Germinated-Soy Milk as a Healthy Diet to Induce High Antioxidant Enzymes in Breast Milk

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Abstract – The high metabolism rate in breastfeeding mothers causes oxidative stress that depresses the Catalase (CAT) and Glutathione Peroxidase (GSH-PX) activities. This study aimed to explore the effects of Germinated-Soy Milk (GSM) on CAT and GSH-PX activity in plasma and breast milk, and Body Mass Index (BMI) of breastfeeding mothers. The subjects were fifty breastfeeding mothers, with 0-6 months feeding period, with the age of 20-35 years, good health condition, and signed the informed consent. They were divided into 2 groups with 25 women for each group. Group I had GSM as the intervention, and group II had a placebo, all interventions were conducted for 2 months. The blood and breast milk samples were drawn intravenously at the baseline, the first and the second month after the intervention. The activities of CAT and GSH-PX in plasma and breast milk and BMI were measured. The average activities of CAT in plasma (P=0.005) and breast milk (P=0.019) were significantly increased as well as the GSH-PX activity in plasma, but the BMI was decreased (P<0.05). The GSM increased the activity of antioxidant enzymes in breast milk higher than in plasma and was able to immediately normalize the body weight. GSM is a recommended healthy diet for breastfeeding mothers.

Key words: breast milk, catalase, glutathione peroxidase, germinated soy milk, BMI

1. Introduction

The increase of metabolic rate and body weight in breastfeeding mothers could cause oxidative stress and leading to immunodeficiency [1]. This increases mothers’ vulnerability to various diseases. The high metabolic rate in pregnant and breastfeeding mothers causes oxidative stress [2]. Free radicals are always formed in the body as they are the side products of the normal metabolism [3, 4]. However, the antioxidants in breastfeeding mothers cannot compensate the free radicals formation resulting in oxidative stress. Thus, there is an imbalance condition between the production of Reactive Oxygen Species (ROS) and the cellular antioxidant defense system in breastfeeding mothers [5]. As the fetus’ antioxidant status highly depends on the mother’s antioxidant status, oxidative stress can affect the babies’ growth and development.

In addition, the increase of body weight can also cause immunodeficiency in breastfeeding mothers [1] as it lowers the enzymatic antioxidant status such as Catalase (CAT) and Glutathione Peroxidase (GSH-PX) [6]. The increase of body weight of breastfeeding mothers causes infiltration of macrophages into adipose tissues, and then produces various biologically active molecules and releases inflammatory cytokines. The macrophages will be accumulated in adipose tissues that lead to overlapping functions. In this case, adipose cells are not only served as energy storages but also as endocrine glands which secrete a number of inflammatory cytokines to maintain the energy balance. Many researchers explained that cytokine is not only produced by adipose cells, but also by various activated immune cells, such as T-cells, B-cells, monocytes [7] and macrophages [8]. It is
understandable that in the breastfeeding mothers with increased body weights, the higher the number of activated immune cells, the greater the size of adipose cells and the level of inflammatory cytokines. Therefore, it is critical to normalize the body weights and maintain the mothers’ antioxidant status of the breastfeeding mothers.

A good daily intake of lactating mothers consists of sufficient amount of antioxidants is essential for their own antioxidants status and finally, their baby since the baby strongly depends on its breast milk quality. Breast milk is the only food for newborn up to 6 months old. Consuming foods rich of antioxidants can minimize inflammation and maintain the immune status. Breast milk contains some immunological compounds and nutrition, such as immunoglobulin, protein, nitrogen non-protein, fats, vitamins and minerals [9].

