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UTILIZATION OF TERONG BELANDA (*SOLANUM BETACEUM* CAV.) PEELS EXTRACT AS ANTIOXIDANT IN RATS UNDER OXIDATIVE STRESS CONDITION

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ABSTRACT
The present study was conducted to determine activity of terong belanda (*Solanum betaceum* Cav.) peels extract to superoxide dismutase and its activity in inhibiting lipid peroxidation in the liver of rats. *In vivo* test used five groups; (1) a control group, (2) a stres group, (3) treatment with extract followed by stress condition, (4) treatment with α-tocopherol followed by stress condition and (5) treatment with extract and α-tocopherol followed by stress condition. The stress condition was achieved by five days fasting accompanied by swimming activity for 5 min/day. The extract and α-tocopherol were administrated at a dose of 60 mg/Kg/BW/day for seven days. The extract treatment increased SOD activity by 15.96% and decreased MDA (*malondialdehyde*) level by 20.99%. The combination of extract and α-tocopherol treatment gave the best results which increased SOD activity by 44.55% and decreased MDA level by 47.06%.

KEYWORDS: *Solanum betaceum* Cav., α-tocopherol, superoxide dismutase, malondialdehyde.

INTRODUCTION
Lifestyle changes and environmental degradation may indirectly cause several problem, such as of polluted environment, high ultraviolet radiation, exposure to pollutants and other free radicals that lead to the appearance of oxidative stress. Oxidative stress is an imbalance state, which the free radicals is produced more than antioxidants in the body. Free radical is a molecule that has an unpaired electron in their outer orbital and usually in the form of hydroxyl radical (• OH), peroxyl radical (• OOH) and superoxide ions (O2 •).

The presence of free radicals can damage the macromolecules in cells such as carbohydrates, proteins, DNA and fats. The damage can cause various pathological conditions to cells, tissue and organ in both human and animals. Furthermore, it can lead the cell to death and triggering various degeneratif diseases.[3,4]

A chain reaction known as lipid peroxidation is caused by hydroxyl radicals in the body. It involves a complex proccess between the reaction of unsaturated fatty acids, a phospholipid constituent of cell membranes, and reactive oxygen compound to form the hydroperoxide.[5]

Lipid peroxidation breaks fatty acids chain into various toxic compounds that are harmful to the cells as malondialdehyde (MDA), ethane and pentane.[6]

Animal and human body normally its own system to prevent the formation of free radicals by three endogenous antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat). Moreover, SOD is one of the endogenous antioxidants that catalyzes the dismutation reaction of superoxide anion (O2) free radical that forming hydrogen peroxide and molecular oksigen.[1]

Increase of oxidative stress condition can be triggered by several factors, such as fasting and doing exercise.[7,8] Oxidative stress condition causes an increases the production of free radicals excessively that can reduce endogenous antioxidant enzymes activity and cause cell damage. Therefore, an exogenous antioxidant is needed, such as vitamins, β-carotene, polyphenols and flavonoids, to enhance the endogenous antioxidant enzymes activity and prevent cell damage.

Several vitamins such as vitamin A, C and E can act as antioxidants (tocopherol). According to the recent research, the addition of 60 mg/ kg body weight during 7 days affect the increase of SOD and decrease MDA in

rat's liver tissue.[9] Another secondary antioxidants that has antioxidant property is flavonoid. Flavonoid in *Solanum betaceum* has been an interest for many researchers due to its potential as an antioxidant.[10]

This study aims is to determine the activities of *Solanum betaceum*'s peel extract against superoxide dismutase and its activity in inhibiting lipid peroxidation reaction in liver tissue of mice under stress conditions.

