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Antioxidant Activity of Cocoa Powder on The Changes of *Super Oxide Dismutase* (SOD) And *Malonaldehyde* (MDA).

Tamrin[1*], Teti Estiasih[2], Ansharullah[1], Anugrawati Cintya[2], Syair[3], Abdul Rahman[3], Rahayu M[3], Abdu Rahman Baco[1], and Muhidin[3].

[1]Department of Food Science and Technology, Faculty of Technology and Agriculture Industry, Halu Oleo University, Kendari Southeast Sulawesi, Indonesia.
[2]Department of Technology of Agriculture Production, Faculty of Agriculture Technology, University of Brawijaya Malang, East Java, Indonesia.
[3]Department of Agrotechnology, Faculty of Agriculture, Halu Oleo University, Kendari Southeast Sulawesi, Indonesia.

ABSTRACT

Excess production of free radicals in the body my trigger oxidative stress conditions, leading to the formation of malonaldehyde (MDA). The oxidative stress then may further cause the damage to the cells and tissues, giving rise to degenerative diseases. An intake of nutrients containing antioxidants, such as cocoa powder may retard the stress. This research was aimed to study the antioxidant activity in cocoa powder in vivo using Wistar rats (*Rattus norvegicus*). Oxidative stress in rats was conditioned by using used cooking oil. Then, a cocoa powder with concentrations of 10% and 20% was mixed in the feed. The results showed that the addition of cocoa powder on feed had reduced the levels of MDA and had increased the levels of antioxidants of SOD (Super Oxide Dismutase) in the tissues of the mice. The addition of cocoa powder of 20% had a better impact than that of 10% on lowering the MDA and increasing the SOD.

Keywords: Cocoa powder, antioxidant, oxidative stress, SOD, MDA

Corresponding author

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INTRODUCTION

Polluted air, exposure to ultraviolet light, and pesticide residues on foodstuffs may have a role as pro-oxidant. This condition then may increase the production of *reactive oxygen species* (ROS) in the body. The increase of ROS may cause oxidative stress conditions. Oxidative stress can be seen from tissue damage caused by oxygen free radicals in the entire biological membrane that are by attacking the proteins, lipids, or fats, nucleic acids and gliko-conjugate [1]. Free radicals do not have an electron pair, so that these free radicals will achieve stability by attacking the nearest molecule to look for electron pairs. Because of the activity of free radicals can damage the molecular shape and causes the cells destroyed macromolecules. Macromolecular cells most vulnerable to attack by free radicals are unsaturated fatty acids such as poly unsaturated fatty acids long (PUFA) [2,3]. The double bond carbon - carbon unsaturated fatty acids (PUFA) weaken the bond carbon - hydrogen, and facilitate the transfer of hydrogen by free radicals, and free radicals can separate hydrogen atoms and formed the radical lipid, which oxidized to produce a lipid peroxyl radical [4].

The body can naturally forming antioxidants (as a response to the formation of free radicals) include super oxide dismustase (SOD). However, many sources prooxidant in the daily activities can lead to the production of free radicals in the body can be higher than an antioxidant that may occur by oxidative stress. Improved conditions of oxidative stress on the body can lead to various degenerative diseases (eg cancer, diabetes, hypertension, and heart). Therefore, it is necessary nutrition for the body of a functional food sources that contains antioxidants. The functional food expected to anticipate shortages and increase the activity of antioxidant enzymes SOD in the body [3,5,6].

Some experts explain that consuming cocoa beans processed products (and product derivatives) that contain antioxidants can provide health benefits. Antioxidant procyanidin (a dimer of catechin) of cocoa has been shown to have biological activity relevant to defense against free radicals (oxidants), vascular health, prevention of tumor, and immune function, as well as demonstrate protection against protein damage peroxynitrite-mediated vitro [7,8,9]. Other benefits are the improvement of cardiac function and relief of angina pectoris, nervous system stimulation, facilitate digestion and kidney and improve bowel function. In addition, cocoa has been used to treat anemia, mental fatigue, tuberculosis, fever, gout, kidney stones, and even sex drive [10,11]. Epidemiological data show that intake of cocoa daily, regularly reduces the risk of coronary heart disease, stroke and inversely related to cardiovascular risk and have a beneficial role in arterial function [12].

