The Effects of Ginseng Java Roots (*Talinum Paniculatum*) extract on Malondialdehyde (MDA) levels in Male White Sprague Dawley Rats with Forced Swimming Test Model

Adies Riyana¹, Prof. Dr. Ambar Mudigdo dr., Sp.PA(K)², Brian Wasita dr., Ph.D, Sp.PA²*
¹Nutrition Science, Postgraduate Program, Universitas Sebelas Maret, Ir. Sutami Street No. 36 A, Kentingan, Jebres, Surakarta, Central Java 57126, Indonesia
²Department of Anatomical Pathology, Faculty of Medicine, Universitas Sebelas Maret, Ir. Sutami Street 36 A, Kentingan, Jebres, Surakarta 57126, Indonesia

*Corresponding author: brianwasita@yahoo.com

Abstract. Introduction : Cell and tissue damage can be caused by chemical species which known as free radical. Free radical is a highly reactive molecule and in excessive amount it can cause an oxidative stress which causes numerous chronic diseases. The ginseng java plant (*Talinum Paniculatum* (Jacq) Gaertn) contains various types antioxidants which can neutralize free radicals, such as flavonoid, saponin, alkaloid, tannin, quinone, and provitamin A. Aims: This study aims to analyze the effects of ginseng java roots (*Talinum Paniculatum*) extract on Malondialdehyde (MDA) levels in male white Sprague Dawley rats with a forced swimming test model. Methods: This study used 30 Sprague Dawley rats which were assigned into 5 different groups, such as the negative control group, positive control group, ginseng roots extract dose of 0.35 mg/200g rats body weight/day group, ginseng roots extract dose of 0.70mg/200g rats body weight/day group, and ginseng roots extract dose of 1.40 mg/200g rats body weight/day group. On the 28th and 35th day the Sprague Dawley rats were treated with a Forced Swimming Test model. The MDA level was measured on the 28th and 35th day using TBARS assay method and statistical analyze by Kruskall Wallis test with p value less than 0.05 was considered significant. Results: This study showed that the administration of ginseng java roots extract dose of 0.35 mg/200g rats body weight/day, dose of 0.70mg/200g rats body weight/day, and dose of 1.40 mg/200g rats body weight/day were significantly lowering the MDA level (p<0.001). In addition, there were significant differences between treatment groups with various doses (p<0.001). Conclusion: The treatment of ginseng java roots (*Talinum Paniculatum*) extract significantly lowered the MDA level in the Sprague Dawley Rats with Forced Swimming Test model.

Keywords : MDA, ginseng java roots extract, forced swimming test
1. Introduction
Cell and tissue damage can be caused by chemical species which is known as free radical. Free radical is a highly reactive molecule and in excessive amount it can cause an oxidative stress which causes numerous chronic diseases. Excessive physical activity increases the occurrence of oxidative stress and malondialdehyde (MDA) levels in the blood (serum). It can be used as an indicator of oxidative stress. The higher level of MDA (µmol/l) indicates that the process of oxidative stress tends to increase. In conditions of oxidative stress, free radicals lead to lipid peroxidation in the cell membrane generating lipid. Cell membranes play an important role as enzyme or receptors, so if oxidative stress occurs on the cell membrane it will lead to loss of cellular function and cell death [1,2].

Malondiadehide (MDA) is formed during homolytic decomposition of lipid peroxidation in cell membranes which generates more free radicals. Over production of oxygen free radicals in the cell membranes can cause various metabolic and cellular disorders. Free radicals can damage cells by damaging these cell membranes. Free radicals also cause cell membrane lipid peroxidation. Lipid peroxides is a well known example of oxidative damage in cells. Lipid peroxidation will affect membrane fluidity, permeability to different substances, and membrane function. MDA levels also increased during high intensity exhaustive exercise (80% d.d 95% maximum repetition) compared to low intensity exercise (20% s.d 35% maximum repetition) which is marked by an increase in lipid hydroperoxide significantly [3].

The ginseng java plant (Talinum Paniculatum (Jacq) Gaertn) which is also known as Som Java or Kolesom is one of the most popular herbal plants and has been used as a raw plant material for drugs that have become more widely available commercially substituting for the Korean or Chinese ginseng. The whole plant of ginseng java can be used as an ornamental plant. In addition, it can also be used as a medicinal herb in the treatment of minor ailments, skin infections, cancer, liver and reproductive disorders to increase resistance to stress and fatigue [4-9].

The ginseng java plant (Talinum Paniculatum (Jacq) Gaertn) contains high antioxidants level such as ginsenosides, phenol acids, flavonoids, saponins and tannins. Ginseng Java is also rich in vitamins and minerals such as vitamin C, iron, manganese, and zinc which could act as an exogenous antioxidant obtained from the diet and has an important role in reducing oxidative stress and cell damage [8,10-12].

