

EFFECT OF GREEN TEA (CAMELLIA SINENSIS) ON ACTIVITY OF SUPEROXIDE DISMUTASE (SOD)

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ABSTRACT

Background: Oxidative stress can be triggered by exposure to psychological stress. Oxidative stress can be reduced by the consumption of exogenous antioxidants, green tea is considered able to reduce oxidative stress. The purpose of this study to examine the effect of green tea steeping on superoxide dismutase (SOD) activity in male rats strain Wistar which exposed to psychological stress.

Materials and Methods: This study used Randomized Post Test Only Group Design. This experimental laboratories study used 25 male *Wistar Strain* white rats (*Rattus Norvegicus*) as experimental animals and grouped into 5 groups. Normal control group (K1), stress control group (K2) and P1 treatment group for 1 hour after green tea, P2 examination 6 hours after administration of green tea and P3 examination 24 hours after administration of green tea. Previously rats given psychological stress 1 x 24 hours in the form of sleep disturbance guard pattern, so that can occur oxidative stress. Provision of steeping green tea shortly after the treatment of psychological stress with a single dose of 3 , 6 ml per rat. Statistical analysis used Multivariate Analysis of Variance (Manova) test.

Result:The result of this study, based on statistical analysis showed that there were differences SOD activity increased in 1sthour, to increase again in the 6th hour and maximum increasedin 24th hour after given of green tea steeping.

Conclusion: A single dose of green tea steeping in animals which undergo oxidative stress has proven to neutralize oxidative stress in all 1st hour after given of green tea steeping and its antioxidant activity remains for up to 24 hour.

Keywords: Oxidative Stress, Green Tea, SOD

1.0 Introduction

Psychological stress is often faced by people with a body rhythm disorders, one of which is to undergo reverse phase guard bed. The shifts in the times that cause the pressure and the burden of life to unfavorable social, economic and environmental conditions can increase the risk of psychological stress s. Based on Riskesdas (2013) data, the prevalence of psychological stress or known as emotional mental disorder in Indonesian population about 14 million people. Prevention is essential to reduce morbidity and mortality. Oxidative stress can be reduced by increasing the production of antioxidants that can be obtained from outside the body to help the effectiveness of antioxidants from the body (endogenous).

Psychological stress is often faced by people with a body rhythm disorder, one of which is to undergo reverse phase guard bed (Maramis, 2009). Shifting the days leading to high pressures and burdens of living on social, economic, and unfavourable environment can increase the risk of psychological stress. Prevention is very important in order to reduce morbidity and mortality. Oxidative stress can be reduced by increased production of antioxidants which can be obtained from outside the body to assist in the effective work of the antioxidants in the body (endogenous). One ingredient that can give effect to suppress oxidative stress processes are green tea (*Camellia sinensis*) where green tea is considered to have the ability to increase superoxide dismutase (SOD) activity.

Antioxidants are needed to strengthen the body's resistance in preventing oxidative stress. Antioxidants work by preventing tissue damage caused by free radicals and prevent the formation of free radicals. Antioxidant supplements are considered less favorable for human health, so antioxidants derived from nature are preferred in their use. In addition to endogenous activity already formed in the body, antioxidants derived from food are preferred (Lobo et al, 2010).

Green tea has the highest antioxidant activity characterized by low IC₅₀ (0.487 µg / ml) when compared with other tea types (black tea: 5,405 µg / ml and oolong tea: 5,005 µg / ml) (Widowati et al, 2015). Green tea contains polyphenols, which are the active substances of green tea. Benefits of tea contained in the bioactive component of polyphenols, optimally contained in tea leaves are still young and intact. Catechin is the dominant compound of polyphenols, and consists of epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin (EGC), Epigallocatechin gallate (EGCG), Gallocatechin (GC) (Ningrat, 2006).

Antioxidants in certain levels can inhibit or slow down the damage caused by the oxidation process (Sayuti, 2015). The human body does not have large amounts of antioxidant reserves, so that if free radicals in the body are over-exposed then the body needs exogenous antioxidants, one of which is the natural antioxidant coming from outside (Sayuti, 2015). One of ingredient that can exert an effect on oxidative stress is green tea (*Camellia Sinensis*) where green tea is considered to have the ability to increase SOD activity because of its antioxidant properties. *Polyphenols* in green tea contain the most dominant flavonoid compounds among other green tea components (about 75%) of the total *polyphenols* in green tea (Shabri and Rohdiana, 2016). The most important flavonoid is *catechin* (about 20-30%) of all leaf dry weight (Anjarsari, 2016), where (EGCG) is the most active compound in the *catechin* component compared to other compounds.

