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Effects of Soy-Germ Protein on Catalase Activity of Plasma and Erythocyte of Metabolic Syndrome Women

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Oxidative stress always accompany patients with metabolic syndrome (MS). Several researchers reported that soy-protein is able to decrease oxidative stress level. However, there is no report so far about soy-germ protein in relation to its potential to the decrease oxidative stress level of MS patients. The aim of this study was to explore the potential of soy-germ protein on activity of catalase enzyme in blood's plasma as well as erythrocytes of MS patients. Double-blind randomized clinical trial was used as an experimental study. Thirty respondents were included in this study with MS, normal level blood sugar, low-HDL cholesterol but high in triglyceride, 40-65 years old, Body Mass Index > 25 kg/m^2, live in Purwokerto and agreed to sign the informed consent. They were randomly grouped into 3 different groups, 10 each: Group I, was given special milk that contains soy-germ protein and Zn; Group II, soy-germ protein, while Group III was placebo; for two consecutive months. Data were taken from blood samples in 3 different periods i.e. 0, 1, and 2 months after treatment. Two months after treatment, there was an increase from 5.36 to 20.17 IU/mg (P = 0.028) in activity of catalase enzyme in blood's plasma respondents who consumed milk containing soy-germ protein with or without Zn. A similar trend of catalase activity, but at a lower level, was also noticed in erythrocyte; which increased from 88.31 to 201.11 IU/mg (P = 0.013). The increase in activity of catalase enzyme in blood's plasma was 2.2 times higher than that in erythrocytes.

Key words: catalase, erythrocytes, plasma, metabolic syndrome women, soy-germ protein

INTRODUCTION

Metabolic syndrome (MS) which specifically characterized by obesity has always two or more risk factors such as type-2 diabetes mellitus, hypertriglyceridemia, low level of HDL, and high blood pressure. Factually, there is a strong relationship between obesity and high possibility of oxidative stress as well as degree of inflammation (Festa *et al*. 2001; Keaney *et al*. 2003). This is possible because in a condition of obesity there will be an increase in glucose transport to adipose tissues. Endothelium cells in this tissue will then speed up the increase of glucose uptake through glucose transporter, so there will a hyperglicemic situation in the blood leads to the increase in NADPH oxidase and production of mitochondrial reactive oxygen species (ROS). A further effect of this situation is a condition called oxidative stress that activate inflammation signal. The activated-endothelium cells will then pull pro-inflammation macrophage (Noronha *et al*. 2005). Weisberg *et al*. (2003) stated that those infiltrated-macrophage to adipose tissues are the main source of cytokinin inflammation. Infiltrated-macrophage to the adipose tissues will also contribute to the increase of NADPH oxidase activity as well as production of ROS in that particular tissue.

High level of oxidative stress can be seen from low activity of antioxidant enzymes like catalase. Recently, Winarsi *et al*. (2011) reported that activity of catalase enzyme within the metabolic syndrome patients was 6.42 UI/mg protein. This number was lower than that noted from healthy people at the same age (Winarsi *et al*. 2013). A similar trend was noted from activity of SOD enzyme, indicated that these patients were low in status of Zn component in their blood (Winarsi *et al*. 2011). This situation will contribute to the progressivity of atherosclerosis (Toshima *et al*. 2000; Libby *et al*. 2002; Noronha *et al*. 2005) and gradually metabolic syndrome will develop into vascular disease. Therefore, activity of catalase enzyme must be increased both intracellular and extracellular.

Some researchers reported that protein of soy bean seeds is potentially used as an antioxidant source (Hermansen *et al*. 2001; Bazzolia *et al*. 2002;

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Winarsi *et al*. 2005). Especially when the seeds are germinated, because it contains more isoflavone than that of natural seeds (Winarsi *et al*. 2010; Song *et al*. 2003). In this situation, antioxidative character of soy-germ protein toward diabetes mellitus type 2 patient was stronger than that of protein from soy bean seeds (Winarsi *et al*. 2009). However, there is no report of the potential of soy-germ protein on the activity of catalase enzyme in metabolic syndrome patients. This study aimed to explore the potential of soy-germ protein on the activity of catalase enzyme in the blood's plasma and erythrocytes of the metabolic syndrome patients.