Antioxidants in cocoa beans have decreased a lot during the process of post-harvest handling (primary processing) as well as the secondary processing. The levels of the antioxidant catechins in cocoa powder are only between 1-2% only [9]. The antioxidant *catechins* from the cocoa bean roasting process can be improved by vacuum [13]. Results reported that the evaluation of antioxidant activity using DPPH radical shown that increased antioxidants in beans or cocoa powder would cause an increase in antioxidant activity [14]. Based on that evaluation it is necessary to test further the antioxidant activity of cocoa powder using the In vivo animal experiments. The main emphasis the use of experimental animals, among others is to study the ability of antioxidants counteract free radicals in the metabolic system of living creatures [15].

MATERIALS AND METHODS

Sample preparation

Cocoa used in this study cocoa powder processed [16]. Dried cocoa seeds were collected from the plantation in Lasusua, North Kolaka, Southeast Sulawesi. The seeds were processed into powder using a hydraulic press, powder grinder, the 80-mesh screen, and some supporting tools. The cocoa powder was roasted using a vacuum frying device to obtain dried cocoa powder.

The tools used for the analysis include scale analytic "Denver Instrument M 310", the glasses, spectrophotometers 20D Plus "LaboMed", UV mini-1240 "Shimadzu", cuvette, micro-centrifuge "Jovan A14", micropipette "Soccorex", tip, vortex, tube 2ml, micro-hematocrit, incubators "WTB Binder", Shimadzu UV-1601. For the maintenance of mice, among other tools used mouse cage, where to eat mice, rats drinking places, sawdust. Then, the apparatus used to make the rat feed were, among others, digital scales, basins, spatulas, measuring cups, meat grinder, trays, and cabinet dryer.

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Stage In Vivo

The experimental animals used were white rats (*Rattus norvegicus*) Wistar strain aged 2 -3 months weighing 150-250 grams [17]. Animal testing in healthy, active and kept in a cage with a period of adaptation one week before treatment is given. Furthermore, to create conditions of oxidative stress in rats is given intake of cooking oil (used oil that has repeatedly been used for frying). Used cooking oil is used as a source of free radicals due to cooking oil that has repeatedly been used had a peroxide value above 100 meq Kg^{-1}.

Mice were fed as much as 20 grams and drink water ad libitum. Standard feed made from Comfeed PARS and flour with a ratio of 2: 1 and the addition of cocoa powder according to treatment (10 and 20%). The added water mixture is then formed into pellets. Every day the rest of the feed mice weighed, and body weight of rats were weighed every three days.

The treatment was done within 4 (four) groups, namely: (1) positive control group/control stress oxidative, giving cooking diet + standard feed for 6 weeks; (2) negative control group, giving a normal diet (food standard) for 6 weeks; (3) The treatment group used cooking diet Giving + standard + cocoa powder feed 10% of the weight of feed for 6 weeks; (4) Provision of diet treatment group cooking oil + standard + cocoa powder feed 20% of the weight of feed for 6 weeks. Each treatment was repeated 5 times.

Testing Activities of SOD and MDA levels

Animals were then analyzed levels of MDA (malonaldehyde) and its SOD activity. MDA is the main product the result of free radical reactions with phospholipids, are abundant in the circulation and which are manufactured in constant proportion to the lipid peroxidation that occurs, so it is a good indicator to see the speed (rate) of lipid peroxidation in vivo [18,19]. The steps of the testing that is animal blood serum taken for analysis. Blood sampling is in retro orbital plexus of the eye. This analysis is done at weeks 0, 1, 2, 3, 4, 5, and 6. The examination conducted by reacting blood serum-reagent, such as TCA, Na-Thio, HCl, and distilled water with a specific composition. Total MDA analyzed using spectrophotometric method at a wavelength of 532 nm [20,21].