MATERIALS AND METHODS

Preparation *Solanum betaceum* Cav peels extract

A total of 1 kg *Solanum betaceum*'s rind that has been finely macerated with ethanol in ± 24 hours. Then the filtrate is collected and evaporated with a rotary vacuum evaporator to obtain a concentrated extract of ethanol. Water and ethanol (7:3) then added to the concentrated extract and evaporated again to obtain the extract water. Further, the water extract was processed into three phase partition which firstly by n-hexane to obtain aqueous extracts and n-hexane extract (EH). Secondly, the aqueous extract was partitioned with chloroform to obtain aqueous extracts and extract of chloroform (EK) and then the water phase partitioned with n-butanol to obtain extracts of n-butanol (EB). Finally, the N-butanol extract is subsequently evaporated to obtain a thick extract of *Solanum betaceum*'s rind that used for in vivo tests.

Animal experiments and sampling

Twenty five *Wistar* rats with an average body weight ± 250 g has been used as experimental animal in this research. The mice are divided into 5 groups and each group consisted of 5 mice, after 2 weeks of adaption to its environment (Table 1.1). Oxidative stress is triggered by fasting, ad-libitum water intake and swimming exercise for 5 minutes/day. Then α-tocopherol and sample's extract injected with a dose of 60 mg/ kg body weight per day. Natur-E that contains 100 IU of D-α-tocopherol was used in this as α-tocopherol source. After the treatment, the experiment mice's liver was taken and analyzed to determine the levels of SOD and MDA.[11]

Tabel 1.1. The treatment group rats and the type of treatment given

Groups	Treatment		
	α-tokoferol (7 day) 60 mg/Kg/Bw/day	**Extract (7day) 60 mg/Kg/Bw/day**	**Stres (5day)***
K	-	-	-
S	-	-	+
TS	+	-	+
ES	-	+	+
TES	+	+	+

information:

+ : threated *T : α-tokopherol*

- : without threatment *S : stres*

** : Fasting 5 days and swimming 5 menit/day* *E : terong belanda peels extract*

Analysis of the activity of superoxide dismutase (SOD) in rat liver

The SOD activity measurements in this study followed the procedure of Wijeratne et al. (2005) with slight modifications. A total of 0.06 mL of the supernatant from the liver is reacted with a mixture of 2.70 mL of 50 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10); 0.06 mL of 10 mM xanthine; 0.03 mL bovine serum albumin (BSA) 0.5% and 0.03 ml of 2.5 mM NBT. Then, the addition of xanthine oxidase (0.04 units). After 30 minutes of treatment, the absorbance then measured at 560 nm wavelength. The PBS that contain 11.5 g / L KCl and is used as negative control.[12]

Analysis of lipid peroxidation levels (malondialdehida = MDA)

Liver tissue sample preparation is performed according to Singh et al. (2002).[13] Moreover, the analysis of MDA performed according to the method that has been done Capeyron et al. (2002) and Suarsana et al. (2011) with slight modifications. The sample of liver is chopped in cold conditions, then centrifuged at 4000 rpm for 10 minutes. For the measurement of MDA, a total of 0.5 mL of the supernatant was added 2.0 mL to cold HCl (0.25 N) containing 15% TCA (thricloroacetic), 0.38% TBA (thio barbituric acid) and 0.5% BHT (butyled hydroxytoluene). The mixture was heated at 800C for 1 hour, then centrifuged at 3500 rpm for 10 minutes. Absorbance was measured with a spectrophotometer at a 532 nm wavelength. *1,1,3,3-tetraethoxypropane* (TEP) has been used as standard solution for this study.[14,15]

Data analysis

Data were analyzed by analysis of variance test (ANOVA) using a completely randomized design (CRD), then continued with Duncan test Multi Range Test (DMRT) with a 95% confidence level.

RESULTS AND DISCUSSION

The activity of superoxide dismutase (SOD) in the liver.

Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide anion radicals (O2 • -) to hydrogen peroxide (H2O2) and molecular oxygen (O2). It is a metaloenzim that active with minerals such as copper (Cu), zinc (Zn) and manganese (Mn). Analysis of superoxide dismutase activity in rat liver sample shows that the tress group (S) has the lowest SOD activity in the

amount of 45.81% (Figure 1). The low SOD activity can be caused by no antioxidant intake during the stress exposure.

