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Javanese Ginseng Root Extract (*Talinum Paniculatum* (*Jacq*) *Gaertn*) to Increase Superoxide Dismutase Activities in White Male Sprague Dawley Rats by Using a Forced Swimming Test Model

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Abstract: Continuously performing heavy activities will damage tissues and cells and cause oxidative stress. Fortunately, this damage can be reduced by giving antioxidants to reduce free radicals. This study uses the forced swimming test model to determine the effect of Javanese ginseng root extract on superoxide dismutase levels in male white Sprague Dawley rats. The samples were 30 white Sprague Dawley rats divided into five groups: the negative control group, the positive control group, treatment group 1 receiving Javanese ginseng extract at a dose of 0.35mg/200g BW/day, treatment group 2 receiving a dose of 0.70mg/200g BW/day, and treatment group 3 receiving a dose of 1.4mg/200g BW/day. On days 28 and 35, the rats were treated with the FST model. Meanwhile, on days 28 and 35, SOD levels were measured using the xanthine-xanthine oxidase method. The effect was analyzed using statistical analysis. The administration of Javanese ginseng extract at doses of 0.35 mg/200g BW rats/day, 0.70mg/200g BW rats/day, and 1.40 mg/200g BW rats/day could significantly increase SOD levels ($p < 0.05$). The administration of 1.40 mg/200g BW rats/day could increase a SOD level up to normal and give the same effect as the administration of xanthine ($p > 0.05$) does. In conclusion, the administration of Javanese ginseng extract significantly increases SOD levels in the rats by using the FST model. This study suggests to increases the potential of Javanese ginseng root as a natural-based antioxidant supplement by investigating human subjects, especially athletes and bodybuilders.
Keyword: Forced swimming test; javanese ginseng root; superoxide dismutase.

INTRODUCTION

Physical activities and adequate nutritional intake influence health status. Regular physical activities can prevent degenerative diseases like coronary heart disease, diabetes mellitus, and osteoporosis. However, if physical activities are done excessively, they will disrupt the balance and work systems of the body's organs. The increased metabolism requires more oxygen because insufficient oxygen triggers the body to perform anaerobic metabolism, buildups lactic acid in the muscles. The accumulation of lactic acid produces free radicals in the form of reactive oxygen species (ROS)[1] Exhausting-physical activities trigger the body to produce too much ROS, which causes excessive oxidative stress, fatigue, and muscle cell damage[2].

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To reduce the adverse effects of oxidative stress conditions, the body requires antioxidants from outside, especially from food intake. Antioxidants from food include vitamin A, vitamin C, vitamin E, flavonoids, and saponins. Antioxidant ability is characterized by increased activities and levels of antioxidant enzymes, namely superoxide dismutase (SOD). SOD enzymes protect against body cells and prevent inflammation due to free radicals. Moreover, SOD promotes superoxide anion radical dismutase (O_2) to be hydrogen peroxide (H_2O_2) and oxygen (O_2)[3].

Food is a source of antioxidants, especially from various plants such as fruits, vegetables, spices, tea, enzymes, and proteins[4]. Indonesia is a tropical region with various plants to improve health, one of which is Javanese ginseng (*Talinum paniculatum*), a popular plant among Indonesians. The community commonly uses this plant as a cooking spice, medicine for various diseases, and a stamina booster. Javanese ginseng contains alkaloids, flavonoids, saponins, tannins, provitamin A, and other nutrients functioning as anti-inflammatory, antioxidant, central nervous stimulation, and blood circulation[5]. The flavonoids in Javanese ginseng contain antioxidants that inhibit free radicals and improve the performance of the brain's nerves and cognitive abilities[6].

There has been researched by Kim et al. on 2010, who have proven that the administration of Korean red ginseng extract can increase intracellular SOD enzyme activity[7], and research by Menezes et al. on 2021, showed that ginseng leaves extract has the potential to have antioxidants to counteract free radicals and superoxide radicals[8]. These two studies only analyzed the effect of ginseng on antioxidant potential in vitro. However, we researched the effect of Javanese ginseng on SOD levels as a biomarker of oxidative stress in experimental animals. So, this research aims to determine the effect of Javanese ginseng extract on SOD levels in male Sprague Dawley rats using the forced swimming test model.

MATERIALS AND METHODS

Preparation of Javanese Ginseng Extract

This study used Javanese ginseng obtained from the Herbadream Company, Sragen, Central Java. Javanese ginseng was cleaned, cut into small pieces, and dried for two weeks. Afterwards, Javanese ginseng extract was made using the maceration method. The extraction process was carried out at the Food Technology and Engineering Laboratory of Universitas Gadjah Mada[5].