RESULTS AND DISCUSSION

Data showed that the dried cocoa beans contain catechins 3.20% and decreased into 2.90% after processed into nib (cocoa nib). After the cocoa nib roasting into a powder, the catechins levels increased to 5.1% (13-Tamrin *et al.*, 2012). Based on the results of animal studies using a strain of Wistar rats treated cocoa powder, MDA of blood serum of mice in the fifth week showed different levels as shown in Figure 1.

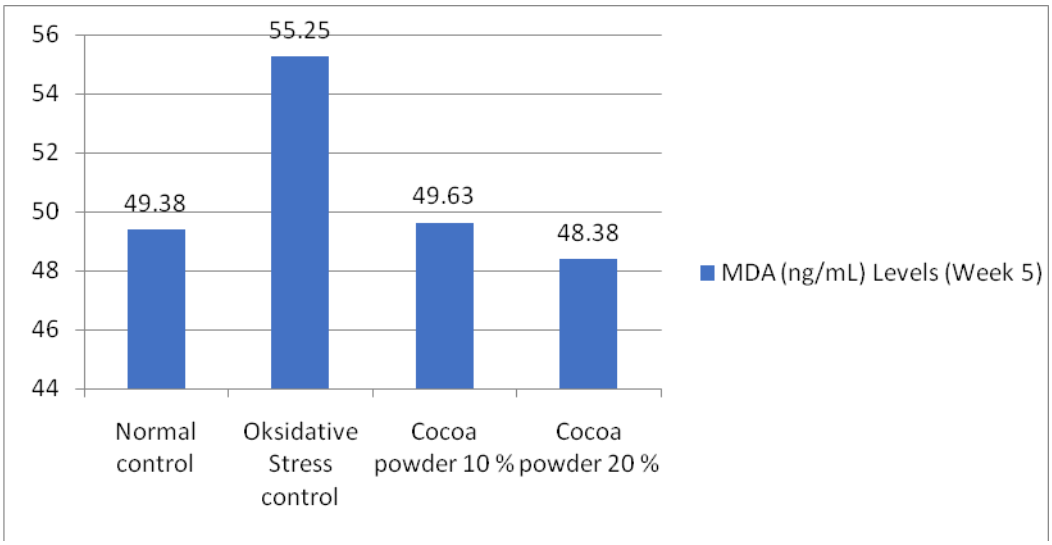

Figure 1: MDA levels of blood serum of Wistar rats at week 5

Figure 1 shows that the mice were conditioned in oxidative stress and untreated cocoa powder seems to have the highest levels of MDA than the other three groups of mice. The condition describes the content of free radicals used cooking oil is very high. The peroxide value from waste cooking oil ranged from 101.04 to 106.39 Meq kg^{-1} [17]. The high peroxide value is an indicator of damage to the used cooking oil, which supports the formation of free radicals, which can cause oxidative stress and if prolonged oxidative stress can cause tissue damage [22]. MDA is one of the aldehyde compounds resulting from the breakdown of lipid peroxides. Lipid peroxides are the result of a free radical oxidation reaction with the lipid membrane in the cell tissues of the body or with acid-unsaturated fatty acids, which form the end product MDA. Therefore, MDA is the end product of lipid peroxides in the body due to the presence of free radical reactions. Free radicals are reactive molecules with unpaired electrons and are produced continuously in the cell, either intentionally or as a byproduct of metabolism. The series of oxidation-reduction reactions in the transformation of the metabolism of proteins, carbohydrates, and fats in the mitochondrion called oxidative phosphorylation. The results of products such as oxygen and its derivatives such as superoxide and hydroxyl radicals [6,23,24].

It in conditions of excessive free radicals is very dangerous because it can cause damage or abnormalities in both biochemical and physiological processes in the cell. The condition can lead to metabolic aberrations that can lead to damage and cell death. Even under conditions of free radicals can damage DNA, proteins, and fats that can lead to some degenerative diseases in humans and animals [2,25].