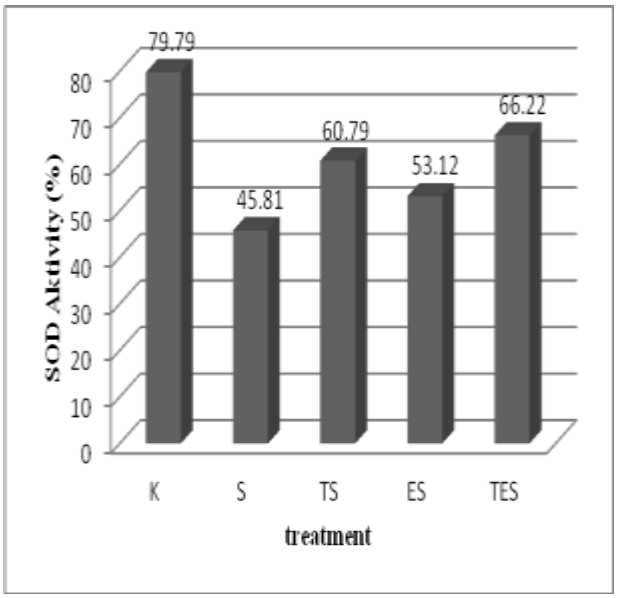

Figure 1. The enzyme activity of superoxide dismutase (SOD) (%) in the liver of the rat treated as K (control), S (stress), TS (given tocopherol and stress), ES (given the extract and stress) and TES (given tocopherol, extracts and stress)

According to the test, fasting and exercising (swimming) can cause oxidative stress conditions. The condition occurs due to heavy physical activity leads to increased metabolism and oxygen consumption that lead into an increasing of the ROS production.[16] An increasing metabolism and oxygen consumption in the body can be caused by the demand of energy during heavy exercise which the greater the energy produced in the body, the more ROS is produced.[2,17]

ROS as $\bullet O_2^-$ can be formed by xantin dehidrogenase (XD) to xantin oksidase (XO). This condition can be triggered by hypoxemia when the oxygen demand has not been fulfilled during heavy exercise.[18] The higher production of free radicals, the more free radicals that can not be neutralized by antioxidant systems in the body. The existence of ROS in the body can harm the endogenous antioxidant defense system, superoxide dismutase (SOD) and cause the decrease of superoxide levels in the body.

Graphic 1 shows that the highest SOD activity possessed by the control group (K) which is 79.79%. It is because of this group has been freed from any stress exposure so it does not form free radicals in the body. In normal conditions, the body's own endogenous antioxidant defense system in the form of superoxide dismutase. It is showed by the high activity of superoxide dismutase in the control group who did not receive any stress exposure.

On one hand, the ES group which only received the extract has the lowest SOD activity amongst the the groups (53.12%). On the other hand, the highest SOD activity was came from the group that received tocopherols and extracts treatment (TES) that is equal to 66.22%. However these results suggest that both have a good activity to counter the free radicals, because both of them has active compound that reacted and prevent the free radical formation in the body.

The mechanism of synergism between tocopherols and *Solanum betaceum*'s rind extract can be assumed similar to the synergism between vitamin E and vitamin C. These mechanism is activated when there is a lipid peroxidation caused by free radical attack that forms a new free radicals. Furthermore, free radicals will be captured by forming a radical tocopherol tocopherol. The tocopherol radical then be stabilized by the active compounds that contained in *Solanum betaceum*'s rind extract and form a secondary short-lived radicals that are less harmful to the body.[19]

The result of statistical test using Duncan Test indicates that between the control group (K), tocopherol-stress (TS), extract-stress (ES) and tocopherol-extract-stress (TES) results was significantly different from the control group (S). The results of these analysis show that the treatment of stress had a negative impact on animal ie, increased levels of free radicals in the body which is characterized by decreasing the activity of superoxide dismutase.