GSM has been well known as a drink rich in antioxidants, as it contains protein, amino acids, and isoflavones that are higher than that of non-germinated soymilk [10]. Some researchers reported that soy isoflavone is a source of antioxidant [10, 11, 12], and anti-inflammation [13, 14, 15]. Therefore, it is expected that isoflavone level in GSM has higher antioxidant properties than that in soy. Antioxidant status of a person can be represented from the activity of the Superoxide Dismutase (SOD), CAT, and GSH-PX enzymes. In the situation where a pro-oxidant level is balanced with antioxidant, these enzymes could neutralize the pro-oxidants. However, Kumar et al. [3] stated that total SOD level in breast milk is five times lower than that in the blood plasma. There is no evidence whether the low level of SOD enzyme in breast milk is parallel to the low level of CAT and GSH-PX. In addition, there are no data shows the effects of GSM on the activity of CAT and GSH-PX enzyme in plasma and breast milk in breastfeeding mothers. Therefore, it is critical to explore the effect of antioxidant contained in GSM on the activities of CAT and GSH-PX enzymes in plasma and breast milk, and body weight of lactating mothers.

2. Materials and methods

This study is a Randomized Clinical Trial with double blind where the authors and the subjects did not know which product was given. The ethical clearance was approved by the ethics committee of the Medical Faculty of Diponegoro University, Semarang, Central Java, Indonesia.

Subjects

The subjects were fifty breastfeeding mothers with 0-6 months breastfeeding period, with the age of 20-35 years old, in a good health condition, live in Purwokerto, Central Java, Indonesia, and signed the informed consent.

Germinated-Soy Milk (GSM) preparation

The GSM was prepared by germinating the soy seeds overnight. Germinated seeds were then cleaned and washed, and then they were blended and sieved. The GSM was then added by 10% sugar, 1% salt and 8 pieces of pandanus leaves. The placebo drink was prepared in a similar way but it only contains milk powder instead of germinated-soy milk (Table 1) [4].

| Compound      | Germinated-soy milk (%) | Placebo (%) |
|---------------|-------------------------|-------------|
| Water         | 80.13±0.001             | 81.43±0.001 |
| Ash           | 0.07±0.002              | 0.09±0.00   |
| Protein       | 13.77±0.001             | 11.47±0.001 |
| Lipid         | 0.94±0.001              | 0.78±0.0012 |
| Carbohydrate  | 5.08±0.00               | 6.22±0.00   |

Grouping research subjects and intervention

All subjects were divided into two groups with 25 for each group. The group I had GSM in their daily diet while group II had placebo only. Both groups consumed 150 ml/day for two consecutive months.
Blood and breast milk samples

Blood and breast milk samples were taken three times at 0, 1 and 2 months after the intervention. All samples were drawn in the morning before breakfast time. Three milliliters of blood was drawn intravenously as a sample using a Venoject tube containing EDTA and then centrifuged at 0.503 x g for 10 minutes to separate the plasma. Three milliliters of breast milk was taken for sample manually by the subjects. The breast milk sample was also taken in the morning before breakfast time, and then centrifuged at 0.503 x g for 10 min at 4°C. The lipid layer on the surface was removed, while the skimmed milk fraction obtained was drawn as the sample test.

Determination CAT and GSH-PX activity

The antioxidant enzymes activity in plasma and breast milk was measured by specific kits of Biovision Assay and Us.cn Life Science Inc., respectively. Elisa reader Labotron LB-6200 was used to read the data.

Body weight was measured as BMI

Body Mass Index (BMI) is a ratio of body weight in kilograms to height in squared meters. The subjects’ weights were measured using Camry weight scale with 0-130 kg scale while the height was measured using Microtoise with 0-200 cm scale. The weight and height measurement and the blood sampling were taken at the same time.

3. Statistical analysis

The data was analyzed using one-way ANOVA with repeated measures and paired samples t-test. Differences between means were considered significant at P<0.05.

4. Result and discussion

The Effect of GSM on CAT and GSH-PX activities in plasma and breast milk

After consuming GSM, the average rate of CAT activity in plasma in the intervention group was significantly increased from 85.69 to 94.02 mU/mL (P< 0.05) and then 106.54 mU/mL (P< 0.05), at the baseline, first and second month, respectively. However, there was no significant different found in the placebo group (P> 0.05). A slight different trend was found at the CAT activity in breast milk of breastfeeding mothers as there was no significant increased at the first month (39.59 mU/mL to 50.22 mU/mL, P> 0.05) but significantly rose at the second month (56.25 mU/mL, P< 0.05). However, stagnancy was shown in the placebo group showed (Fig 1).