MATERIALS AND METHODS

This study was a continuation of Winarsi *et al.* (2012) which used double-blind randomized clinical trial for two consecutive months. Total respondents of this study were 30 women with metabolic syndrome. A preliminary survey was done in the polyclinic of internal disease of the Margono Soekarjo Hospital, Purwokerto, to select respondents. All respondent candidates were then visited at their home and received motivation and explanation about their disease and its development. Respondent's criteria were as follows: sugar blood content was higher than normal (fasting > 100 mg/dL; 2 h postprandial > 126 mg/dL; anytime > 200 mg/dL), hypertriglyceridemia (>150 mg/dL), low-HDL cholesterol (<50 mg/dL), age between 40 to 65 years old, Body Mass Index (BMI) > 25 kg/m^2 (Table 1), live in Purwokerto, and agreed to sign informed consent. Respondents were then divided into 3 (three) different groups, each group consist of 10 patients. Group I, was given functional milk which based on soy-germ protein plus Zn; group II, was given functional milk which based on soy-germ protein without Zn; and group III was given placebo milk (Table 2); each group was treated for two consecutive months.

Blood sampling was taken 3 times as in a baseline phase, 1 and 2 months after treatment; for this purpose sample was taken intravenously at the amount of 2 mL using a venoject tube with EDTA. In order to measure the activity of catalase enzyme, blood sample was then separated from its plasma and erythrocyte. Activity of catalase enzyme in the erythrocyte was done using the upper layer of erythrocyte solution after grounded and centrifuged to split the solution into two different layers.

Measurement of Catalase Enzyme Activity in Plasma or Erythrocyte. Lysate was initially prepared by addition of 800 µL 0.5% Triton X-100 solution into 200 µL blood's plasma/erythrocyte. Following to this was preparation of a standard solution that would be used to measure all samples. Stock solution was prepared by diluting 10 µL catalase in 50 mL of phosphate buffer. A standard solution was made from 0.5 mL of stock solution into 9.5 mL of phosphate buffer (1/20) and 0.5 mL stock solution into 19.5 mL phosphate buffer (1/40). Ten µL of lysate was then fixed with 12.5 mL phospate buffer. A chemical reaction will be started after addition of 1 mL H_2O_2. All solutions were then mixed using vortex at slow motion, then placed in a spectrophotometer at 240 nm wavelength at the interval of 15, 30, 45, and 60 sec for measuring any change in absorbance. The A_{240} value ranged from 0.02-0.10 (Winarsi *et al*. 2006). Calculation:

$$\frac{A_{30s} - A_{60s}\ \text{measurement} \times 87.44^*}{A_{30s} - A_{60s}\ \text{standard 1/20}}$$

Table 2. Chemical composition of the products given to the respondents

(%)	A	B	C
Water	4.79 ± 0.08	4.6 ± 0.008	4.5 ± 0.003
Ash	22.68 ± 0.07	22.57 ± 0.09	22.4 ± 0.006
Fat	1.11 ± 0.01	1.02 ± 0.003	1.04 ± 0.003
Protein	42 ± 0.05	39.47 ± 0.09	36.72 ± 0.037
Carbohydrate	29.39 ± 0.04	32.27 ± 0.02	35.03 ± 0.32
Total	100 ± 0.005	100 ± 0.003	100 ± 0.03

A: functional milk based on soy-germ protein +Zn; B: functional milk based on soy-germ protein without Zn; C: placebo. Data were shown as an average values with +Standard Error, 3 replications (Winarsi *et al*. 2012).

Table 1. Respondent's profile in a baseline

	I	II	III	P =
Age (year)	50.2 ± 2.0	51.5 ± 2.3	49.5 ± 2.3	0.81
BMI (kg/m2)	31.09 ± 1.1	31.23 ± 0.7	33.33 ± 0.99	0.21
Blood's glucose (mg/dL)	221.2 ± 15.36	213.7 ± 6.8	223.3 ± 8.83	0.81
Triglyseride (mg/dL)	236.4 ± 37.9	204 ± 22.8	212 ± 30.4	0.74
HDL (mg/dL)	45.19 ± 3	48.73 ± 2	45.87 ± 2.3	0.61
Blood's pressure, Sistole (mmHg)	156 ± 9	149 ± 8	154 ± 6	0.81
Blood's pressure, Diastole (mmHg)	106 ± 2	108 ± 7	102 ± 4	0.71

I = group that was given soy-germ protein + Zn; II = Group that was given soy-germ protein without Zn; C = placebo. Data were shown as mean ± Standard error; n = 10 (Winarsi *et al*. 2012).

87.44 = a conversion factor, titration of catalase according to the recipe 13.99 x 10^{-3} UI/mL x dilution factor (1/6250).