This study aims to determine the effect of ginseng java root extract on MDA levels in male white Sprague Dawley rats with Forced Swimming Test Model. The effect of ginseng java root extract to MDA levels as a biomarker of oxidative stress has not been widely studied, so it still needs further research.

2. Materials and Methods

2.1 Preparation Extract Ginseng Java
Ginseng java root used in this study was obtained from a company that provides various types of herbal plants from Herbadream Company and domiciled in Sragen, Central Java. After harvesting, the ginseng java was cleaned, cut into small pieces and dried for 2 weeks. Ginseng java root extract is made using the maceration method. The extractions process was carried out at the Laboratory of Food Technology and Engineering of Gadjah Mada University.

2.2 Animals and Grouping
The sample size is calculated based on the guidelines of the WHO concerning Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicine for each group of at least 5 with a reserve of 10% (1 tail). Reserves in each group are needed in anticipation of rats dropping out at the time of the study.

The adaptation process was held during the 7th day, after that experiment animals were randomized and divided into 5 treatment groups. The total sample was 30 white rats, the treatment group was divided into negative control groups, positive controls, treatment group 1 were given a ginseng extract
dose of 0.35 mg / 200gBW, treatment group 2 were given a ginseng extract dose of 0.70 mg / 200g BW, treatment group 3 were given a ginseng extract dose of 1.40 mg / 200g BW. Ginseng java roots extract is given orally once a day for 35 days in a row. Measurements of experimental animal body weight are carried out every 7 days.

2.3 METHODS

This study was a randomized experimental trial with Post Test Control Group Design. This study used male (Sprague Dawley) rats 2 months old (weight 150-200g) as experimental population.

Forced swimming tests were carried out in accordance with previous research with various modifications. The male white rats were placed in a container filled with water with the temperature maintained ranging from 25°C±1°C. One hour after the dosing, all rats underwent a weight-loaded swimming test with a piece of lead (approximately 5% of each rat’s body weight), which was attached to their tail’s root. The rats were defined exhausted with failure to rise above the water within 10 seconds and no movements in all four legs, the body was bent, the tail is stretched and the head is left underwater [13,14,15].

On the 28th and 35th day, experimental animals were challenged by the forced swimming test to determine the effect of Javanese ginseng root extract on oxidative stress due to excessive physical exercise. But before being approved for the forced swimming test, the experimental animals were drilled to adapt to swimming without loads for 3 days for 20 minutes / day. After swimming, the rats are dried with a towel and put into the cage [16].

Measurements MDA levels were examined using the TBARS method. A total of 0.75 mg of phosphoric acid was added to the 13 mg polypropylene tube, then 0.05 mg of blood plasma sample was added. 0.25 mg of 40mM Thiobarbituric acid (TBA) solution was then added to the mixture, followed by 0.45 mg of water then mixed well and tightly closed. After being heated in a water bath for 60 minutes at a temperature of 100°C, the mixture is then cooled to reach 30°C, then put into the C18 Sep-Park column. Before use, the column is washed with 5 mg of methanol and water and then discarded. Next the sample mixture is put into the column and also removed. TBA is then eluted from the column by adding 4 mg of methanol and stored in cuvet. Color density was read by a spectrophotometer at a wavelength of 532 nm. As a standard, 1.1.3.3 tetraetoksipropana (TEP) was used.

2.4 Statistical Analysis

All collected data were presented mean ± standard deviation. Before running statistical analysis, homogeneity and normality data were analysed using Saphiro Wilk tests and statistical analyze by Kruskall Wallis and Mann Whitney test with p value less than 0.05 was considered significant.

3. Results

The general condition of all rats was normal but 1 rat was died during the trial. The effects of ginseng java root extract on the MDA levels with the forced swimming test on the 28th day are presented in Table 1. This study results that MDA levels of KP1, KP2, KP3 after exhaustive swimming was 21.42 (µmol/l), 15.22 (µmol/l), 11.17 (µmol/l) respectively and it significantly decreased compared with the positive and negative control. While the effects of ginseng java root extract on the MDA levels of KP1, KP2, KP3 after exhaustive swimming on the 35th day was 21.00 (µmol/l), 13.83 (µmol/l), 9.75 (µmol/l) respectively and it significantly decreased compared with the positive and negative control. In Table 2, presented data of post hoc analysis to determine the comparison between each group to assess which group showed the greatest effects. The results showed that there were significant difference between groups either on the 28th day test or 35th day test (Table 2). It showed that the administration of ginseng java root extract can lower the MDA level in various doses and is significantly different compared to the control groups (p<0.005).
Table 1. Effects of Ginseng Java Root Extract on the MDA Level Sprague Dawley White Rats with Forced Swimming Test on the 28th and 35th Day

| Groups      | MDA (µmol/l) Mean Rank | p*  |
|-------------|------------------------|-----|
|             | 28th day | 35th day |    |
| K-          | 27.50     | 26.50     |    |
| K+          | 3.50      | 3.50      |    |
| KP 1        | 21.42     | 21.00     | <0.001* |
| KP 2        | 15.22     | 13.83     |    |
| KP 3        | 11.17     | 9.75      |    |

