

EFFECTS OF BEAN JUICE (*Phaseolus vulgaris* Linn) FOR INCREASING SUPEROXIDE DISMUTASE ACTIVITY IN PSYCHOLOGICAL STRESS RATS

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<https://doi.org/10.32827/ijphcs.5.5.88>

ABSTRACT

Background: Psychological stress is often suffered to people with body rhythm disorders, such as in sleep disorders upside down people. Psychological stress can trigger oxidative stress. Oxidative stress can be minimized by antioxidant activity. Beans as antioxidants are considered to reduce oxidative stress. The aim of this study was to examine the effectiveness of bean juice in increasing SOD activity in male white rats (strain wistar) exposed by psychological stress.

Materials and Methods: This study is an experimental research, with Randomized Post Test Only Group Design. There were five groups in this study, control group, stress control group, and 3 treatment groups. Treatment groups were exposure by psychological stress, which was given by inverted sleep guard pattern for 1 x 24 hours. These treatment groups were given bean juice dose 4,5 g/ml. Blood from each treatment group was taken after 1 hour, 6 hours, and 24 hours to measure SOD activity. MANOVA (Multivariate Analysis of Variance) Test was used to analyze the difference of SOD activity in five treatment groups.

Result: The results showed that SOD activity can be increased by providing bean juice in the first hour after psychological stress exposure. The highest SOD level was found after 24 hours giving bean juice. SOD activity can increase due to the content of flavonoids in beans that can fight free radicals and increase endogenous antioxidants activity through scavenging mechanism.

Conclusion: A single dose of bean juice in this study, can increase SOD activity after one hour giving by bean juice and its antioxidant activity persisted until 24th hour. It showed that bean juice can work as an antioxidant by increasing SOD activity.

Keywords: Oxidative stress, Psychological stress, Bean juice, *Phaseolus vulgaris* Linn,

1.0 Introduction

Psychological stress is often suffered to people with body rhythm disorders, such as in sleep disorders upside down people. Yani's research (2014) showed a significant relationship between shift work patterns and the incidence of depression, anxiety and stress due to decreased quality of sleep and decreased quality of relationships with family or friends. Continual sleep patterns disorder will cause psychological stress (Hawari, 2016). Psychological stress can trigger the occurrence of oxidative stress, and causes some degenerative diseases. Acute stress conditions, such as physical stress or psychological stress will affect the Hypothalamus Ptiutari Adrenal (HPA) Axis system in the body. Stimulus in the hypothalamus leads to increase secretion of adrenal hormones and cortisol hormone (Kestin et al, 1993). When adrenaline is secreted, there is an enhancement in heart rate and blood vessel constriction (vasoconstriction). Free radicals occur due to vasoconstriction and blood turbulence that occur continuously and blood glucose activity are increased. Katekholamine (noradrenaline and adrenaline) as a source of free radical formation, formed by autooksidation in complex reactions (Sozmen, 1998).

The imbalance between free radical formation and antioxidant activity in the body causes oxidative stress (Yoshikawa, 2002). Oxidative stress in the body causes degenerative diseases (Pham Huy et al, 2008). Oxidative stress is caused by oxidative reacting free radicals in the body, especially the lipid peroxidation reaction producing malondialdehyde (MDA) as a marker of oxidative stress. Increased free radicals or reactive oxygen species (ROS) cause decreased endogenous antioxidants in the body, one of which is an enzymatic superoxide dismutase (SOD) enzyme that inhibits super oxide (O₂⁻) (Del Rio, 2005).

The body needs antioxidants that can protect the body from free radical attacks, which can trigger oxidative stress. Beans (*Phaseolus vulgaris linn*) components can give effect to suppress oxidative stress process. These components lead beans to increase SOD, GPx, and CAT activity in diabetic rats model which is given by beans juice (Venkateswaran *et al.*, 2002). Beans have antioxidant activity that can provide protective action due to lipid peroxidation and to enhance the defence of cellular antioxidants.

This study aims are to analyse the role of antioxidants contained in bean juice (*Phaseolus vulgaris Linn.*) in increasing SOD activity. This study explains how psychological stress mechanisms decrease endogenous antioxidants one of them SOD, and beans as exogenous antioxidants can increase the activity of SOD.

2.0 Materials and Methods

This study is an eexperimental research, with Randomized Post Test Only Group Design. Samples of this study were 25 white male rats strain wistar (*Rattus norvegicus*) and divided into 5 groups. Control group (K1), stress control group (K2), P1 treatment group for examination after 1 hour giving bean juice, P2 treatment group for 6 hours after giving bean juice, and P3 treatment group after 24 hours after giving bean juice. All groups, except control group were inverted sleep guard pattern for 1 x 24 hours as psychological stress exposure. Before treatment, 2-3 month old white male rats weighed with an average weight of 150-200 grams, and in good health. Beans are obtained from traditional markets, made by juice with

extractor machine. Bean juice dose 4,5 g/ml was given to five treatment groups, then blood samples from each group was taken after one hour, six hours, and twenty four hours giving by bean juice. MANOVA (Multivariate Analysis of Variance) was used to analyze the difference activity of SOD in each group.