RESULTS

Catalase Activity of Blood's Plasma. At the baseline phase, catalase activity of blood's plasma of women with metabolic syndrome was 5.29 IU/mg in average. This level did not differ among these respondents of the groups (P = 0.83). A similar condition was noted for all respondents after being tretaed for 1 month (P = 0.25). However, a significant increase was noted after the respondents were treated for 2 months, i.e. 20.17 IU/mg (P = 0.018). This high level of catalase enzyme activity was particularly noted when respondents consumed milk based on soy-germ protein plus Zn and a similar trend was also noted from group which taken milk based on soy-germ protein without addition of Zn (P = 0.47) (Figure 1). It can be concluded that addition of Zn compound did not increase the activity of catalase enzyme in human's blood plasma.

Catalase Activity of Erythrocyte. At a baseline, it was noted that activity of catalase enzyme in the erythrocyte of women with metabolic syndrome was 72.57 IU/mg. This level was similar among those selected respondents (P = 0.84). After being treated for a period of one consecutive month, there was a trend to increase but the different was not statistically significant (P = 0.12). However, after being treated for another one month, the activity of catalase enzyme in erythrocyte was significantly different from that at the baseline (from 88.31 to 201.11 IU/mg; P = 0.013) especially in the group which was treated with milk based on soy-germ protein plus Zn. A slight increase (88.31 to 166.14 IU/mg; P = 0.04) was noted from another group which consumed milk based of soy-germ protein but without addition of Zn (Figure 2). However, the data of catalase enzyme in erythrocyte were not statistically different (P = 0.50), which means that addition of Zn had also no effect on the activity of catalase enzyme in erythrocyte of women with metabolic syndrome.

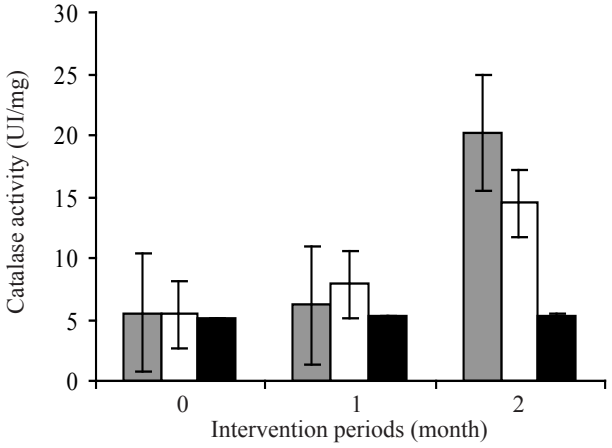

Figure 1. Effect of soy germ protein on catalase activity of plasma of metabolic syndrome women. ▨ A, soy germ protein + Zn; □ B, soy germ protein; ■ C, placebo.

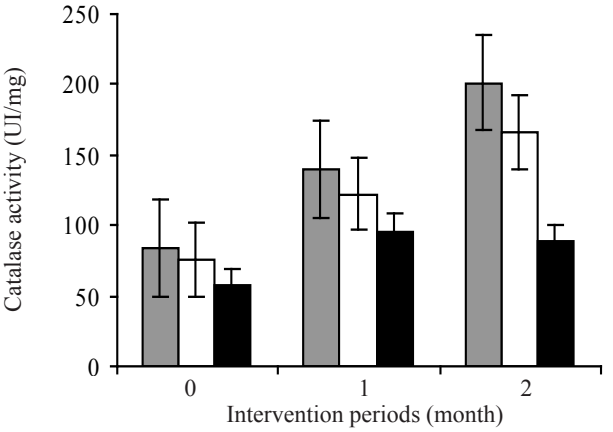

Figure 2. Effect of soy germ protein on catalase activity of erythrocyte of metabolic syndrome women. ▨ A, soy germ protein + Zn; □ B, soy germ protein; ■ C, placebo.

DISCUSSION

Some researchers have reported that oxidative stress has a strong relationship with Body Mass Index (BMI) (Olusi 2002; Keaney *et al*. 2003), where higher value of BMI is followed by higher level of oxidative stress. This was also noted from all respondents of the current study which have a BMI of 31.88 kg/m^2, where at this level of BMI, the activity of catalase enzyme is low. Mahadev *et al.* (2004) found NOX_4 which plays a significant role in forming the H_2O_2 (a strong oxidizing agent) to easily transformed into ROS within the adipose cells of obese patients. Therefore, higher BMI level might existed with higher NOX_4 level and H_2O_2 compound being formed.