Experimental Animals and Grouping

The samples of this research were 30 male rats. The adaptation process was carried out on day seven by randomly dividing the samples into five groups: the negative control group (P1), the positive control group receiving xanthin as an antioxidant (P2), treatment group 1 receiving 0.35mg/200g BW/day of Javanese ginseng extract (P3), treatment group 2 receiving 0.70mg/200g BW/day of Javanese ginseng extract (P4), and treatment group 3 receiving 1.4mg/200g BW/day of Javanese ginseng extract (P5). The determination of the dosage of Javanese ginseng extract in this study was based on the usual doses consumed by humans and converted to experimental animals[9]. The dosage in each treatment group is based on research by Riyana, Mudigdo, and Wars (2019)[5]. Javanese ginseng root extract was administered orally daily for 35 days[10]. Meanwhile, the body weight of experimental animals was measured once every seven days.

All stages of this research have followed animal ethics in managing experimental animals. This research has received ethical approval from the Ethics Commission of the Faculty of Medicine, Sebelas Maret University Surakarta, Certificate Number 290/UN27.6/KEPK/2019.

Mice Preparation and Testing Method

Before undergoing the forced swimming test, the rats weighed about 5% of their weight with a piece of tin. The tin was attached to the root of the rats' tails. An hour after dosing, all rats were forced to swim in containers filled with water at a temperature of 25°±1°C. The forced swimming test was conducted by placing white male rats in containers filled with water at a temperature of 25°±1°C. One hour after dosing, all rats underwent a heavy-weight swimming test with a piece of tin (about 5% of each mouse's body weight), which was attached to the root of their tails. Rats were defined as exhausted if they failed to rise above the water in 10 seconds, all four legs did not move, their bodies were bent, the tails were stretched, and the heads were left underwater[11,12].

On days 28 and 35, the experimental animals were challenged with a forced swimming test to determine the effect of Javanese ginseng root extract on oxidative stress due to excessive-physical exercises. However, before this study had been approved for the forced swimming test, the experimental animals were drilled to adapt to swimming without weights for 20 minutes/day for three days. After swimming, the rats were towel-dried and put in a cage[5,13]

The next step was the SOD-level examination. The 0.06 ml plasma was reacted with 2.70 sodium-carbonate buffer consisting of 0.1mM EDTA (pH 10), 0.06 ml xanthine 10mM, 0.03 ml bovine serum albumin (BSA) 0.5%, and 0.03 NBT 2.5MM. Then, xanthine oxidase (0.04 units) was added. The 61 absorbances formed after 30 minutes and were measured at a wavelength of 560 nm. SOD levels (%) were calculated using the formula (W/A) x 100%. A is the absorbance of the sample solution, and B is the absorbance of the control solution[14,15].

The mice used in this study did not die and remained healthy because they had been acclimatized for one week to adapt to the environment. Mice were also given standard feed from PT Comfeed and aquadest ad libitum.

Statistical Analysis

The collected data were presented by mean ± standard deviation. Before running statistical analysis, homogeneity and normality data were analyzed using Shapiro-Wilk tests. The results of the analysis showed that the data were not normally distributed ($p<0.05$) and had a homogeneous variance ($p>0.05$). The Wilcoxon test was used to determine the difference in the effect of each treatment group between the 28th and the 35th day. The Mann-Whitney test analyzed the difference in the effect of the five treatment groups. Then a Post-Hoct test was carried out to determine which two groups had different effects.

RESULTS AND DISCUSSION

SOD levels were measured using the xanthine-xanthine oxidase method. The results of this measurement are presented in Table 1. Groups P2, P4, and P3 significantly increase SOD levels. In contrast, the other groups do not show a significant increase; for example, group P1 decreases the SOD level. The highest mean increase in SOD levels is found in groups P2, P5, and P4, respectively.

On day 28, the treatment groups do not show a different mean of SOD levels (p = 0.293). In contrast, on day 35, all treatment groups show a significantly different mean of SOD levels. This indicates that the forced swimming in Sprague Dawley rats results in a different mean of SOD levels.

