Improvement of immune and antioxidant status of COVID-19 vulnerable groups using mung bean sprout yoghurt

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Abstract

This study aimed to determine the effect of mung bean sprout yoghurt (MungbeS-Yo) on plasma CRP and SOD levels of healthcare workers. MungbeS-Yo was prepared by mixing green bean sprout milk and skim milk, sucrose, and Lactic Acid Bacteria (LAB). The subjects of this study were 20 female healthcare workers, randomly divided into 2 groups. Group-1 was given MungbeS-Yo, while group-2 was given Skim-Yo (as control), as much as 200 mL/day for 2 months. MungbeS-Yo is prepared from 70 parts of mung bean sprouted milk, 30 parts skim, 0.5% LAB, and 10% sugar. Blood samples were taken before and after the intervention. Blood plasma was tested for CRP and SOD levels. CRP levels decreased from 5.41 to 3.61 mg/L (P = 0.037), while SOD levels increased from 88.86 pg/mL to 176.05 pg/mL (P<0.044). MungbeS-Yo has the potential to improve the immune status and enzymatic antioxidant status of plasma susceptible groups infected with Covid-19. Thus, MungbeS-Yo can help raise the immune level against infection.

1. Introduction

Healthcare workers are the front line in handling COVID-19 patients. Therefore, healthcare workers are required to have excellent physical and psychological conditions. However, the high workload and the anxiety of being infected, create a big stressor. Stress is closely related to a decrease in immune status, a self-defense response against viruses. Nakata (2012) and Maydych (2019) added that stress affects the inflammatory response, characterized by increased levels of C-Reactive Protein (CRP). Shivpuri et al. (2012) reported that interpersonal stress is greater in women than men are. Therefore, it is very reasonable if female healthcare workers are one of the vulnerable groups for COVID-19. It has been reported that 32.9% of healthcare workers were infected with Covid-19 due to direct contact with patients and 22.7% were infected due to direct contact with co-workers of healthcare workers (Huang et al., 2020; Vandercam et al., 2020). Considering the number of Covid-19 sufferers is still increasing, it is important to make efforts to improve the immune status of healthcare workers, they can continue their services to patients.

Currently, the right medicine has not been found to

defend the body during a pandemic like today. Possibly, going back to nature is a wise step to overcome this. Some experts say that immune status is closely related to antioxidant status. Therefore, the role of antioxidant-rich food products being able to overcome oxidative stress by increasing the activity of antioxidant enzymes and improving the performance of the immune system by suppressing inflammatory markers (Middleton et al., 2000; Winarsi et al., 2010; Winarsi et al., 2012; Winarsi et al., 2013; Winarsi et al., 2016).

One type of legume that is rich in antioxidants is green beans. Besides being known to be rich in nutrients, green beans also contain oligosaccharides, which often cause flatulence. To minimize the levels, it is necessary to soak and germinate. Germination reported Winarsi et al. (2019) can remove beany flavour, increase antioxidant content, digestibility (Sokrab et al., 2012) and total phenolic content (Guo et al., 2012; Winarsi et al., 2020) whose antioxidant activity can protect the body from oxidative stress (Winarsi et al., 2016). The activity of protease, lipase, and carbohydrase increases, causing the content of amino acids, fatty acids, and glucose in sprouts to be higher than in ungerminated
beans. Simple compounds such as glucose, amino acids, fatty acids, vitamins, and minerals are important nutrients for the growth of lactic acid bacteria (LAB), a bacterial species in the manufacture of yoghurt. Mung bean sprouted yoghurt prepared from mung bean sprouts and skim milk, which is fermented using LAB, contains 1,273.11 mg GAE/L, 41.40% protein, and 12.17% fibre, and L. acidophilus in MungbeS-Yo as much as $6.56 \times 10^7$ CFU/mL, it is called rich functional drink antioxidants and probiotics (Winarsi et al., 2021).

The problem is, can MungbeS-Yo improve the immune status and antioxidant status of healthcare workers? This study aimed to explore the benefits of MungbeS-Yo on improving the immune status and antioxidant status of healthcare workers.

2. Materials and methods

This study used a true experimental design. This study has gained ethical approval from the Komisi Etik Penelitian Kesehatan (KEPK) of the Faculty of Health Sciences, Jenderal Soedirman University, with a reference number: 253/EC/KEPK/I/2021.