It was noted in the current study that soy-germ protein was able to increase the activity of catalase enzyme in both blood's plasma and erythrocyte. Winarsi *et al*. (2010) reported that soy-germ protein contains isoflavone (glicytein) which is higher than that of protein of soy seeds. Hubert *et al*. (2005) stated that glicytein has the largest potential of being used as a radical scavenger compared with other isoflavones, and this may contribute to lower H_2O_2 level at the preliminary phase and therefore, this compound will never be transformed into hydroxyl radical. This will lead to a lighter task of catalase enzyme because there is no radical to be cleared in the blood.

Lu *et al*. (2008) also reported that isoflavone in soy-germ could increase the glutation level in male Sprague-Dawley mice. Glutation, a tripeptide consists of amino acids cystein, glycine, and glutamic acid, is an antioxidant that participates directly in a process of neutralizing free radicals or ROS while keeping exogenous antioxidant in a good form. Furthermore, glutation was also noted to participate in detoxification of various xenobiotic both organic and inorganic through a step called direct conjugation. By optimalizing function of macrophage in balancing oxidative degeneration, especially those related with expansion of monoclonal of lymphocyte, glutation is also an important component in human immune response. Glutation can also stabilize mitochondrion membrane to reduce apoptopsis in lymphocyte cells, and deficiency of glutation, therefore, can lead to a condition called hemodialysis and oxidative stress. However, an opposite situation existed, because it was noted that activity of catalase enzyme in erythrocyte was higher than it was predicted, that might be related with an increase of glutation level. Beside isoflavone, peptides which were formed as a result of soy protein hydrolysis along germination period might also affected activity of catalase enzyme. Dia *et al*. (2009) observed five healthy men at the age of 18-25 years old, who were treated with 50 g of soy protein in their daily intake. At the fifth day, respondents were checked for lunasin peptide content in their blood's plasma, at 30 min and 1 h after consuming their daily diet, and data showed that there was an increase of lunasin peptide from 66.0 $\pm$ 25.4 to 71.0 $\pm$ 32.8 ng/mL. Several researchers stated that lunasin peptide could help to promote health status. Hernández-Ledesma *et al*. (2009) reported that lunasin peptide is able to suppress the level of oxidative stress through blocking of activity in oxidation of linoleic acid and radical scavenger 2,2'-azinobis (3-ethylbenzothiazoline-6-asam sulfonat) (ABTS). Lunasin peptide was also noted to be able to suppress the release of pro-inflammation of TNF-α cytokin (tumor necrosis factor-α) and interleukine-6. Galvez *et al*. (2000) stated that in an exogenous application of lunasin, this compound could suppress transformation of fibroblast cells in mice that were deliberately induced by a carcinogenic material. Lunasin was also selectively induced apoptosis especially to those transforming cells, by blocking anacetylation of histon. This findings proved that the lunasin has an anti-inflammation and anti cancer characters, because of its strong antioxidant property. Based on this finding, lunasin might be contribute to the increase of catalase enzyme activity in both the blood's plasma and erythrocytes of metabolic syndrome patients.

In a general, amino acids with base, acids, or neutral character do not show its capacity as an antioxidant component. However, tryptophan, tyrosine, cysteine and homocysteine are also having antioxidant capacity in physiological concentration (Meucci and Mele 1995). An increase of activity of catalase enzyme might be also supported by the role of such amino acids contained in the soy-germ protein, as it was reported by Krajckovikova-Kudlakcova *et al*. (2002).

Along the process of soy seed germination, there is protein degradation to become free amino acids. Apart from isoflavone content level, increasing activity of catalase enzyme is also related with the availability of free amino acids in the soy-germ protein. Amino acids is the main component of all cells to do protein synthesis including catalase protein. As stated by Higashi and Shibata (1964), catalase enzyme consisted of 19 different amino acids, and most of them are available in the soy-germ protein. Higher protein for enzyme being produced would therefore increase activity of that particular enzyme.

According to Atmaca (2004) sulphur is an important component for human body because it plays an important role in amino acids, protein and other bio-molecules formation. Sulphur is also an important component for flavoenzyme that contains Fe, for example catalase. Although most of nuts do not contain or contain low sulphur amino acids like methionine and cysteine, but these two amino acids are higher in soy than that of other nuts. In this case, catalase enzyme might be affected by the content of methionine and cysteine in the soy-germ protein to increase its activity.

Potential of soy-germ protein as an antioxidant is mainly due to its own components like isoflavone, peptide and several amino acids. In this study, data showed that activity of catalase enzyme in either blood's plasma or erythrocyte increased, though the level of increment in blood's plasma was higher than that of in erythrocyte. It might be because of this enzyme has an intracellular character, therefore its activity in erythrocyte is higher than that of extracellular, though the increase in erythrocyte only 12.7% in compared with 27.3% in blood's plasma.

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