![Catalase activity of lactating mothers.](image)

*Fig 1. Catalase activity of lactating mothers.*
In addition, the GSH-PX plasma activity was significantly increased from 137.43 to 143.06 ng/mL (P< 0.05). The GSH-PX level in the breast milk in the intervention group was also significantly increased from 32.63 to 63.98 ng/ml (P< 0.05). However, the GSH-PX level both in plasma and breast milk in the placebo group were only slightly increased (Fig 2).

The increased activity of CAT and GSH-PX presumably related to the isoflavone contained in GSM. The intake of protein-rich GSM plays an important role in improving the work of isoflavone. Winarsi et al. [12] reported that all soy-based food products contain isoflavones and capable of increasing CAT and GSH-PX enzyme activity in the lymphocyte cells of premenopausal women. One of the isoflavones found in GSM is glycitect, the most potential radical scavenger among isoflavones followed by genistein and daidzein [16]. They can reduce free radicals and suppressing the production of ROS. Thus, the present of isoflavones in GSM was responsible for the significant increase of CAT and GSH-PX activities in plasma.

In addition, the high level of germinated soy's isoflavones caused the significant elevation of CAT and GSH-PX activities in the breastmilk. Although the level of isoflavone in breast milk was not measured, according to Jahan-mihan [17] consuming soy products can increase the level of isoflavone in breastmilk. The isoflavone glycitect levels contained in the germinated-soy is 1.5 to 7 times higher than in soybean [10, 18]. The level of Glycitin in soy and soy-based foods isoflavones is only approximately 5-20% of the total isoflavone. Thus, the antioxidant effects of isoflavones are not only able to affect CAT and GSH-PX activity in plasma but also in breast milk.

Isoflavones stimulating the CAT and GSH-PX in various ways including scavenging the free radical compounds, chelating metal ions that activate redox reaction (chelate redox-active metal ions), and increasing the expression of metallothionein. Soy isoflavones have a low level of estrogenic-like activity as they can bind the receptors of alpha and beta estrogen. As the highest estrogenic-like activity among all isoflavones, Glycitin also has a scavenging activity on reactive oxygen.

Isoflavones bond the amino acids of the germinated-soy proteins as antioxidants. The role of isoflavones as antioxidants takes place through two mechanisms, namely as a hydrogen ion donor and a free radical scavenger [19]. As an electron donor, isoflavones cut the chain reaction by donating an H atom for the unpaired electrons, so the number of free radicals was reduced. Along with the reduction of free radicals, it directly increased the antioxidant status of the subjects. Thus, the high level of glycitect in GSM was responsible for the increase of CAT and GSH-PX activity in the plasma and the breast milk of the breastfeeding mothers. However, there is a need to build the evidence about glycitect as the literature exploring glycitect is fewer compared to other isoflavones, such as genistin.

In addition, the increased of CAT and GSH-PX enzyme activity was not only due to the presence of isoflavones but also due to the availability of free amino acids in the germinated soy milk.
protein. GSM increased the antioxidant level of the subjects by supplying an adequate amount of amino acids. During its germination, proteins from the soy seeds are degraded to free amino acids. Amino acids are important components for protein synthesis in all cells, including for the formation of protein CAT and GSH-PX enzymes. Ashokan et al. [20] reported that CAT enzyme consists of various amino acids as well as GSH-PX, such as Glx, Gly, Ala, Leu, Phe, and Arg, where most of them present in GSM. The GSM is believed to be able to increase the supply of amino acids required for CAT and GSH-PX enzyme synthesis [21], so that the production of these enzymes in the subjects who consumed GSM is higher than those who consumed the placebo. It can be assumed that the more the protein enzymes being synthesized, the higher the enzymes activity is. Therefore, more free radicals can be removed and decline the oxidative stress level.