Green tea is processed in a special way. After picking, the tea leaves will be fumigated. The process will dry the tea leaves, but not to change the color of the leaves at all. This is the condition that causes water steeping tea leaves still look light green. The process of green tea in its manufacture is then proven to maintain various nutrient content, including antioxidant polyphenols greater than black tea and red tea (Marie et al, 2005).

In general, antioxidants work by donating an electron to an oxidant compound so that the activity of the oxidant compound can be inhibited. Exogenous antioxidants have the ability to donate electrons and can function as reducing agents that can chelate metal ions and reduce the potential of free radicals in the body (Kesuma and Rina, 2015). Based on its function and its working mechanism, polyphenols in which flavonoids, catechins and EGCGs are secondary antioxidants that work by chelating metals that act as pro-oxidants, capture radicals and prevent chain reactions. Secondary antioxidants act as binders of metal ions, oxygen traps, decomposers of hydroperoxides into non-radical compounds. The metal chelating compound which forms σ -metal bonds is effective as a secondary antioxidant because only these compounds can decrease redox potential and stabilize oxidized forms of metal ions.

2.0 Materials and Methods

This research is a type of research of *Experimental Laboratories* (Laboratory Experiments), where this research will be conducted in the laboratory, with the intention to control the potentially disruptive external variables. In this study used animal try white male *rats wistar strain* given exposure to psychological stress, so that experiencing oxidative stress, because oxidative stress is a condition that wants to be influenced by the provision of green tea.

The research design used is *Randomized Post Test Only Control Group Design*. With this design allows the researchers to measure the effect of treatment / intervention with the experimental group were determined simply, by comparing the control group and the experimental group. This study using *Wistar strain* male rats were divided into 5 groups. Normal control group (K1), stress control group (K2) and P1 treatment group for 1 hour after green tea, P2 examination 6 hours after administration of green tea and P3 examination 24 hours after administration of green tea. Previously rats given psychological stress 1 x 24 hours in the form of sleep disturbance guard pattern, so that can occur oxidative stress. Provision of steeping green tea shortly after the treatment of psychological stress with a single dose of 3 , 6 ml per rat. Statistical analysis used Multivariate Analysis of Variance (Manova) test.

Determination of green tea dose using a comparison table of animal body animal try with humans, where the dose of mice weighing 200 grams is 0.018 human dose. Based on previous research the safe dose for humans in consuming green tea is 2 cups per day (Sesso, 2015), where in 1 cup of green tea is proportional to 100 ml of water and contains 1 gram of green tea powder, then 2 cups is proportional to 2 grams of tea powder green. For that in this study using a dose of 2 cups steeped green tea *single dose* . The conversion dosage of the volume

amount of green tea steamed for mice equivalent to 100 ml ~ 0.018 x 100 ml is 1.8 ml of steaming green tea. For 2 cups of green tea is equivalent to 3 , 6 ml steeping green tea. So made green tea from 2 grams of green tea powder brewed in 200 ml of water with a temperature of 70 ° C.

steeped green tea used is 100% steamed green tea from green tea leaf shoots and processed by certain methods to produce pure green tea green tea powder with a smooth green tea 200-250 mesh (very smooth). Physical form of powdered green tea is called matcha. Matcha is processed from steamed green tea export grade and is green when brewed. Green tea leaves are obtained from Kaanan Chakra tea plantation in Bandung, West Java, Indonesia. Green tea powder is brewed in volume 3 ml in mice for 1 x gift per sonde. Serum SOD examination was performed using Enzyme Linked Immunosorbent Assay (ELISA) and Human Super Oxide Dismutase (SOD) ELISA Kit E0918Hu (Rich S et al, 2007; Myin K et al, 2017). Expressed in units of U / mL.

3.0Result

The lowest meanof SOD activity wasshowed in stress control group (K2) that were 89.63 ± 1.014 U/ml, while themean of normal control group (K1) were 91.31 ± 1.030 U/ml. The mean of SOD activity werebegun to increase inP1 and P2 group, and the highest mean of SOD activity in P3 group.

Table 1: The MeanDifference of Superoxide Dismutase (SOD) in the Control Group and The Treatment Group of Rats

Group	n	SOD (U/ml)
		Mean \pm SD
K1	5	91.31 ± 1.030
K2	5	89.63 ± 1.014
P1	5	92.92 ± 1.229
P2	5	93.33 ± 1.229
P3	5	94.22 ± 1.097

SOD activity in all groups had a normal distribution ($p > 0,05$), using the *One Sample Kolmogorov-Smirnov test*. The normal control group (K1) with p value = 0.970, the stress control group (K2) $p = 0.677$, the treatment group (P1) $p = 0.999$, the treatment group (P2) $p = 0.999$ while in the treatment group (P3) $p = 0.989$. *Levene* homogeneity test using *Test*, MDA showed homogeneous data on the entire group of test animals with a value of $p = 0.945$ ($p > 0, 05$).