3.0 Result

3.1 SOD Activities Between Groups

The lowest average SOD activity was found in group K2 (89.6340 U/ml), this group was given by psychological stress treatment without giving bean juice. Control group had SOD activity amount 91.3166 U/ml. SOD activity in treatment group (P1) was increased to 392,2337 U/ml, and is higher than positive control group (K2). On the other hand, SOD activity in treatment group (P2) amount 92,9897 U/ml and (P3) amount 93.4021 U/ml. the average activity of SOD was almost equal to the average of SOD control group (K1).

Table 1. Average of SOD Activity (U/ml) and Standard Deviation of Control Groups and Treatment Groups

Groups	n	Superoxide Dismutase (SOD)
		(U/mL)
Mean ± SD		
K1	5	91,3166 ± 1,03046
K2	5	89,6340 ± 1,01496
P1	5	92,2337 ± 1,36595
P2	5	92,9897 ± 1,25323
P3	5	93,4021 ± 1,07026

Information:

K1: without treatment

K2: psychological stress treatment

P1: Psychological stress + green bean juice + checks 1 hour after bean juice

P2: Psychological stress + green bean juice + check up 6 hours after bean juice

P3: Psychological stress + green bean juice + 24 h after checking of bean juice

The mean difference of SOD activity between treatment groups is shown in Figure 1 below.

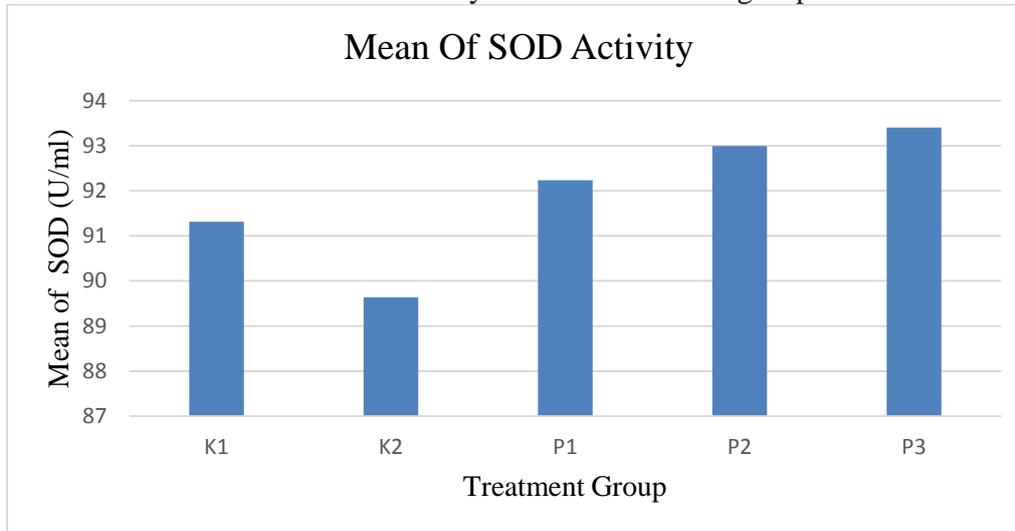


Figure 1. The Average Activity of Super Oxide Dismutase (SOD) (U/ml) In All Experimental Groups

3.2 Different in SOD Activities Between Groups

MANOVA test used to test SOD activity, and it showed that the significance value 0.000 ($p < 0,05$), so it can be concluded that there is significant effect after giving bean juice in all groups. Based on the Post Hoc test with LSD, showed that all groups were significantly different (p value $< 0,05$). The result of LSD test on SOD activity can be shown in table 2 below.

Table 2 P Value of LSD Test for SOD Activity

Groups	K1	K2	P1	P2	P3
K1	-	0,032*	0,224	0,033*	0,010*
K2	0,032*	-	0,002*	0,000*	0,000*
P1	0,224	0,002*	-	0,313	0,125
P2	0,033*	0,000*	0,313	-	0,579
P3	0,010*	0,000*	0,125	0,579	-

Description: *) = significant.

There was a difference of SOD activity between Control group (K1) and Positive Control Group (K2) which exposed by psychological stress treatment, that had significant value $p = 0,032$, treatment group P2 had significant value $p = 0,033$ and group treatment P3 had significant value $p = 0.010$. There was a difference between Positive Control Group (K2) and all treatment groups P1, P2 and P3, significant values with treatment group 1 (P1) $p = 0,002$, treatment group 2 (P2) $p = 0,000$ and treatment group 3 (P3) significant $p = 0,000$. While between the treatment groups P1, P2, and P3 there was no significant difference.

