



Intake Flavonoid Glycosides of Fruit *Solanum Betaceum* in Its Activity as a Candidate of Anti-Stress Oxidative

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ABSTRACT

Objective: Oxidative stress due to an imbalance between free radicals and antioxidants in the body can be overcome by intake exogenous antioxidants, such as flavonoid glycoside constituent of *Solanum betaceum*. This study aimed to prove the effect of flavonoid glycosides from an *n*-butanol extract of *Solanum betaceum* as an antioxidant. **Method:** The experiment was done using a true experimental randomized completed with posttest only control group design. Twenty-four rats were divided into four groups, with each group containing six rats. Group 1 (Po) was the control; group 2 (P1) was given the maximum physical activity; group 3 (P2) was given ethanol extract, with the maximum physical activity; group 4 (P3) was given *n*-butanol extract, with the maximum physical activity. The maximum physical activity was achieved after swimming activity for 90 minutes/day for 5 days. The ethanol and *n*-butanol extract were administered orally at a dose of 50 mg /Kg BW /day for 5 days, respectively. After 5 days of treatment, all rats were then euthanized to obtain their livers to be analyzed to achieve SOD and MDA data. All of the data were analyzed using ANOVA to obtain the treatment differences with the control by statistically significance level of $\alpha = 0,05$. **Results:** Antioxidant activity test results on Wistar rats given maximum physical activity showed that the intake of ethanol and *n*-butanol extract of terong belanda at a dose of 50 mg /Kg BW caused higher SOD activity, and significantly lower MDA levels ($p < 0,05$) **Conclusion:** Based on the results of this study, it could be concluded that as the flavonoid glycoside extract of *n*- butanol terong belanda (*Solanum betaceum*) was proved, it can prevent oxidative stress through SOD, and thereby decrease the level of MDA liver of Wistar rats.

Key Words: Solanum Betaceum Cav, SOD, MDA, Liver Wistar Rats.

eIJPPR 2018; 8(6):1-7

HOW TO CITE THIS ARTICLE: Ida Ayu Raka Astiti Asih, Ida Bagus Putra Manuaba, Ketut Berata, Bagus Komang Satriyasa, Ketut Tunas (2018). "Intake Flavonoid Glycosides of Fruit *Solanum Betaceum* in Its Activity as a Candidate of Anti-Stress Oxidative", International Journal of Pharmaceutical and Phytopharmacological Research, 8(6), pp.1-7.

INTRODUCTION

The decline in the environmental quality indirectly causes humans to be constantly faced with the problems related to the polluted environment including high ultraviolet radiation, exposure to pollutants and other free radicals that cause oxidative stress. Oxidative stress is a state of imbalance between free radicals (pro-oxidants) and antioxidants in the body.

Exposure to free radicals that occurs at any time causes the community to become more protective, so that there has been a tendency to consume antioxidants in the form of herbal supplements as a preventive measure because it has been considered more practical. The usage of medicinal plants has not been an emerging issue; it has been recorded in the history of many cultures [1]. The antioxidant properties of medicinal plants must be evaluated by several methods because these plants contain complex phytochemicals [2]. Antioxidants are

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 August 2018; **Revised:** 17 November 2018; **Accepted:** 22 November 2018



categorized in two groups of synthetic and natural [3], and they are able to act as contributors to hydrogen radicals, or can act as free radical acceptors so that they can delay the initiation stage of free radical formation.

One of the free radicals that often attacks unsaturated fatty acids in the body is hydroxyl free radicals. Hydroxyl radicals can cause chain reactions known as fat peroxidation. Fat peroxidation is a complex process due to the reaction of unsaturated fatty acids which make up phospholipid cell membranes with reactive oxygen compounds to form hydroperoxide [4]. Fat peroxidation causes the breakdown of the chain of fatty acids into various compounds that are toxic to cells such as malondialdehyde (MDA), ethane and pentane [5].