Manova test in all groups of experimental animals against SOD activity showed $p = 0.000 < 0, 05$, so that it can be stated that there is a significant influence after administration of green tea on a whole group of experimental animals to the activity of SOD. The mean difference in SOD activity in each group were shown in Figure 1 below.

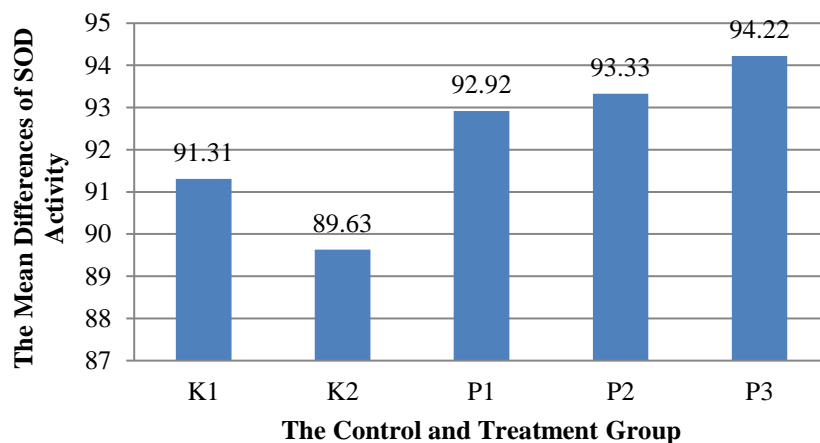


Figure 1: Graph of SOD activity mean (U/ml)

The graphs in the figure above show that between the groups (K1) and (K2) there is an average difference in SOD activity, as well as between groups (K1) with P1, P2 and P3. The differences were evident in the stress control group (K2), where the mean SOD activity was lower than all the study groups. The mean between treatment groups P1, P2, and P3 did not differ significantly between groups.

The statistical test performed on SOD activity data showed that the average of SOD activity was increased at all treatments with almost equal improvement with the normal control group and in the P3 treatment the maximum SOD activity exceeded the normal control group. In the group given only exposure to psychological stress (K2), SOD activity showed the lowest average among all study groups. Based on the data obtained, the provision of green tea in all groups of experimental animals that experience oxidative stress due to exposure to psychological stress can increase the activity of SOD. To find out which groups of different meanings, then tested *LSD*, significant if $p < 0,05$. The test results can be seen in the following table 2:

Table 2: The Comparison of SOD Activity with *LSD* Post-Hoc

Group	K1	K2	P1	P2	P3
K1	-	0.028*	0.035*	0.010*	0.001*
K2	0.028*	-	0.000*	0.000*	0.000*
P1	0.035*	0.000*	-	0.568	0.081
P2	0.010*	0.000*	0.568	-	0.223
P3	0.001*	0.000*	0.081	0.223	-

there was a difference between the normal groups (K1) and the stress group (K2) with *p value* 0.028, as well as between the normal groups (K1) and the treatment group (P1) *p value* 0.035, with (P2) *p value* 0.010 and with (P3) *p value* 0.001 shows a significant number. Furthermore, there was a significant difference between stress group (K2) and all treatment groups P1, P2, P3 with *p value* 0,000, 0,000, and 0,000 respectively.

All treatment group values (P1, P2 and P3) obtained results that were not significantly different (not significant). This explains that SOD is strongly influenced by the doses of steeping green tea used, so the tea used to decrease oxidative stress depends on the dependent dose to lower blood glucose, so it may be necessary to use higher doses to obtain a significant difference in the treatment group in the next study.

4.0 Discussion

The most critical antioxidants that can improve oxidative stress pressure effect is the enzyme superoxide dismutase (SOD). Representative three-dimensional structure of an enzyme that is this protein catalyzes changes superoxide into hydrogen peroxide and oxygen. Superoxide is a free radical molecule that has unpaired electrons. Therefore it is very reactive and can injure the reactivity of molecules in the body. Because it has an important role, SOD has become an alternative way to minimize tissue damage caused by free radicals. SOD is an enzyme with ramifications (branching) which extended (Nurhayati, et.al., 2011).

