

# Effect of Red-Fleshed Pitaya (*Hylocereus Polyrhizus*) to Increase Glutathione Peroxidase Levels in Male Rats (*Rattus Norvegicus*): The Induced Oxidative Stress

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**Abstract.** High intensity physical activity requires large amounts of antioxidants. Exhausting physical activity can decrease glutathione peroxidase levels in response to increasing production of free radicals. Glutathione peroxidase levels to eliminate free radicals and reduce oxidative damage. This study aims to know the effect of antioxidant red-fleshed pitaya extract to increase glutathione peroxidase. The subject of this research are 16 male rats, age 3 months with average weight of 200 gr, divided into 2 groups. Group 1 (OS-RFP) : the induced oxidative stress and given aquadest, group 2 (OS+RFP) : the induced oxidative stress and given 100 mg/kgWB of red-fleshed pitaya extract. Oxidative stress is given was swam for 20 minutes, thrice a week for 3 weeks. The glutathione peroxidase levels in the group 1 higher than the group 2. There were significant differences in the increasing glutathione peroxidase levels ( $p=0.000$ ) between group 2 and group 1. This research shows that glutathione peroxidase levels have increased in oxidative stress groups given red-fleshed pitaya extract. Red-fleshed pitaya extract has to increase glutathione peroxidase levels that is can eliminate oxidative stress.

**Keywords:** Oxidative stress, glutathione peroxidase, red-fleshed pitaya, rat..

## 1 Introduction

Exhausting physical exercise increases oxygen needs due to the increase of metabolism in the body. The rise of oxygen uptake in contracting muscle leads to ischemia reperfusion which causes one electron to detach from respiratory chain and forms free radicals (Power *et al.*, 2008; , Sahlin *et al.*, 2010).

In physiologist condition, the free radicals production will be balanced by the production of endogenous antioxidant in the body like glutathione peroxidase (GPx). Glutathione peroxidase is a natural antioxidant in the form of an enzyme, produced in the body, with a strong effect and as the first defense against attack from free radicals (Gomes *et al.*, 2012).

The study done by Harahap *et al.* (2018) stated that a regular and controlled dosage of exercise, like weight training, based on the ability of each individual can eliminate the free radicals and increase of glutathione peroxidase levels, more over improve the performance of a 200-metre runner (Harahap *et al.*, 2018).

Antioxidant activity depends on the intensity of exercise as exhausting physical exercise

can decrease of glutathione peroxides levels in response against increasing of free radicals and to eliminated of free radicals and reduce oxidative damage. However, during high intensity physical activity, the free radicals production can be greater than glutathione peroxides which results in oxidative stress (George and Osharechiren, 2009; El Abed *et al.*, 2014).

Actually free radicals, including ROS (*Reactive Oxygen Species*), important for normal body healthy in reduce inflammation, phagocytosis and regulate the muscle tone of blood vessels and organs. However, if the balance of the free radicals and antioxidant is disturbs which results in oxidative stress (Valko *et al.*, 2007; Cooper *et al.*, 2002; Marciniak *et al.*, 2009; Castrogiovanni, 2012). Oxidative stress cause heavy training has damages cells, muscles fatigues and decreases antioxidant levels (George and Osharechiren, 2009; Kurkcu *et al.*, 2010; Azizbeigi *et al.*, 2013).

To determine whether there is an increase in oxidative stress among others by analyzing the endogenous antioxidant content namely glutathione peroxides. The increased oxygen consumption during exercise will activate glutathione peroxides enzymes to move hydrogen peroxidase ( $H_2O_2$ ) from the cell. The affinity of glutathione peroxides against  $H_2O_2$  is stronger than catalase. The location of glutathione peroxides is in mitochondria and cytosols to protect cells from free radicals that can damage lipid membranes, proteins and nucleic acids (Urso, 2013).

The body needs exogenous antioxidants to eliminate and prevent oxidative stress (Gomez *et al.*, 2009). The sources of exogenous antioxidant are Vitamin E and C also beta-carotene. External antioxidant from food or supplement can to fight the excess of free radicals. Proanthocyanidin from grape seed was given to rats for 2 weeks. It lowers down Malondealdehyde levels, increases superoxide dismutase and glutathione peroxides activities significantly, moreover reduces fatigue after physical activities (Belviranli *et al.*, 2006).

Red-fleshed pitaya (*Hylocereus polyrhizus*) is one type of fruits that is unique with a lot of benefits. The fruit is recently popular among the people in Indonesia and appears as natural antioxidant. Several invitro studies have shown that red-fleshed pitaya extract has the power as antioxidant (Sani *et al.*, 2009; Nurul, 2014). This study aims to know effect antioxidant red-fleshed pitaya extract to increase glutathione peroxides in male rats the induced oxidative stress.

## **2 Methods**

### **2.1 Animal**

The subject of this research are 16 male rats, age 3 months with average weight of 200 gram. Rats were acclimatized and maintained for 1 week to adapt in a groups of 4 rats per cage made of plastic material (30 x 20 x 10 cm) which is covered with fine wire mesh.

### **2.2 Material and Reagents**

This study using red-fleshed pitaya extract obtained from methanol extraction process. Maceration technique was used and the extract was concentrated with air-drying method. Glutathione peroxidase levels were examined using ELISA method and spectrophotometry wavelength of 450 nm with mouse Glutathione peroxidase Elisa kit, catalog E1483Mo for glutathione peroxidase levels analysis.