Based on Figure 1 also shows that the addition of cocoa powder of 10% and 20% in the diet of mice provides changes to the levels of MDA are formed. The addition of 10% cocoa powder showed decreased levels of MDA thus approaching the body tissues of normal mice (without treatment used cooking oil). The data illustrate the antioxidants in cocoa powder could inhibit free-radical oxidation of waste cooking oil so that the formation of MDA is also inhibited. Furthermore, the addition of 20% cocoa powder feed on mice showed lower levels of MDA from the group with the addition of cocoa powder of 10% and a group of normal mice. This condition could indicate increased levels of cocoa powder in feed mice also increase the levels of antioxidants. In conditions of a high antioxidant number of hydroxyl groups alleged to be higher, so the ability to stabilize free radicals in the body tissues of mice was higher than 10% cocoa powder levels.

In addition to inhibiting free radical reactions, the cocoa powder containing antioxidants also affect the levels of superoxide dismutase (SOD) in the body tissues of mice. SOD is an intracellular enzymatic antioxidant. That play an important role in the protection of cells against oxidative stress, ROS, and indirectly to maintain the balance of some toxic oxygen species [22,25,26,27]. The SOD is mostly working intracellular mitochondria and the cell cytoplasm. SOD parse highly reactive superoxide anions into hydrogen peroxide (H_2O_2) and oxygen. In humans, there are three forms of the enzyme Cu, ZnSOD, EC-SOD, and MnSOD. While red blood cells (erythrocytes) contain only human Cu, which acts as an enzyme ZnSOD first-line defense against ROS [28]. In detail, SOD content data based on the analysis of blood serum of experimental animals can be seen in Figures 2 and 3.

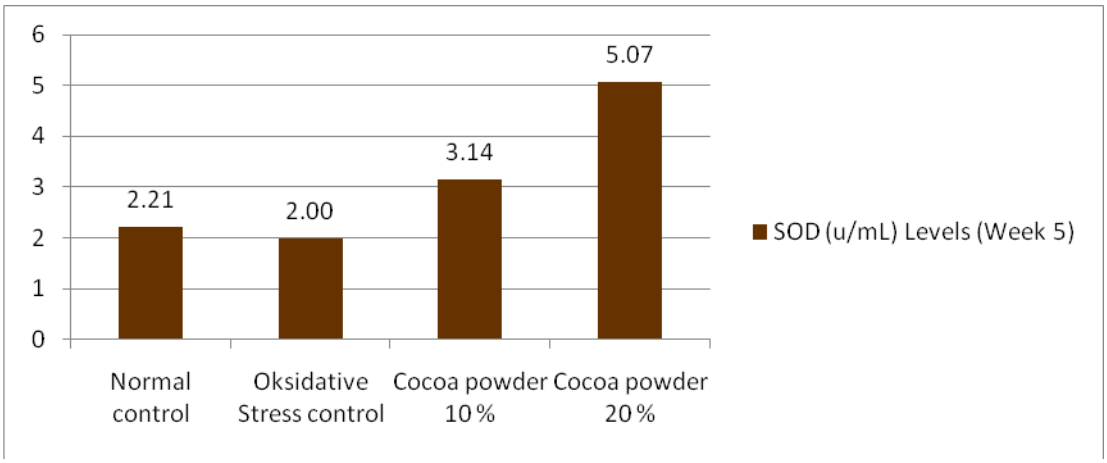

Figure 2: Levels of SOD from blood serum Wistar rats at week V

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Figure 2 shows that the group of mice treated with cooking oil without the addition of cocoa powder revealed that SOD levels low. Low levels of SOD describe the low activity of SOD enzyme. It can be a proof of the high oxidative stress by excessive free radical production. The conditions suppress the SOD enzyme, so it is not able to eliminate many free radicals are formed. Thus, it assumed that if the high oxidative stress will increase lipid oxidation marker. Lipid peroxide is a marker of oxidative damage (oxidative injury) presented with elevated levels of MDA. This is supported by the data in Figure 1 which shows the levels of MDA in groups of mice treated with cooking oil without the addition of cocoa powder is very high compared to the other three groups of mice that is 55.25 ng / mL in the fifth week. In conditions of high levels, MDA as a toxic compound may disrupt membrane integrity and cell function, which further causes cell damage [29].