Superoxide dismutase (SOD) is one of the endogenous antioxidants that plays a role in catalyzing free radicals of superoxide anions into hydrogen peroxide and oxygen molecules [6]. Chevion [7] reported that stressful conditions due to maximum physical activity can increase oxygen consumption, due to an increase in metabolism in the body. Increased use of oxygen, especially by contracting muscles, causes an increase in electron leakage from mitochondria which will become Reactive Oxygen Species (ROS). Oxygen used in the body's metabolic process during heavy physical activity can cause an increase in the production of free radicals that are highly reactive to the cells or surrounding cell components. Increased levels of free radicals under stress conditions in rat liver and kidney tissue have been reported by Wresdiyati [8] as indicated by a decrease in the content of intracellular antioxidants such as superoxide dismutase.

Terong belanda (*Solanum betaceum*) is a fruit that has nutrients and vitamins that are very important for the health of the human body such as anthocyanins, carotenoids, vitamins A, B6, C, and E, and are rich in iron, potassium, fiber, and minerals [9]. Previous studies have isolated 3 flavonoid glycosides from terong belanda *n* butanol extract with IC₅₀ 70.11ppm (a strong antioxidant) [10]. The three flavonoid glycosides quercetin-3-O-rhamnoside, Rutine, and Kaempferol-3-O-rutinoside are substituted sugar in C-3 atoms. The presence of sugar substituents in C3 atoms that have a galoyl group-like structure has made them potent antioxidants [11].

Based on the results of these studies, the *in vivo* testing of intracellular prolyl antioxidant superoxide dismutase was

done, which included superoxide dismutase activity and malondialdehyde content in Wistar rats liver tissue with maximum physical activity.

MATERIALS AND METHOD

List of chemical material used:

EDTA (*Ethylene Diamine Tetraacetic acid*), BSA (*bovine serum albumin*), NBT (*Nitro Blue Tetrazolium*), PBS (*Phosphate Buffered Saline*), TCA (*trichloroacetic*), TBA (*thio barbituric acid*), Xantin, BHT (*butylated hydroxytoluene*), TEP (*1,1,3,3-tetraethoxypropane*) and cytochrome c

Experimental animals and sampling

In this study, the sampling process was adjusted to the method carried out by Wresdiyati [12]. The experimental animals used were 28 male Wistar strain rats with an average body weight of ± 250 g. After the rats were adapted for 2 weeks to their environment, they were grouped into 4 treatment groups; each group consisting of 7 rats. Oxidative stress was given by swimming activities until almost drowning/day (1.5 hours). The rats were given *ad libitum* feed and drinking water. Ethanol extract and *n* butanol extract (flavonoid glycosides) 2 mL at a dose of 50 mg /Kg/ BW/day were given to the rats using a sonde. After the treatment according to the design, the rats were then sacrificed and dissected to take rat liver tissue from each treatment group. Rat liver tissue samples were then analyzed to determine SOD activity and MDA levels using the TBARS method. All the experimental works with the animal were carried out after obtaining approval from the organization of Animal Ethics Committee (ethical Clearance) No:313/KE-PH-Lit-3 /VIII/2017.

Analysis of rat liver SOD activity

Rat liver SOD activity was determined using the colorimetric method by the Superoxide Dismutase Kit (Biovision, K335-100). The samples of enumerated liver rats were then dissolved in PBS containing 11.5 g / L KCl, and then centrifuged at a speed of 1000 rpm for 10 minutes at 4°C. The lysate of the liver formed was transferred into a new tube and stored at -80°C, until ready for the analysis. The amounts of solution which were added to the sample, and the blanks 1,2 and 3, can be seen in Table 1.

Table 1. Amount of each solution for sample Blank 1, 2 and 3

	Samle	Blank 1	Blank 2	Blank 3
Sample solution	20 µL	-	20 µL	-
ddH ₂ O	-	20 µL	-	20 µL
WST working solution	200 µL	200 µL	200 µL	200 µL
Enzym working solution	20 µL	20 µL	-	-
Dilution Buffer			20 µL	20 µL

All solutions were homogenized and incubated at 37 ° C for 20 minutes, then the absorbance was measured at a wavelength of 450 nm using a microplate reader.