Table 1. The Average SOD Levels of Treatment Groups on White Sprague Dawley Rats with Forced Swimming Test

Groups	SOD Levels (%)			Pb
	Day 28	Day 35	Δ SOD	
P1	22.01±7.23	17.92±5.94	4.09±1.42	0.026*
P2	22.64±3.96	72.64±5.17	50.00±7.43	0.028*
P3	25.15±6.49	39.25±5.87	13.96±10.21	0.068
P4	28.30±4.30	61.64±6.92	33.33±10.64	0.027*
P5	25.78±5.69	72.33±4.87	46.54±6.82	0.027*
p^a	0.293*	0.001*		

Description:
P1 (negative control): Aquades; P2 (positive control): Xanthine; P3: 0.35mg/200g BW of Javanese ginseng extract; P4: 0.70mg/200g/200g BW; P5: 1.40mg/200g BW
*: significant (< 0.05); p^a: Kruskal Wallis; p^b: the Wilcoxon signed rank test

Table 2 presents data on different SOD levels between the two treatment groups on day 35. The data show significantly different SOD levels in all pairs of treatment groups (p < 0.01), except for pairs in groups P2 and P5 (p > 0.01).

Table 2. Post-Hoc Analysis of Effects of Ginseng Java Root Extract on SOD Levels of White Sprague Dawley Rats with Forced Swimming Test on Day 35

Group 1	Group 2	*P*
P1	P2	0.004*
	P3 (0.35mg/200g BW)	0.006*
	P4 (0.70mg/200g BW)	0.004*
	P5 (1.40mg/200g BW)	0.004*
P2	P3 (0.35mg/200g BW)	0.006*
	P4 (0.70mg/200g BW)	0.013*
	P5 (1.40mg/200g BW)	0.936
P3	P4 (0.70mg/200g BW)	0.006*
	P5 (1.40mg/200g BW)	0.006*
P4	P5 (1.40mg/200g BW)	0.013*

Description:
P1 (negative control): Aquades; P2 (positive control): Xanthine; P3: 0.35mg/200g BW of Javanese ginseng extract; P4: 0.70mg/200g/200g BW; P5: 1.40mg/200g BW
*: Significant on day 35 (< 0.05).

This study has revealed that the administration of 0.35mg/200g BW, 0.70mg/200mg, and 1.40mg/200mg BW of Javanese ginseng root extract added with xanthine to white Sprague Dawley rats can significantly increase SOD levels. Meanwhile, the rats that do not receive Javanese ginseng root extract show a decrease in SOD levels.

The results of this study are supported by Kim et al. on 2010, who have proven that the administration of ginseng extract could increase intracellular activities of the SOD enzyme[(7)]. Meanwhile, Menezes et al. on 2021 suggest that Javanese ginseng extract potentially has antioxidants to counteract free radicals and superoxide radicals[(8)]. Other studies have also revealed that Javanese ginseng contains secondary metabolites, such as saponins, flavonoids, and tannins. The flavonoids in Javanese ginseng have antioxidant activities[16].

Moreover, this study has revealed that the administration of 1.40mg/200g BW of Javanese ginseng extract (P5) could increase SOD levels in rats by 46.54% to 72.33%. Meanwhile, Harun et al. on 2017 have found that the normal SOD level in Sprague Dawley rats is 81.45±5.53. The administration of Javanese ginseng extract with lower doses of 0.35mg/200gr BW and 1.70mg/200gr BW insignificantly increases SOD levels[17]. Thus, 1.40mg/200gr BW of Javanese ginseng extract can increase SOD levels even though these levels have not yet reached normal. The limitations of this study are the researchers did not compare the treatment group with the normal control group who were not given any treatment, and the dose of Javanese ginseng was not correct, so that the therapeutic effect that occurred was no better than the xanthine effect in the positive control group. This study employs a small dose of Javanese ginseng extract, so the expected therapeutic effect is less optimal. 500 g/mL Javanese ginseng can provide 98.45% free radical scavenging effects, and 500 g/mL Javanese ginseng can provide 97.01% of superoxide radicals in the xanthine/xanthine oxidase systems[18.]

On day 28, the five groups do not show significantly different SOD levels; in contrast, on day 35, they show significantly different SOD levels. This finding denotes that the administration of Javanese ginseng extract requires a certain duration to bring a therapeutic effect. Furthermore, Table 2 summarizes significantly different SOD levels in the treatment groups, except for the pair of P2 and P5. This finding asserts that the administration of Javanese ginseng extract at 1.40mg/200mg BW of white rats brings nearly similar effects to the administration of xanthine.

CONCLUSION

The administration of Javanese ginseng extract with doses of 0.35mg/200mg BW/day, 0.70mg/200mg BW/day, and 1.40mg/200mg BW/day could increase superoxide dismutase enzyme activities in white male Sprague Dawley rats by using the FST model on day 35. A dose of 1.40mg/200mg BW/day has a similar effect as xanthine. This study suggests that the development of sciences and technology increases the potential of Javanese ginseng root as a natural-based antioxidant supplement by investigating human subjects, especially athletes and bodybuilders.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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