Figure 2 also shows that the group of mice treated with the addition of cocoa powder 10 and 20% in the feed have higher SOD levels of a group of normal mice (without treatment). It can be a picture of the influence of cocoa powder is added to the feed to the activity of SOD mice. SOD activity can be judged by its ability to inhibit the reaction catalyzed by superoxide radicals. The activity of an enzyme (SOD) is proportional with concentration of the enzyme [30,31]. In Figure 2 is seen that in the group of rats that feed is added 20% cocoa powder had the highest levels of SOD. The conditions reinforce the notion that the high levels of nutrients (including antioxidants) in feed can increase the activity of SOD enzymes in the body tissues of mice. Other research reported that a high nutrient could increase the activity of SOD [32]. The high activity of SOD enzymes provides the maximum ability to catalyze the change of superoxide into hydrogen peroxide and oxygen to inhibit the formation of MDA. This is corroborated by the low levels of MDA are formed in groups of mice treated with the addition of cocoa powder of 20% (Figure 1).

The addition of the cocoa powder in feed mice turned out to provide an increase in SOD enzyme activity after a period of six weeks. The increase was exceeded increased activity of SOD in the group of normal mice, and a group of rats treated used cooking oil. In detail, SOD from blood serum of mice after six weeks of the maintenance period can be seen in Figure 3.