Based on statistical analysis, the activity of SOD in the control group and the treatment group stress was significant ($p < 0.05$), but there are significant differences between each treatment (P1, P2 and P3). Antioxidants can be used as a therapy in reducing oxidative stress. Antioxidants from outside (exogenous) as a natural ingredient indispensable in helping the antioxidant activity of the (endogenous) in counteracting free radicals cause oxidative stress. Green tea with active ingredients that inhibit free radicals is expected to reduce the risk resulting from oxidative stress.

Green tea is refreshing drink raw materials that are believed to prevent, treat and eliminate the symptoms of the disease and has been used for thousands of years. There had done much research on the healthful benefits of green tea. Chemicals in green tea greatest role in shaping taste and special properties are polyphenols (Rohdiana, 2009). Polyphenols are recognized to have antioxidant activity which is very strong (Yi-fan, Jia-shun, G, 2014). Its ability to capture free radicals is 100 times more effective than vitamin C and 25 times more effective than vitamin E (Hagermae, 2002).

Based on the results obtained, it is stated that there are significant differences between the normal control group (K1) and stress control group (K2) with three treatment groups (P1, P2 and P3) that causes SOD increased after were given of green tea. SOD associated with protein synthesis. Psychological stress stress on SOD protein synthesis, causing SOD becomes lower. This can be evidenced by decreased SOD activity due to rats that only were given psychological stress (K2). Because of the psychological stress, it can decreased the synthesis of endogenous antioxidants.

At the same time, SOD activity increased in groups of P1, P2 and P3 after were given green tea steeping. That occurrence had supported by the previous study which described that the antioxidant activity of green tea has the structure of phenolic unique, which can donate electrons or protons into free radicals and show the resonance of electrons delocalisation. The study showed the effect of green tea is associated with upregulation of genes for SOD and catalase. In rats, catechin derived from polyphenols can protect epidermal cells against SOD

and catalase changes caused by radiation damage. In other systems, green tea can also activate the expression of other antioxidant enzymes (Li, et.al., 2007).

Psychological stress significantly causing SOD decreased, then after were given green tea, SOD could be increased. It had known that SOD was an endogenous antioxidant that can increase SOD activity, and at the same time, the body will be protected more strongly than their exposure to free radicals to the next activity. Through the results of this study, can be applied by the activities or jobs required higher SOD as protection against oxidative stress (e.g. security, the night shift, and conditions or job that requires one to stay up).

According to Tsao (2010) in his research explains that polyphenols in green tea are able to induce other antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase (SOD). Similarly, Yukman Li (2007) explains that green tea may activate the expression of other antioxidant enzymes. In this case, the explanation can be proven through this study because of the increased activity of SOD after administration of steeping green tea.

SOD's mechanism of action actually depends on metallic minerals such as Manganese (Mn), Zinc (Zn) and Copper (Cu) in order to work. Therefore, these minerals must be available in sufficient quantities, if you want to inhibit the emergence of degenerative diseases (Kesuma and Rina, 2015). When viewed from the content of green tea itself, it contained several types of minerals including minerals needed by SOD to work, so indirectly green tea is also able to transfer minerals needed SOD to increase work activities. Green tea contains 4-5% mineral per leaf dry weight.

As the above conditions takes place, then the SOD activity would decreased. However, when given green tea, the SOD activity also increased in 1st and 6thhour, and further increased up to the 24th hour after consuming green tea steeping. That is, someone who was in a state or night work shift, when consuming green tea will be ready to move up to the next 24 hours due to the protection of the SOD were higher. All values of the treatment group (P1, P2 and P3) to get results that are not much different (not significant). It was clear that the SOD were strongly influenced by dose green tea steeping was used. So, the green tea used to reduce oxidative stress depends on the dose.

5.0 Conclusion and recommendation

There is difference in oxidative stress of Superokside Dismutase (SOD) activity after administration of green tea sediments in wistar strain white rats exposed to psychological stress. A single dose of green tea steeping in rats which undergo oxidative stress is proven to neutralize oxidative stress in 1st hour after is given of green tea and its antioxidant activity can remains up to 24 hours. There was an effect after the administration of green tea sediments in male white rats of wistar strains exposed to psychological stress on Superokside Dismutase (SOD) activity.

In a vulnerable group of people suffering from sleep disorders that cause psychological stress resulting in the production of oxidative stress in the body, green tea as functional food ingredients are expected to be used as exogenous antioxidants that help maintain and overcome the state of oxidative stress due to irregular sleep patterns.

It may be necessary to use a higher dose of green tea steeping to get a significant difference in the treatment group at the next.

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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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