Based on the data obtained, the provision of green bean juice in male white strains wistar who experience oxidative stress due to exposure to psychological stress can increase the activity of SOD in all treatment groups given exposure to psychological stress and bean juice.

4.0 Discussion

This study aims to analyse the role of antioxidants contained in bean juice (*Phaseolus vulgaris* Linn.) in increasing SOD activity. Based on the results of this study, it can be concluded that the treatment of reverse guard sleep patterns can cause psychological stress in the experimental groups, which are marked by decreasing of SOD activity significantly in group K2. SOD activity in group K1 did not have significant difference with group P1 that is given bean juice and measured activity of SOD after 1 hour. It showed that the provision of bean juice can increase SOD activity and restore to a normal circumstances due to psychological stress since 1 hour after giving bean juice.

The results according to the theory that SOD is an antioxidant enzyme that plays an important role in protecting cells from damage caused by Reactive Oxygen Species (ROS) (Azadmanesh & Borgstahl, 2018). SOD performs a bio protective role by converting superoxide to oxygen (O₂) and hydrogen peroxide (H₂O₂) by cyclic oxidation and reduction reaction to active metals (Azadmanesh & Borgstahl, 2018).

The flavonoid content of the beans will counter the superoxide anion, suppress the production of ROS and increase the activity of SOD enzymatic antioxidants (Baba, et al., 2007). The provision of *Phaseolus vulgaris* juice (PPEt: 200 mg/kg body weight) increases GPx, SOD, CAT, Gluthatione-S-Transferase in the liver and kidney of diabetic rats induced by streptozotocin significantly (Venkateswaran, 2012) Endogenous antioxidants such as SOD, CAT, and GPX are able to block reactions in free radical chains when overproduced or when cellular antioxidant defense systems break down. These three enzymes can counter free radical activity resulting in cellular and neurological damage such as Reactive Oxigen Metabolite (ROM), super anion oxide (O₂⁻), hydroxyl radicals (⁰OH), and hydrogen peroxide (H₂O₂) (Sahu, et.al., 2013).

SOD activity has increased, which indicates the body's adaptation reaction in overcoming oxidative stress. Provision of bean juice increased the activity of SOD significantly starting from the 1st hour after the granting of bean juice and increased the maximum until the 24th hour. It is very useful for the protection of the body against free radical attacks repeatedly. The role of flavonoids and arginine in bean juice strongly supports increased activity of SOD as an endogenous antioxidant. Where SOD activity increases then the body will be protected stronger than the presence of free radical exposure to the next activity. Arginine acts as a precursor of the formation of Nitric Oxide (NO) as a vasodilator of blood vessels that can decrease oxidative stress. Flavonoids will scavenge the presence of superoxide anions, thus suppressing ROS production and increased SOD enzymatic antioxidant activity.

Based on these analyses, it can be concluded that bean juice (*Phaseolus vulgaris* Linn) as exogenous antioxidants can increase activity of endogenous antioxidant enzyme SOD through antioxidant activity on beans. Provision of bean juice dose 250 grams/day in humans converted to experimental animals to 22,5 g/kg BW proved to significantly increase the activity of SOD since 1 hour after the provision of bean juice. Even at 24 hours after the provision of bean juice, the average SOD activity was higher than the 1st and 6th hours after giving bean juice.

5.0 Conclusion and recommendation

Provision of bean juice (*Phaseolus vulgaris linn*) dose of 4,5 gr/ml successfully increases SOD activity as an endogenous antioxidant due to psychological stress. Bean juice (*Phaseolus vulgaris linn*) can overcome oxidative stress due to ppsychological stress exposure. Bean juice can effectively increase SOD activity from the first hour after giving bean juice with a safe dose for health. This effect can last up to 24 hours after the consumption of bean juice.

Further research is needed on the provision of beans to determine endogenous antioxidant activity in the next stage (catalase and glutathione peroxidase) that work to overcome free radical attack on oxidative stress conditions.

Acknowledgement

The ethical clearance of this research had been accepted and proof with ethical approval certificate from Health Research Ethics Committee Faculty of Public Health Airlangga University. Authors thank to colleague, Public Health Nutrition Department, Faculty of Public Health, Airlangga University and Biochemistry Laboratory, Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya for their support and cooperation. So, there's no conflict of interest in this research.

Declaration

Author(s) declare that there's no conflict of interest in this research

Author's contribution

Author 1: Concept, idea, data collection, statistical analysis, and manuscript writing

Author 2: Concept, idea, literature review and manuscript writing

Author 3: Concept, idea, literature review, and manuscript writing

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