2.1 Production of MungbeS-Yo

Mung beans were germinated for 10 hrs, blended until a smooth liquid was obtained called mung bean sprout milk. Mung bean sprout yoghurt was prepared by mixing green bean sprout milk and skim milk in a ratio of 70:30. As much as 10% of sucrose was added and pasteurized at 80°C for 10 mins, cooled to 45°C, then inoculated with 0.5% LAB starter of the total volume, and incubated at room temperature of 27-35°C for 24 hrs (Winarsi et al., 2021).

2.2 Research subjects

A total of 20 female healthcare workers became the research subjects with the criteria as followed aged 30-50 years old, had central obesity, inflammation, and oxidative stress did not leave the research site for two years and were willing to sign informed consent. The subjects were randomly divided into two groups, with a total of ten people in each group.

2.3 MungbeS-Yo intervention to research subjects

The first group received a MungbeS-Yo as much as 200 mL/d of regularly for two months, while the second group was given a placebo (Skim-Yo).

2.4 Blood sampling

Blood samples were taken twice (0 and 2 months after the intervention), with a total of 3 mL samples for each blood sampling. The blood sample was centrifuged at 3,000 rpm for 10 mins, and the plasma portion was separated from the erythrocytes. Blood plasma was examined to determine the SOD levels (using Abbkine SOD ELISA) and CRP levels (using Calbiotech High Sensitivity C-Reactive Protein CRP ELISA).

2.5 Data analysis

Data were analyzed with the help of the SPSS Statistics 17.0 program. All data were tested for Shapiro-Wilk normality. Differences in plasma CRP and SOD levels before and after the intervention were analyzed using the Paired t-test.

3. Results and discussion

Subject characteristics include a description of age, BMI, CRP and SOD levels initial (Table 1). Healthcare workers have an age range between 30-50 years, are obese, and have CRP levels above normal. According to Kratz et al. (2012), the normal threshold for CRP levels is 0.2 – 3.0 mg/L. This condition proves that healthcare workers experience inflammation, and it is possible that the levels will continue to increase, due to frequent contact with various patients, including Covid-19 sufferers in their work area. CRP levels are generally not associated with viral infections, but in fact, high CRP levels are closely related to the length of hospitalization of patients (Jeon et al., 2017). This proves the important role of CRP levels in respiratory viral infections, such as the coronavirus that attacks the respiratory tract.

Table 1. Subject characteristics

| Variable            | MungbeS-Yo (n = 10) | Skim-Yo (n = 10) | p*          |
|---------------------|---------------------|-----------------|-------------|
| Mean±SD             | Mean±SD             |                 |             |
| Age (y)             | 38.86±3.49          | 38.14±4.88      | 0.758       |
| BMI (kg/m²)         | 30.96±3.87          | 28.61±4.16      | 0.297       |
| CRP level (mg/L)    | 5.41±1.93           | 6.31±2.99       | 0.559       |
| SOD level (pg/mL)   | 88.87±16.19         | 96.15±25.42     | 0.239       |

*Independent t-test. MungbeS-Yo: the group that was given mung bean sprout yoghurt, Skim-Yo: the group given skim yoghurt as a control

CRP levels are also used as a prognostic marker for pneumonia in COVID-19. The plasma CRP concentration increased to 11.47 mg/L in the mild infection group and even increased to 23.4 mg/L in the moderate to severe infection group. Elevated CRP levels are used as an early indicator of nosocomial infection and help detect the severity of COVID-19 regardless of age (Chen et al., 2020). CRP is considered a biomarker that easy and inexpensive to measure, compared to other markers (Armani and Becker, 2005). A decrease in CRP levels indicates an improvement in immune status.

Among inflammatory markers, CRP is the most
sensitive marker that can predict future cardiovascular disease events (Chen et al., 2010). Various factors influence inflammatory markers, including nutrition, antioxidants, physical fitness and exercise. Physical activity has been reported to reduce the inflammatory marker CRP (Meyer et al., 2007; Pyne and Pyne, 2013). According to Sproston and Ashworth (2018), CRP is the most sensitive marker of acute inflammation, because its levels can increase up to 1000 times during infection. CRP synthesized by hepatocytes, in response to increased levels of IL-6, IL-1, and TNF- released by inflamed tissue. CRP plays a key role in activating the complement system, inducing the expression of adhesion molecules, increasing phagocytosis and macrophages, as well as leukocyte activation and opsonization (Armani and Becker, 2005).