Note:
K-: Exercise forced swimming test model only; K+: Xantin+ Exercise forced swimming test model; KP1: Exercise forced swimming test model + Javanese ginseng root extract 0.35mg/200g BW; KP2: Exercise forced swimming test model + Javanese ginseng root extract 0.70mg/200g BW; and KP3: Exercise forced swimming test model + Javanese ginseng root extract 1.40mg/200g BW.
* : kruskall walliss test

Table 2. Post-hoct analysis of Effects of Ginseng Java root extract on the MDA level of the white Sprague Dawley Rats with a forced swimming test on the 28th and 35th Day

| Group 1       | Group 2       | p1* | p2** |
|---------------|---------------|-----|------|
| Positive control | KP1 (0.35 mg/200 gBW) | <0.001* | <0.001** |
| Negative control | KP2 (0.70 mg/200 gBW) | <0.001* | <0.001** |
|                | KP3 (1.40 mg/200 gBW) | <0.001* | <0.001** |
| Positive control | KP1 (0.35 mg/200 gBW) | <0.001* | <0.001** |
|                | KP2 (0.70 mg/200 gBW) | <0.001* | <0.001** |
|                | KP3 (1.40 mg/200 gBW) | <0.001* | <0.001** |
| KP1 (0.35 mg/200 gBW) | KP2 (0.70 mg/200 gBW) | 0.002* | <0.001** |
| KP2 (0.70 mg/200 gBW) | KP3 (1.40 mg/200 gBW) | <0.001* | <0.001** |
| KP3 (1.40 mg/200 gBW) | 0.002* | 0.165 |

Note:
K-: Exercise forced swimming test model only; K+: Xantin+ Exercise forced swimming test model; KP1: Exercise forced swimming test model + ginseng java root extract 0.35mg/200g BW; KP2: Exercise forced swimming test model + ginseng java root extract 0.70mg/200g BW; and KP3: Exercise forced swimming test model + ginseng java root extract 1.40mg/200g BW.
P1: p-value of 28th day test
P2: p-value of 35th day test
* : Mann-Whitney test

4. Discussion
The results showed that high intensity physical activity can cause an increase in oxidative stress characterized by an increase in MDA levels. MDA is the end product of lipid peroxidation processes.
During physical activity, ROS will form as a by-product of a phosphorylated oxidation energy form (ATP) in the electron transport chain in the mitochondria. The phosphorylated oxidation process requires O2 to bind hydrogen to form water, but not all O2 consumed (around 4% -5%) changes to ROS [17].

The antioxidant compounds of ginseng java root extract can lower MDA levels of rats with a forced swimming model test. The decrease was influenced by the antioxidant content of ginseng java root extract which can inhibit free radicals by suppressing the lipid peroxidation process. The phytochemical compound such of saponins, alkaloids, tannins, flavonoids, and other compounds can physiologically facilitate circulation in the central nervous system or blood circulation in the peripheral nerves. Flavonoid compounds can suppress free radicals and stabilize ROS because it will be oxidized by radicals, resulting in a more stable, and less-reactive radical. While tannin compounds have a cooling effect and can coat the tissue below, so that nerve cells are protected from harmful external stimuli. Vitamin C can efficiently prevent the formation of superoxide, hydrogen peroxide, radical hydrosils, peroxyl radicals and oxygen radicals. Vitamin C is more effective in inhibiting fat peroxidation by peroxyl radicals than other plasma components such as α-tocopherol. Vitamin C can prevent membrane peroxidation by increasing tocopherol activity and preventing cell damage due to oxygen radicals [18,19].

Our results showed that the forced swimming test model has proven to increase serum MDA levels in male white rats given by ginseng java root extract for 28 and 35 days which was consistent with previous reports by Qi Bin et al (2014). Study conducted by Wang et al (2010) also concluded that ginseng polysaccharide also affects MDA and GPx levels [14].

5. Conclusion
To our knowledge, this study is the first trial to investigate the effects of ginseng java root extract on MDA levels in male white Sprague Dawley rats with a forced swimming model test. Treatment of ginseng java root extract improves one of biochemical markers for oxidative stress condition, that was the MDA levels. The administration of ginseng java root extract was proven to significantly decrease the serum MDA level and suppressed the oxidative stress condition. According to the data, these findings indicate that ginseng java root extract is a safe ingredient and can be used as a dietary antioxidant agent and showed the greatest effect with dose of 1.40 g/200 BW of rats.

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