Levels of (MDA) in the liver

The MDA test results show that the (S) group has the highest MDA levels compared to other treatment groups in the amount of 6.29 nmol/g. The results of analysis of MDA each treatment group are presented in Figure 2.

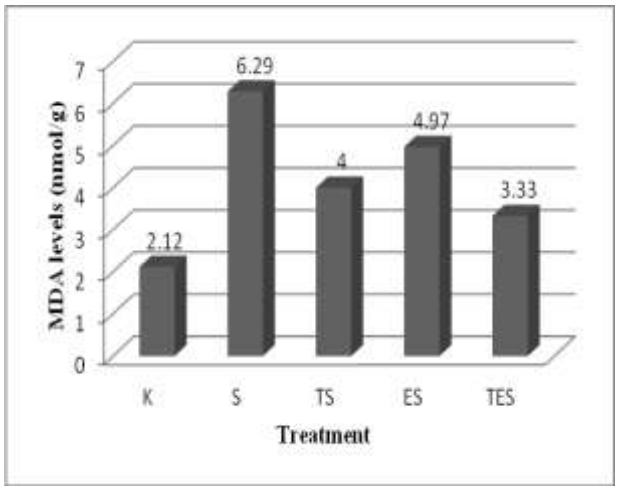

Figure 2. level of malondialdehyde (MDA) (nmol/g) in the liver of the rat treated as K (control), S (stress), TS (given tocopherol and stress), ES (given the extract and stress) and TES (given tocopherol, extracts and stress)

The MDA in (S) group can be caused by oxidative stress because of fasting and heavy exercise. The body requires energy-producing compound at the time of fasting, but Liver's glycogen is only able to provide glucose for several hours. Therefore gluconeogenesis will occurs in the liver that require another substrates from another tissue, which is can be from the amino acid glikogenik and lipids.[9,20]

In normal conditions, the catabolism of fatty acids occurs in the mitochondria by β-oxidation.[21] Increase of β-oxidation process will occur in peroxisomes when fasting, which is a minor pathway of β-oxidation process on the normal state normal. Based on research conducted by Wresdiyati and Markita (1995), the condition of stress can trigger the increased number of peroxisomes impact on improving oxidation in peroxisomes. Increase of activity of β-oxidation in peroxisomes causes an increase in the number of free radicals as a second product of the metabolism process.[7]

The graphic 2 informs that the control group (K) has the lowest MDA level that is equal to 2.12 nmol/g protein. MDA generated by the control treatment group is lower than the other treatment groups. This indicates that the amount of free radicals formed in the body is low that cause the stress levels of MDA is very low.

The (ES) group who only received the extract has the highest MDA levels among the groups (4.97 nmol/g). MDA levels is indicated by the lowest tocopherol treated groups combined with extracts (TES) that is equal to 3.33 nmol/g protein. It is because both of them might be have a synergistic activity in counteracting free radicals that resulting ower levels of MDA. These combination can be used as an alternative to reduce levels of MDA in the body due to oxidative stress. Previous research states that a combination of several types of antioxidants often provide better protection (synergism) on the process of lipid peroxidation compared with one type of antioxidant.[22]

Based on the statistical analysis result by Duncan test α = 0.05, the results indicates that between the control group (K), tocopherol-stress (TS), extract-stress (ES) and tocopherol-extract-stress (TFS) significantly different from the treatment of stress (S). The results of the analysis show that the treatment of stress had a negative impact on animal ie, increased levels of free radicals in the body which is characterized by increased levels of MDA.

CONCLUSION

In summary, either *Solanum betaceum*'s rind extract or α-tocopherol may increase the activity of SOD and decrease MDA levels in rat liver tissue. The combination of *Solanum betaceum*'s rind extract with α-tocopherol provide the best effect.

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