### 2.3 Statistical Analysis

Data were analyzed with statistics and presented in form of means and standard deviation, tables and figures. Each data obtained is first determined by the distribution of the Normality test. If the data is normally distributed, an independent t test is performed.

## 3 Results

Table 1, the results of examination the glutathione peroxides levels are normally distributed. The mean glutathione peroxides level at the OS+RFP group was higher compared to the OS-RFP group (32,24±2,43 vs 24,69±4,69) (tabel 2). This shows that glutathione peroxides levels an increase significant at the oxidative stress groups given red-fleshed pitaya compared to the oxidative stress group without given red-fleshed pitaya (figure 1).

**Table 1.** Normality test of the glutathione peroxides.

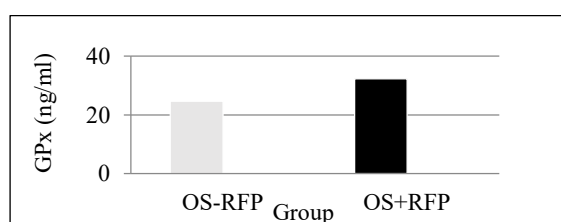
Parameter	Groups	Normality test	
		statistic	p
Glutathione peroxides	OS-RFP	0.865	0.135*
	OS+RFP	0.895	0.263*

Note: \* = signifikan (  $p > 0,05$ ), SD : standard deviation

**Table 2.** Effect of Red-fleshed Pitaya to Increase Glutathione Peroxidase levels.

Group	Mean	SD	p value
OS-RFP	24.69	4.69	0.000*
OS+RFP	32.24	2.43	

Note: \* = signifikan (  $p < 0,05$ )



**Fig. 1.** Glutathione peroxides levels in OS-RFP group and OS+RFP group

## 4 Discussion

The results of this study are found that exhausting physical exercise as a cause of oxidative stress when combined with giving red fleshed pitaya extract every day during exercise can increase glutathione peroxidase levels. Red fleshed pitaya extract with a dose of 100 mg/ kgBW is better in increasing glutathione peroxidase levels.

The study showed that red fleshed pitaya extract has the potential as an exogenous antioxidant which can eliminate free radicals those formed during exhausting physical exercise are evidenced by increased glutathione peroxidase levels.

Free radicals are compounds or atoms where the outer orbitals have unpaired electrons so that they are highly reactive to surrounding cells. Reactive compounds look for pairs, by attacking and binding to the molecular electrons around them and if these compounds meet new radicals, new radicals will form again and so on so that a chain reaction will occur (Kelsey and Bloomer, 2009).

Damage in biological molecules is affected by this oxidative stress (Finaud et al., 2006; Andersson et al., 2009; Kothari et al., 2010). Phosphorylation oxidation due to exhausting physical exercise is the main internal factor causing oxidative stress (Kurkcu et al., 2100).

Despite the effects of exercise on health benefits, many studies reported during exhausting physical exercise there is an ischemia-reperfusion process that causes the production of oxidative due to release it of electrons from the respiration chain. During exhausting physical exercise, the release of free radicals can increase in mitochondria and cause oxidative stress (Daniel et al., 2010; Escribano et al., 2010). Oxidative stress causes DNA damage, loss of protein structure function such as membrane enzymes and receptors, and structural damage from the lipid cell layer (Guzel et al., 2007; Abruzzo et al., 2013; Liu et al., 2013). The research by Norouziyan et al. in active women it has been found that Total Anti-Oxidant Capacity and Glutathione after exercise can be increase. This shows that exercise can be stimulating the body's ability to increase antioxidant (Norouziyan et al., 2014). Glutathione peroxides is an endogenous antioxidant that functions as free radicals catcher by releasing its own electrons to prevent oxidation from occurring and destroying other molecules. Glutathione peroxides is called as scavenger enzyme to hydrogen peroxide, found especially in mitochondria.

The high and low glutathione peroxides levels determine its activities as antioxidant. The higher of glutathione peroxides levels is, the higher the activities of endogenous antioxidant are. On the other hand, when there is a decline in the level, there is also a decrease in the activity (Gomes et al., 2012).

Effectiveness of antioxidant systems in offset free radical production reaches saturated conditions in physical activity with a 70% load of maximal heart rate (Castro et al., 2009), because higher intensity exercise will produce more free radicals (Guzel et al., 2007). In this study, glutathione peroxides levels is observed to have the tendency to increase in the group given heavy physical training and red-fleshed pitaya extract. This happens as a result of red-fleshed pitaya to have the potential as antioxidant that is able to balance the increase of free radicals from heavy workouts.

Therefore, glutathione peroxides level is higher in the group that received red dragon fruit extract than the group that did not. The result from this study aligns with the study done by Bing & Wang (2010) in which stated that Ginkgo Biloba can increase the glutathione peroxidase levels in liver tissue and improve recovering process after a maximum physical activity ( Bing & Wang, 2010).

Another research by Suarsana et al. (2013), the application of isoflavone is able to handle SOD decrease and to prevent MDA level from increasing at heart tissues of rats in oxidative stress condition.

## 5 Conclusions

The conclusion of this study is that red-fleshed pitaya has the potential as antioxidant that is able to eliminate oxidative stress due to exhausting physical exercise. Red fleshed pitaya extract as an antioxidant has an effect on increasing glutathione peroxides in rats induced by oxidative stress.

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