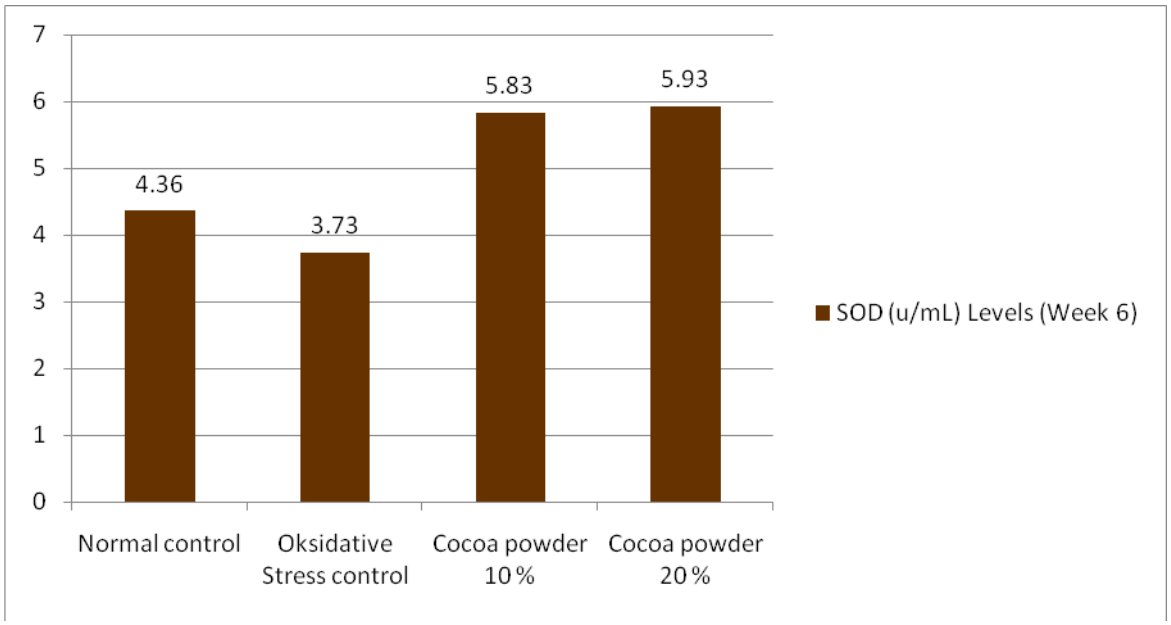

Figure 3: Levels of SOD from blood serum Wistar rats at week VI

Based on Figure 3 is known that increased levels of SOD in the group of rats treated used cooking oil also increased so that the level is approaching the levels of SOD group of normal mice. This increase probably caused in the sixth week of the effects of free radical activity of used-cooking oils has decreased so slowly been able to overcome the body's immune system started normal mice with metabolic conditions. The same thing may also happen in groups of mice with the addition of cocoa powder of 10% and 20% as shown in Figure 3. According to Figure 3 can also be explained that the process of recovery of the metabolism of the effect of free radical activity can be faster with the addition of cocoa powder in feed mouse. Speed the recovery process faster allegedly took place at a level higher cocoa powder. Such conditions can be seen in Figure 3, the activity

of SOD in the addition of the cocoa powder is 20% higher than the addition of 10%, although the difference SOD relatively little of both these treatments, but the data can be highlighted the importance of the intake of foods that contain antioxidants and their role in inhibiting the overproduction of free radicals in the body

CONCLUSIONS

Based on the above it can be concluded that the addition of cocoa powder on rats feed can reduce levels of MDA and increase SOD. The addition of the cocoa powder content of 20% can decrease MDA and increase SOD was higher than 10% cocoa powder.

REFERENCES

[1] Sharma A, Johri BN. Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. Journal Microbiol. Res. 2013; 158 : 243–248.

[2] Sharma AD, Mallick AR, Ghosh AK. Free radicals and their role in different clinical conditions: an overview. International Journal of Pharma Sciences and Research (IJPSR) 2010; 1(3): 185-192

[3] Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008; 4 (2) : 89-96.

[4] Grotto D, Barcelos GR, Valentini J, An-tunes LM, Angeli JP, Garcia SC. Low levels of methylmercury induce DNA damage in rats: protective effects of selenium. Arch Toxicol 2009; 83 (3): 249-254

[5] Surai PF. Antioxidant systems in poultry biology: superoxide dismutase. Animal Nutrition 2016; Vol.1 No.1 (8): 1-17.

[6] Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. J. Pharmacogn Rev. 2010; 4(8):118–126

[7] Shahidi F, Ambigaipalan P. 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects –A review. Journal of functional foods 2015; 18 : 820-897

[8] Kenny TP, Keeni CL, Jones P, Kung HJ, Schmitz HH, Gershwin ME. Pentameric *procyanidin* isoalated from *Theobroma cacao* seeds selectively downregulated ErB2 in human aortic endothelial cells. J. Exp. Biol. Med. 2004; 229: 255-263.

[9] Hannum SM, Erdman JW. Emerging health benefit from cocoa and chocolate. Journal of Medicine Food, 2004; 3(2):73-75.

[10] Hollman PCH, Cassidy A, Comte B, Heinonen B, Richelle M, Richling E, Serafini M, Scalbrt AA, Sies H, Vidry S. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. The Journal of Nutrition. 2011; 141: 989S–1009S.

[11] Corti R, Flammer AJ, Hollenberg NK, Lüscher TF. Contemporary reviews in cardiovascular medicine : cocoa and cardiovascular health. Journal Circulation, 2009; 119:1433-1441.

[12] Paramo JA. A new favourable effect of cocoa on atherosclerosis? Oxford Journal. Cardiovascular Research, 2010; 79 (1): 3–4.

[13] Tamrin, Harijono, Yuwono SS, Estiasih T, Santoso U. The change of catechin antioxidant during vacuum roasting of cocoa powder. J. Nutrition andFood Sci., 2012; 2 (10) :1-5.

[14] Tamrin. Catechins changes and characterization of powders cocoa (*Theobroma cacao* L.) in vacuum roasting. Dissertation. Doctoral Program of Agricultural Sciences - Interests in Agricultural Technology, Graduate Faculty of Agriculture, University of Brawijaya. 2013.

[15] Katz D L, Doughty KK, Ali A. Cocoa and chocolate in human health and disease. Antioxidants & Redox Signaling. 2011; 15(10): 2779-2811.

