds occuring as a result of lipid peroxidation. MDA reflected the degree of damage in cells caused by overproduction of free radicals, whereas SOD was associated with free radical scavenging ability[8]. MDA also reflected the severity of lipid peroxidation, thus it often used as biomarker of oxidative stress.

In this study, MDA level was significantly higher in control-exhausted group (K2) than in baseline/control group (K1). It indicated that exhaustive exercise enhanced lipid peroxidation that lead to oxidative stress, resulting in dramatic increase in MDA level. The result was similar to other studies conducted by Jawi et al. (2014) that showed increase of MDA level in rats after subjected to intensive training. It could be inferred that exhaustive exercise enhanced the production of free radical ($O_2^{\bullet-}$), which further lead to lipid peroxidation[12].

Although there are some debates regarding the source of free radical during exercise, mitochondria has been long considered as the major source of ROS[14]. But as the intensity of exercise increased, the production of ROS mainly from hypoxia condition. During hypoxia, adenosine triphospate (ATP) will decrease due to ATP-dependent calcium ionic pump impairment and activation of Ca-dependent proteases. This activity result in the cleavage of xanthine dehidrogenase to xanthine oxidase (XO). This enzyme catalyze a reaction where hipoxanthine were converted to xanthine then to uric acid and free radical ($O_2^{\bullet-}$)[6]. It explained why ROS production elevated sharply during exhaustive exercise.

Meanwhile, MDA level gradually decreased after the administration of PSP extracts dosage 0.5 mL and 1 mL per day although the decline was not statistically significant in P2. It can be inferred that lipid peroxidation increased in response to exhaustive exercise, but when combined with PSP supplementation, it could play a protective role against oxidative damage. The protective effect of PSP extract was associated with anthocyanins

compound. Anthocyanins possess strong antioxidant activity as it can stabilize the unpaired electron and the ability to chelate transition metal ions (Fenton reaction termination)[15]. The reduction of MDA level in P1 and P2 could be due to scavenging ability of anthocyanin by donating its electron to free radical so the molecule became more stabil.

Anthocyanins can also protect cells from oxidative stress by inducing the activation of nuclear factor erythroid 2 related factor 2 (Nrf2) pathway. The activation of Nrf2 leads to induction of endogenous antioxidant enzymes including SOD, GPx, and inducible nitric oxide synthase[9]. SOD is the first line defense against oxidative stress in living cells. In this study, the notable decline of SOD level in K2 caused by exhaustive exercise increased the susceptibility to oxidative stress. A change in SOD level was then observed in treatment groups (P1 and P2) where SOD level increased significantly after the administration of PSP extracts for two weeks. It can be explained that SOD level was gradually recovered due to high antioxidant properties of anthocyanins in PSP extracts. The mechanisms of activation Nrf2 activation pathway resulted in the increase synthesize of SOD as well as the level of SOD. It was observed that SOD level in P2 was greater than P1, showed that greater dosage of PSP extract could give more protection effect from oxidative stress.

CONCLUSION

The increase production of free radicals during and after exhaustive exercise can exceed the capacity of the endogenous antioxidant defense system and induce oxidative conditions. However, the adminitration of exogenous antioxidant such as PSP may prevent cells from lipid peroxidation and affect the biomarkers of oxidative stress. PSP extract given everyday for two weeks can lower the level of MDA and increase the level of SOD after being subected to exhaustive exercise.

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