CRP levels in blood plasma will increase more than normal levels, after 6 hrs of infection, and even reach their highest levels at 50 hrs after trauma, infection or inflammation. CRP levels decreased exponentially after 18-20 hrs later (Ridker, 2003; Kaski, 2010). High serum CRP levels are common in viral infections, as well as SARS, which is the closest human disease to COVID-19. IL-6 production is also high, even IL-6 production due to SARS is higher than other viruses in general, such as influenza (Okabayashi et al., 2006). Therefore, blockade of cytokines or pro-inflammatory receptors is therapeutic in COVID-19 (Shakoory et al., 2016). Some researchers mention that functional foods containing antioxidants can reduce plasma CRP levels (Winarsi et al., 2016). It was also reported that soybean sprouted milk can reduce CRP levels in pregnant women to reach 74.34%, because of the antioxidant role of isoflavones in it.

3.1 Effect of MungbeS-Yo on health care workers plasma CRP levels

After 2 months of intervention, CRP levels in the MungbeS-Yo group decreased from 5.41 to 3.61 mg/L (P = 0.037) (Figure 1), compared to the control (P = 0.13). The same finding was reported by Salehzadeh (2015) that yoghurt given to athletes could reduce CRP levels. Winarsi et al. (2021) reported that MungbeS-Yo contained 1,273.11 mg GAE/L phenolic, 41.40% protein, and 12.17% fibre.

As antioxidant compounds, phenolics reduce the activity of reactive compounds (Amaretti et al., 2013), chelate transition metal ions, inhibit several enzymes associated with ascorbate autoxidation (Talwalkar and Kailasapathy, 2003), reduce oxidant compounds in the intestine (Azcarate-Peril et al., 2011), induces the transcription of genes involved in glutathione biosynthesis in the intestinal mucosa (Lutgendorff et al., 2009), increases glutathione synthesis in pancreatic cells, and stimulates the action of enzymes in the intestinal microflora (Lutgendorff et al., 2008). MungbeS-Yo with its protein and fiber contents considered useful as a product to support the immune system during the Covid-19 pandemic. Phenolic acids are simple compounds that play a role in reducing the risk of chronic diseases, various types of cancer (Reboredo-Rodriguez et al., 2018), and various viruses (Kaila et al., 1994).

Figure 1. Effect of mung beans sprout yogurt on CRP plasma levels of healthcare workers. Values followed by the same superscript are statistically not significant with α = 5%. MungbeS-Yo: the group that was given mung bean sprout yogurt, Skim-Yo: the group given skim yogurt as a control.

Generally, a product is considered a probiotic, if it contains good bacteria as much as 10⁶-10⁷ CFU/mL. In this study, MungbeS-Yo contained LAB as much as 6.56×10⁶ CFU/mL (Winarsi, 2021). Therefore, MungbeS-Yo deserves to be considered as a probiotic drink. Probiotics are antimicrobials, which can increase lactose absorption, stimulate the immune system, treat and prevent allergies, prevent bacterial infections in preterm birth, inflammatory bowel disease, bladder and ear infections, tooth decay, chronic diarrhoea and travel diarrhoea. Beneficial bacteria of Lactobacillus, Streptococcus thermophilus and Vulgaris, which are often found in yoghurt, can improve the digestive system and have a major impact on health (Salarikia et al., 2013). These bacteria also inhibit the growth and multiplication of pathogenic bacteria.

The decrease in plasma CRP levels may be related to the role of phenolic compounds in MungbeS-Yo. According to Winarsi et al. (2016), isoflavones (a form of phenolic) in sprouted soybean milk is capable to improve the CRP of pregnant women after 2 months of intervention. Isoflavones can suppress plasma IL-6 production in lactating mothers who are given sprouted soybean milk. IL-6 is a pro-inflammatory cytokine, which is secreted by immune cells and other cells that experience inflammation in response to the body's defence against foreign substances. IL-6 stimulates hepatocyte CRP production (Tanaka and Kishimoto, 2014). Once produced, CRP is then released into the inflamed tissue or organ. At the site of inflammation,
CRP performs a defence mechanism by activating the classical complement pathway, bacterial opsonization and complement-mediated phagocytosis (Sproston and Ashworth, 2018). Phenolics reduce CRP levels by inhibiting IL-6 regulation. The inhibition of IL-6 regulation has an impact on suppressing CRP production, and the levels decrease.