[16] Tamrin, Harijono, S.S. Yuwono, T. Estiasih, U. Santos**o.** Various temperature of vacuum and conventional roasting on color alteration and polyphenols content of cocoa powder**.** Journal of Food Science and Engineering 2012; 2(11): 642-651.

[17] Wardhani MR, Estiasih T. Antioxidant effects of dissolved cocoa powder on oxidative stress prevention in Wistar Rats. Journal Pangan dan Agroindustri, 2014; 2 (2): 43-49.

[18] Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. Free Radical Biology and Medicine *2005:* 39(7):841-852.

[19] Kadiiska MB, Hatch GE, Nyska A, Jones DP, Hensley K, Stocker R, George MM, van Thiel DH, Stadler K, Barrett JC, Mason RP. Biomarkers of oxidative stress study IV: Ozone exposure of rats and its effect on antioxidants in plasma and bronchoalveolar lavage fluid. Free Radical Biology and Medicine 2011; 51(9):1636-1642.

[20] Jusman SW, Soewoto H, Sadikin M, Kurniati MMV, Wanandi SI, Abadi P, Prijanti AR, Harahap IP. 2001. Biochemistry Laboratory Experiments. . Widya Medika. Jakarta. 2001.
[21] Mahdi AA, Annaro S, Tripathi S, Nawab A, Mahdi F, Hasan M, Roy R, Khetrapel CL. Correlation of age-related metabonomic changes in 1H NMR serum and urine profiles of rats with cognitive function. The Open Magnetic Resonance Journal, 2008; 1:71-76.
[22] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and in oxidative stress-induced cancer. Mini-review. J. Chemico-Biological Interactions, 2006; 160:1–40.
[23] Sugino N, Nakata M, Kashida S, Karube A, Takiguchi S, Kato H. Decreased superoxide dismutase expression and increased concentrations of lipid peroxide and prostaglandin F2 in decidua of failed pregnancy. Molecular Human Reproduction 2000; 6(7):642-647.
[24] Ozkaya O, Sezik M, Kaya H. Serum malondialdehyde, erythrocyte glutation peroxidase, and erythrocyte superoxide dismutase levels in woman with early spontaneous abortion accompanied by vaginal bleeding. Med Sci Monit. 2008; 14(1):47-51.
[25] Wresdiyati T, Astawan M, Adnyane IKM. Anti inflammation activity of ginger (*Zingiber officinale)* oleoresin on kidney of rats under stress condition. Journal Teknologi dan Industri Pangan, 2003; 14 (2):113-120.
[26] Seif H SA. Changes due to hepatotoxicity and the protective role of some medicinal plants. Review Article. Journal of Basic and Applied Sciences 2016. 5:134–146
[27] Nimalaratne C, Wu J. Hen egg as an antioxidant food commodity: A review. Journal of Nutrients. 2015; *7*(10): 8274-8293.
[28] Mates JM, Jimenez FS, Antioxidant enzymes and their implications in pathophysiologic processes. Frontiers in Bioscience. 1999. 4:339-345.
[29] Winarsi H, A. Yuniaty A, Nuraeni I. Improvement of antioxidant and immune status of atherosclerotic rats adrenaline and egg-yolks -induced using cardamom-rhizome-ethanolic-extract: an initial study of functional food. Agriculture and Agricultural Science Procedia. 2012; 9:264–270.
[30] Batinic-Haberle I, Tovmasyan A, Roberts ER, Vujaskovic Z, Leong KW, Spasojevic I. Therapeutics: latest insights into their structure-activity relationships and impact on the cellular redox-based signaling pathways. Antioxidants & Redox Signaling. 2014; 20 (15): 2372-5415.
[31] Halliwel B, Gutteridge JMC. Antioxidant defences - free radicals in biology and medicine, Oxford: Clarendon Press, 1999. p105-245
[32] Tuminah S. 2000. Radikal bebas dan antioksidan – kaitannya dengan nutrisi dan penyakit kronis. Cermin Dunia Kedokteran. 2000; 128:49-51.