SOD activity calculation formula (%):

$$\text{SOD Act (\% inhibisi)} = \frac{(A \text{ blank 1} - A \text{ blank 3}) - (A \text{ sample} - A \text{ blank 2})}{(A \text{ blank 1} - A \text{ blank 3})} \times 100\%.$$

Analysis of malondialdehyde (MDA)

The liver tissue samples were prepared following the Singh [13] procedure. Furthermore, the analysis of MDA levels was carried out according to the method used by Capeyron [14] and Suarsana [15] with few modifications. The liver was chopped in cold conditions. Homogenate was centrifuged at 4000 rpm for 10 minutes. For measurement of MDA levels, 0.5 mL of clear supernatant plus 2.0 mL of cold HCl (0.25 N) containing 15% TCA (trichloroacetic), 0.38% TBA (thiobarbituric acid) and

0.5% BHT (butyld hydroxytoluene) was used. The mixture was heated at 80°C for 1 hour, then centrifuged at 3500 rpm for 10 minutes. The absorbance was measured by a UV-Vis spectrophotometer at a wavelength of 532 nm. The standard solution used was TEP (1,1,3,3-tetraethoxypropane).

RESULT AND DISCUSSION

Results

In Vivo Antioxidant Activities

The experimental study was carried out using a randomized post-test only control group design. Data from the observations were analyzed statistically using IBM SPSS 23.0 software. The average variable and normal distribution of each group and the variance homogeneity of each variable have been presented in Table 2.

Table 2: Average, normality and homogeneity of variants

	P0	P1	P2	P3	p*
SOD (%)	76,28±2,02	16,33±1,33	49,76±1,01	55,84±1,27	0,569
p**	0,975	0,642	0,056	0,856	
MDA (nmol/g)	1,36±0,10	8,80±1,23	3,95±0,28	2,76±0,27	0,149
p**	0,567	0,113	0,528	0,757	

Information:

P0= Control group (without treatment)

P1=Group with physical activity (swimming for 90 minutes/day until almost drowning)

P2=Group with ethanol extract dose of 50 mg/kgBW and maximum physical activity

P3=group with flavonoid glycosides extract of n-butanol dose of 50 mg/kgBW and maximum physical activity

*=homogeneous data at p> 0.05

**=data is normally distributed at p> 0.05

Superoxide dismutase activity in Wistar rat liver tissue

Superoxide dismutase activity in Wistar rat liver tissue has been shown in figure 1.

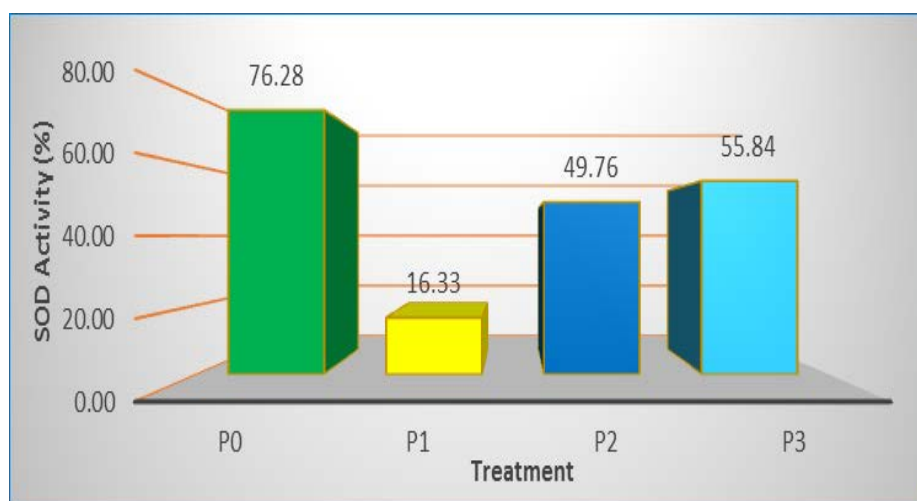


Fig. 1: Superoxide dismutase (SOD) enzyme activity (%) in the liver rats tissue

The results of the comparative analysis of SOD activity after the treatment between groups have been presented in Table 3.