The germinated soymilk in this study contains 13.8% protein or about 13.8 mL protein per 100 mL of GSM. Each subject drank 150-200 mL of GSM in his or her daily intake. If one mL of protein is assumed as equal to one gram of protein, each subject had 20.7-27.6 g protein of GSM per day. The National Research Council (1989) recommended breastfeeding mothers with 70 kg body weight to take one portion of legume (=90 g or 1/2 cup cooked nuts) which contains 7-8 g protein or 15% of the Recommended Dietary Allowance (RDA). In this study, each subject had a 41.4-55.2% of RDA protein from the GSM, this is an ideal amount for promoting functional foods compound. Isoflavones contained in the GSM was 39.1 ppm (=39.1 mg/L). By the same calculation for protein content, each subject in this study consumed about 6-8 mg isoflavones per day. This amount is as recommended for Asian people (3-28 mg/d) [22]. Thus, it can be concluded that the amount of GSM consumed by the breastfeeding mothers was sufficient to provide an adequate level of protein.

In addition, it was found that the CAT and GSH-PX enzyme activity in the breast milk of the breastfeeding mothers was increased after consuming the GSM for two consecutive months. At that time, the baby was at the early age (1.95±0.81 month) so the breast milk volume might be still in full as well as its nutritional contents. Thus, it is reasonable that the effects of isoflavone did not significantly increase the activity of CAT or GSH-PX. However, after the CAT and GSH-PX enzyme activity in the breast milk were significantly increased in the second month. This condition might be related to a low antioxidant status of the subjects due to a longer period of breastfeeding. Yet, the GSM consumption helped them increasing the antioxidant status by providing an adequate amount of isoflavones.

In this case, antioxidants of isoflavones accelerated the metabolisms, digestions, and thereby enhanced the babies’ passive immune. The GSM also enriched the bioactive compounds of the breast milk, giving a positive impact on the babies’ immune status. Breast milk does not only supply the protective factors for newborns, but also facilitates the development, tolerance and response of anti-inflammation factors. In addition, breast milk is a communication tool between the mothers’ immune system and the babies that actively triggers the immune system, maintains body's normal metabolism and provides adequate amount microflora of the babies.

The implication of antioxidant activity in breast milk

The result shows that the activity of CAT and GSH-PX in breast milk was higher than that in plasma. This brings positive impact to the mothers and the babies as it improved the quantity and quality of the breast milk. Although the quantity of the breast milk was not measured in this study, the subjects reported that after breastfed the babies slept tighter and longer than usual. Factually, the babies who consumed GSM slept much longer (1.85 hours) than those in the placebo group (1.25 hours). It can be assumed that the babies felt full and satisfied. Qualitatively, GSM increased the activity of CAT and GSH-PX so that improved the immune status as it reduced the production of inflammatory cytokines [4].

The effect of GSM on BMI

The BMI was significantly decreased after two months in the intervention group (22.77 to 20.64 kg/mm², p=0.02) but there was no changed in the placebo group (p=0.94). The decrease of the mothers’ BMI is related to the proteins contained in GSM. Winarsi et al. [10] reported that GSM contains high protein. One of the protein’s characters is a thermogenic character and gives a full feeling [23] so that it diminished the hunger and willingness to eat. Skovet al. [24], reported that a group of high-protein diet gave a longer satiety than a low protein diet. In addition, the body does not have
protein storages, so it immediately metabolized into energy. This then pressing the sense of hunger so that decreased the mothers weights [4]. This condition is preferred by the mothers as their weight can be recovered as a good as the pre-gestation weight yet they still can producing breast milk optimally. Thus, as a healthy diet, GSM brought not only benefits for the babies but also the mothers.

5. Conclusions
GSM that was consumed by the breastfeeding mothers for 2 consecutive months significantly increased the CAT and GSH-PX enzymes activity in plasma and breast milk. However, the increase of CAT activity in the breast milk was higher (62.28%) than that in the plasma (29.81%). Similarly, the GSH-PX activity in the breast milk was also higher (40.53%) than in the plasma (4.01%). GSM is not only a source of antioxidants, but also capable of losing the breastfeeding mothers' weights, and normalized their BMI levels within 2 months. Thus, this product is suggested to be consumed by breastfeeding mothers as it can help them to provide sufficient amount of breast milk that rich in antioxidants.

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