In addition to phenolics, MungbeS-Yo probiotics also have the effect of improving the level of inflammation. According to Duncan and Flint (2013), probiotics are live microbes which when given in adequate amounts can be beneficial for the health of the digestive tract (Antoine, 2010). The probiotic bacterial species in yoghurt are *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*, used as a starter in the yoghurt fermentation process. It was reported that the amount of LAB in MungbeS-Yo was $6.56 \times 10^7$ CFU/mL (Winarsi et al., 2021), it is suspected that these bacteria contributed to suppressing CRP levels.

Giving probiotics early in life accelerates the formation of a balance of intestinal microflora (Kabir, 2009), thus supporting the formation of a good immune system in the intestinal lumen (Jeppsson et al., 2004). Probiotics are also capable to modulate physiological characteristics in the digestive tract, such as mucosal immunity, mucosal trophicity, and intestinal permeability (Fioramonti et al., 2003). Supplementation of probiotics can stimulate the production of short-chain fatty acids (SCFA), such as butyric acid, which plays an important role in the proliferation of intestinal epithelial cells. Increased production of butyric acid can help expand the absorption area in the intestinal lumen, so that nutrient absorption is more optimal (Ohashi and Ushida, 2009).

SCFA production requires cooperation between organic acid-producing bacteria and other bacteria in the gut, through a cross-feeding mechanism (Van Immerseel et al., 2006). These bacteria hydrolyze undigested carbohydrates into monosaccharides, through anaerobic fermentation in the intestine. The monosaccharides formed are then converted into phosphoenolpyruvate (PEP) via the Embden-Meyerhof-Parnas pathway (glycolysis) and pentose-phosphate, which are then converted into organic acids (Besten et al., 2013). The absorbed SCFA is the main energy source for enterocytes, so it can help meet the energy needs of the host.

According to Hegazy and El-Bedewy (2010), SCFA produced by probiotics can reduce plasma CRP levels, by blocking enzyme synthesis from liver CRP. CRP synthesized by the liver is a response to release factors by fat cells, such as IL-6 (Kinoshita et al., 2015). In their study, Hegazy and El-Bedewy (2010) observed that the consumption of probiotics in patients with ulcerative colitis for 8 weeks, can significantly improve inflammation, by reducing IL-6 levels, TNF- expression, and NF-κB. The decrease in the concentration of IL-6 can indirectly lead to the reduction of CRP production. Decreased CRP concentration is associated with reduced expression of inflammatory factors, in other words, an improvement in immune status.

The plasma C-RP is a marker of systemic inflammation, whose levels are elevated by chronic conditions, including obesity and high waist circumference. In this study, subjects were obese, as indicated by a large BMI > 30 kg/m$^2$ and had a waist circumference of 93.72 cm. However, BMI decreased significantly from 32.99 to 28.95 kg/m$^2$ ($P = 0.043$), and waist size also decreased from 93.72 to 89.43 cm ($P = 0.044$). Thus, the decrease in plasma CRP was supported by a decrease in the subjects' BMI and waist size.

The anti-inflammatory properties of some probiotic strains are thought to work by reducing mucosal inflammation by modulating levels of cytokines and other inflammatory mediators (Choi et al., 2006). Studies on the consumption of probiotic yoghurt containing *L. acidophilus* and *Bifidobacterium* in pregnant women for nine weeks led to a decrease in CRP sensitivity. This reduction in inflammation due to the probiotic MungbeS-Yo may be related to its effect on increasing levels of SOD, which scavenges superoxide and hydroxyl radicals, thereby suppressing plasma CRP expression.