Table 3. Comparative analysis of SOD activity after treatment between groups

Group	Average difference	P*
Control and treatment 1	59,95	0,001
Control and treatment 2	26,52	0,001
Control and treatment 3	20,44	0,001
Treatment 1 and treatment 2	33,43	0,001
Treatment 1 and treatment 3	39,51	0,001
Treatment 2 and treatment 3	6,08	0,001

Description: * = Significance at $p < 0.05$

Malondialdehyde (MDA) content in Wistar rat liver tissue

Malondialdehyde (MDA) content in Wistar rat liver tissue has been shown in figure 2.

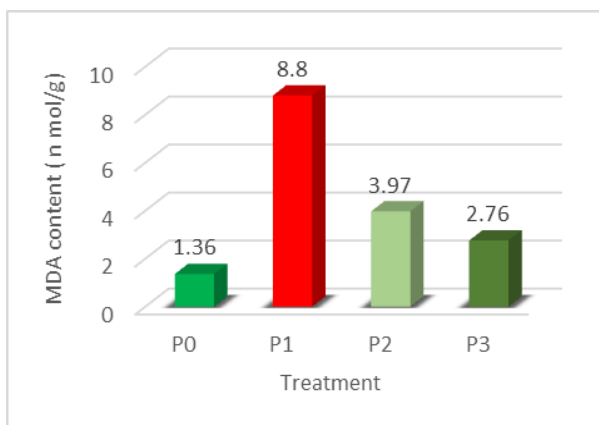


Fig. 2. Content of malondialdehyde (MDA) (nmol/ g) in the liver rats tissue

The results of the comparative analysis of MDA levels after the treatment between groups have been presented in Table 4.

Table 4. MDA Comparative Analysis After Intergroup Treatment

Group	average difference	p*
Control and treatment 1	7,43	0,0001
Control and treatment 2	2,59	0,0001
Control and treatment 3	1,49	0,0001
Treatment 1 and treatment 2	4,84	0,0001
Treatment 1 and treatment 3	6,04	0,0001
Treatment 2 and treatment 3	1,20	0,0001

Description: * = Significance at $p < 0.05$

DISSCUSION

Each treatment group was tested based on the mean SOD. The results of the One-Way ANOVA test on the average SOD activity showed a p-value of 0.0001. This value indicated that the four treatments given to rats had significantly different effects ($p < 0.05$). The analysis of superoxide dismutase activity in rat livers showed that group 1 (P1) had the lowest SOD activity, which was 16.33%. This was caused by the provision of stress treatment without the provision of compounds containing antioxidants in the form of the flavonoid glycosides *n*-butanol extract of terong belanda. The provision of swimming activities until almost drowning to the experimental animals included heavy physical activity which can lead to conditions of oxidative stress. This condition occurred because the heavy physical activity triggered an increase in metabolism and oxygen consumption to increase ROS production [7]. The increase in metabolism and oxygen consumption in the body was caused by increased energy requirements during heavy physical activity. The greater the energy was produced in the body, the more ROS was produced [16, 17].

The highest SOD activity was owned by the control group (P0) which was 76.28%. This happened because the control group was not treated with stress, so that the free radicals were not formed in the body. Under normal conditions, the body has an endogenous antioxidant defense system in the form of superoxide dismutase which was still good. This was demonstrated by the high activity of superoxide dismutase in the control group that did not receive any treatments.

Group 3 (P3) had 55.84% superoxide dismutase activity. When it was to compared with the groups 1 (P1) and 2 (P2), this group had higher superoxide dismutase activity. This was likely due to the presence of flavonoid glycoside compounds in the extract. Flavonoid glycosides from *n*-butanol extract were more capable to increase SOD enzyme activity than ethanol extract. Nilesh [18] reported that the intake of *n*-butanol extract of *Beta vulgaris* L. leaves was more capable to increase SOD enzyme activity of rat liver compared to the intake of ethanol extract. This was probably due to the fact that in the ethanol extract, there were still compounds that were antagonistic to the active substance, thereby reducing its activity.

The presence of flavonoid glycoside compounds can help superoxide dismutase work in the body by counteracting free radicals formed by stress. Flavonoids reduce the compounds that can inhibit various oxidation reactions.

Flavonoid compounds have the potential as antioxidants because they are able to transfer H⁺ ions (HAT) to free radical compounds. The mechanism of free radical reduction reaction carried out by flavonoid contained in *n*-butanol extract resembles the mechanism of superoxide radical reduction by quercetin compounds. Flavonoid glycosides play a role in increasing the activity of SOD enzymes by working directly and indirectly. Directly, the presence of a hydroxyl group allows flavonoids to scavenge free radicals by donating hydrogen atoms (SET-PT) to free radicals and chelating metals (SPLET) which play a role in the formation of ROS [19]. Indirectly, flavonoids are able to increase the expression of endogenous antioxidant genes through several mechanisms such as activation of nuclear factor erythroid 2 related factor 2 (Nrf2) resulting in an increase in SOD enzyme synthesis [20].

Flavonoid glycosides are also able to inhibit enzymes that play a role in ROS formation such as microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADPH oxidase and others [21] so that the amount of ROS decreases and SOD enzyme activity increases.

The results of the test using the Least Significant Difference test (LSD) showed that the highest mean difference was found in group 1 and control. The results of the analysis indicated that stress had a negative effect on the experimental animals, namely increased levels of free radicals in the body which have been characterized by the decreased activity of superoxide dismutase. The

highest increase in SOD activity was found in rats treated with treatment 1 and treatment 3 (flavonoid glycosides *n*-butanol extract), with an increase of 241.94%. Overall, the treatment given to the experimental animals had a significantly different effect. The treatment in group 2 gave an increase in superoxide dismutase activity of 204.71%. The intake of *n*-butanol and ethanol extract helped superoxide dismutase to reduce the number of free radicals formed in the body.

Lipid peroxidation, especially in unsaturated fatty acids, usually produces the final product in the form of malondialdehyde (MDA) through the oxidation by free radicals. Malondialdehyde has been widely used as an indicator of oxidative damage, especially from unsaturated fatty acids [22]. The method used to measure MDA levels was based on the reaction that occurred between MDA and thiobarbiturate (TBA) and formed a bond of TBA-MDA complex which produced red color, and its intensity was measured using a spectrophotometer. The formation reaction in TBA-MDA complex have been presented in Figure 3.

The results of One-Way ANOVA analysis of the mean of MDA levels with a p-value of 0.0001 indicated that the five treatments given to rats had a significantly different effect ($p < 0.05$). The results of MDA analysis showed that group 1 (P1) had the highest MDA content compared to the other treatment groups, which was 8.79 nmol/g. The high content of MDA in group 1 was caused by the conditions of oxidative stress that occurred in experimental animals.

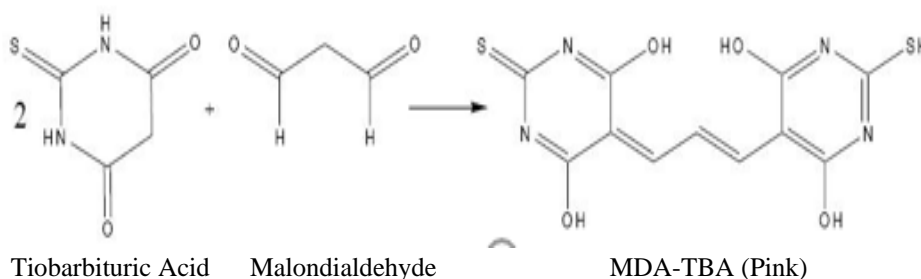
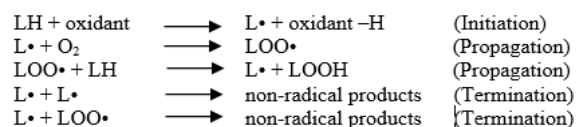