### 3.2 Effect of MungbeS-Yo on plasma SOD levels of healthcare workers

Subjects in this study were obese if their BMI was 25 kg/m$^2$ (WHO, 2000). According to Winarsi et al. (2012), obese women experience high oxidative stress in their bodies. In obese conditions, the wider adipose tissue can lead to hypoxic conditions ($O_2$ deficiency). Chronic hypoxia increases oxidative stress by producing excessive free radicals without compensating for the activity of antioxidant enzymes. The higher the level of oxidative stress in obese patients, the lower the activity of the SOD enzyme. During a pandemic, healthcare workers are at risk of being exposed to the virus due to direct contact with various kinds of patients. Baseline data showed that plasma SOD levels were low. Several researchers reported that antioxidant-rich products could improve the antioxidant activity of SOD (Harun et al., 2017; Winarsi et al., 2016; Setiawan et al., 2016). According to Winarsi et al. (2021), MungbeS-Yo contains phenolic antioxidants of 1,107.80 mg GAE/L, which is expected to increase plasma SOD levels of...
healthcare workers.

After two months of administration of MungbeS-Yo, there was a significant increase in SOD levels from 88.86 pg/mL to 176.05 pg/mL (p = 0.044), as did the Skim-Yo group but not significantly (p = 0.590) (Figure 2). The increase in SOD levels in this study concurred with the findings of Setiawan et al. (2016), who had given sprout soybean juice at a dose of 2.12 g/d for 28 days in rats. This is due to the presence of phenolic compounds that have antioxidant abilities. In this study, subjects were given 200 mL of yocamio, which contains 221.56 mg of phenolic GAE (Winarsi et al., 2021). Rahmawati et al. (2014) proved that the administration of a product with a flavonoid content of 21.46 mg/g in 14 days could increase the SOD activity of rats. Winarsi et al. (2016) also reported that an increase in plasma SOD also occurred in the provision of sprouted soybean milk for 2 months containing 39.1 ppm isoflavones. Isoflavones are able to increase SOD levels by increasing SOD activity, suppressing free radical activity by binding to reactive oxygen species (ROS) and converting them into isoflavone radicals (Winarsi et al., 2016). Thus, the phenolic action of MungbeS-Yo in increasing plasma SOD levels is the same as that of isoflavones.

Mung bean sprout yoghurt is a probiotic drink that has many health benefits. The L. acidophilus content in MungbeS-Yo is 6.56×10⁷ CFU/mL (Winarsi et al., 2021). This is in accordance with the SNI standard (2009) that fermented milk is said to be a probiotic drink if the number of LAB reaches 10⁷ (Utami et al., 2017). Wang et al. (2017) reported that athletes under conditions of high physical stress and oxidative stress can be neutralized by administering Lactobacillus rhamnosus because probiotic bacteria can suppress the production of free radicals, and this is reflected by increasing antioxidant levels in the body. Utami et al. (2017), reported that the administration of probiotic bacteria Lactobacillus casei at a dose of 10.9×10⁷ cells/kgBW/d for 2 weeks increased SOD activity by 50.7%. Probiotics are reported to reduce oxidative stress by neutralizing oxidants in the gastrointestinal tract expressed in antioxidant enzyme activity, stimulating the immune system by suppressing cytokine-induced inflammation and preventing oxidative stress and reducing intestinal pathogenic bacteria (Kleniewska et al., 2016).

MungbeS-Yo contains 17.26% Vitamin C (Winarsi et al., 2021). Based on the age and gender of the subjects, the amount of Vitamin C needed in a day is 75 mg. Vitamin C is a natural antioxidant that works synergistically to neutralize free radicals (Suhail et al., 2011). Several studies have shown that vitamin C supplementation can improve the activity of the SOD enzyme (Suhail et al., 2011; Boudouris et al., 2013; Rafighi et al., 2013), it is possible that the Vitamin C contained in MungbeS-Yo also plays a role in increasing SOD levels of healthcare worker plasma. Mung bean sprouts are the raw material for making yoghurt. Thus, the product obtained by LAB fermentation makes food products have therapeutic benefits, including antioxidant and immunostimulant activity, prophylaxis (preventing infection) against several types of intestinal infections, increasing tolerance to foods containing lactose, and preventing cancer.

4. Conclusion

MungbeS-Yo is a food product fermented by LAB which has the potential as an immune-stimulant by suppressing the level of inflammation, reducing CRP levels by 33.27%. MungbeS-Yo is rich in phenolic antioxidants; which have been shown to increase SOD antioxidant levels by 98.01%. Thus, it is believed that MungbeS-Yo is a probiotic drink product that is useful for improving the immune system of healthcare workers who are susceptible to exposure to COVID-19. Therefore, MungbeS-Yo can help raise the immune level against infection.

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