Fig. 3: Formation Reactions of TBA-MDA Complex [23]

This oxidative stress occurred because of the maximum physical activity (swimming until almost drowning) in the experimental animals. Maximum physical activity caused an imbalance between free radical production and the body's antioxidant defense system. During the maximum physical activity, oxygen consumption throughout the body increased by 20 times, while oxygen consumption in muscle fibers was estimated to increase by 100-folds. This can cause the body to be deprived of oxygen, known as hypoxia. This increased oxygen consumption resulted

in the increased production of free radicals that can oxidize fat in muscle tissues, causing damage to the muscle cells [4].

Increased free radicals in the body were caused by making them have physical activity through swim. Free radicals formed caused oxidative damage and fat peroxidation in the cell membrane, and produced MDA. The mechanism of lipid peroxidation that occurred in the body was as follows



Cell membranes in the body are composed of lipids (LH) which are usually in the form of polyunsaturated fatty acids. The peroxidation process starts from the initiation stage where there is a reaction between free radicals (oxidants) and unsaturated fatty acids (LH) to form carbon radicals (L•) in the form of free fat. The free fat then reacts with oxygen to form peroxy radicals (LOO•). If the peroxy radical reacts again with the other unsaturated fatty acids, lipid hydroperoxide (LOOH) which is cytotoxic and free fatty acid (L•) will be formed, so that a chain reaction will be occurred. The reaction will end through the termination stage, if free fat is formed at the initiation stage or other radicals that are formed at the propagation stage reacting back to the other radicals into non-radical products [4, 24]. The process also forms fat endoperoxide which decomposes to form malondialdehyde [17]. Thus, the high MDA levels in treatment group 1 indicate that the high number of free radicals formed under the conditions of oxidative stress.

The control group (P0) had the lowest MDA content of 1.36 nmol/g protein. MDA produced by the control treatment was the lowest compared to the other treatments. This indicated that the number of free radicals formed in the body that without maximum physical activity, this number would be very low, so that the MDA levels formed were very low. Group 2 had MDA levels of 3.97 nmol/g, while group 3 had MDA levels of 2.76 nmol/g. This group was able to reduce MDA levels in group 1 from 8.80 nmol/g to 2.76 nmol/g. This was likely due to the flavonoid glycoside compounds contained in the extract. Thus, flavonoid glycoside compounds were able to react with free radicals, and break the chain of fat peroxidation. When group 2 was compared to group 3, it turned out that giving *n*-butanol extract produced lower MDA content than ethanol extract. This was probably due to the flavonoid glycoside contained in the *n*-butanol extract which are antagonistic with ethanol extract.

Boligon [25] reported that *n*-butanol extract of *T. catharinensis* leaves was better able to reduce MDA levels in Wistar rats compared to ethanol extract. This was due to the fact that the content of polyphenols and flavonoids per gram of *n*-butanol fraction was higher than that of ethanol extract which has been characterized by an IC₅₀ value of *n*-butanol fraction which was lower than ethanol extract.

CONCLUSIONS

Based on the results of the study, the following can be concluded:

The intake flavonoid glycosides from terong belanda was more capable to increase SOD activity, and reduce MDA content of Wistar rat tissue with maximum physical activity compared to SOD activity and MDA content of liver tissues of Wistar rats which were not given the intake of flavonoid glycosides with a significant difference.

ACKNOWLEDGMENTS

The authors would like to express their thanks to the Directorate General of Higher Education and Center of Research and Community Service Udayana University for funding this study through Hibah Doktor Scheme 2017.

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