## Original article:

The Effect of Turmeric Extract (Curcuma zedoria) on Antioxidant Enzymes Activity Chris Adhiyanto<sup>I</sup>, Lucky Briliantina<sup>I</sup>, Narila Mutia Nasir<sup>2</sup>, Hari Hendarto<sup>I</sup>, Flori R. Sari<sup>I</sup>, Kenyo Sembodro<sup>3</sup>, Sisy Marfani<sup>3</sup>, Salma Maulidia<sup>3</sup>

### Abstract

Background: Indonesia is a country that has diversity of spices and types of cuisine. In the early 70s, the use of MSG (monosodium glutamate) as a flavor enhancer began to be popular in Indonesia. It replaces natural flavor such as sugar, salt or spices because its affordable price compared to natural flavor. MSG as food additive is added to foods that exceed limits and may cause a negative effect to the body. One of the effects is the increasing of free radicals that can lead to various degenerative diseases such as blood vessel disorders and others. White turmeric is a common spice that widely used by Indonesian. Several studies have reported the benefits of white turmeric as an antifungal, antimicrobial and so on. The objective of this research is to find the effects of white turmeric extract in improving endogenous antioxidants to suppress the free radical effects because of the excessive use of MSG. Materials and Method: We measured the activity of SOD, catalase and GSHreductase as endogenous antioxidants in mice treated with MSG and white turmeric. The subjects of this study used 24 Sprague Dawley male rats aged 2-6 months; weight 100-150 grams, which were randomly divided into 6 groups. The control group did not receive any treatment. The MSG group was given MSG (4800mg / kg/ day). Groups 3 and 4 were given MSG and white turmeric extract, respectively (100 mg/kg/day and 200 mg/kg/ day). Groups 5 and 6 were given MSG and vitamin C respectively (250 mg/kg/day and 500 mg / kg/ day) after 14 days of treatment; the activity of enzyme measured by Abcam Enzyme Kit and detected by Spectrophotometer Thermo Scientific Multi-scan Go. Result: The administration of white turmeric extracts will increase SOD and Catalase activity, and reduce GSH-reductase activity. Conclusion: White turmeric extract will help the work of endogenous antioxidant enzymes in counteracting free radicals produced from MSG metabolism.

**<u>Keywords:</u>** White Turmeric extract, Mono Sodium Glutamat, Free radicals, antioxidants endogen

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## Introduction

Monosodium Glutamate (MSG) is a food flavoring enhancer that is widely used throughout the world under various trademarks, containing 78% glutamic acid and 22% salt and water <sup>(1)</sup>. The maximum use of MSG in food is 3.5 mg / day (for body weight 70 kg) or 1 teaspoon <sup>(2,3)</sup>. Kushwaha reported that consumption of 10 mg/kg body weight MSG in experimental animals could cause oxidative stress by showing changes in antioxidant enzyme activity <sup>(3)</sup>.

Oxidative stress can cause damage to various organs. But the body is equipped with a set of

defense systems to ward off free radical attacks namely antioxidants so that it can limit the damage caused by free radicals. These antioxidants are divided into two types, namely endogenous and exogenous antioxidants. Endogenous antioxidants include enzyme systems such as the enzyme Superoxide Dismutase (SOD) which found in mitochondria and cytosols, Glutathione Peroxidase (GPx), Glutathione Reductase (GRx), and catalase (CAT) <sup>(4)</sup>. While exogenous antioxidants, it cannot be produced by the body and it is found in fruits, vegetables, nuts, seeds, and some meat. These foods contain β-carotene, vitamin C and vitamin

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### E etc (4, 5).

White turmeric (*Curcuma zedoaria*) contains essential oils, polysaccharides, and substances that can act as antioxidants, anti-inflammatory, analgesic and hepatoprotection which are curcumin and sesquiterpenes (6,7,8).

This research was conducted to determine the ability of white turmeric extract (Curcuma zedoaria) to protect the body from ROS caused by MSG by measuring changes in endogenous antioxidant activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GRx)

# **Materials and Methods**

The design study of this research is an experimental laboratory. The subject of this study was 24 male Sprague Dawley rats, aged 2-6 months, 100-150 grams, which were randomly divided into 6 groups. Group 1 is a normal group, only given distilled water. Group 2 is a group of mice with 4800 mg MSG/kg/day administration. Group 3 was a group of mice with 4800 mg MSG/kg/day administration then given turmeric extract 100 mg/ kb/day. Group 4 was a group of mice with 4800 mg MSG /kg/day given then turmeric extract 200 mg /kg/day. Group 5 groups of mice with 4800 mg MSG/kg/day were then given and then given vitamin C 250 mg/kg/day. Group 6 groups of mice with the administration of 4800 mg MSG/kg/ day were then given vitamin C 500 mg/kg/day. Before the treatment of white turmeric extract (Curcuma zedoaria) in groups, 3 and 4 were first given MSG at a dose of 4800 mg/kg/day. The same treatment as in the group treated with vitamin C. All groups was carried out for 14 days.

Measurement of catalase, SOD and GR activity was carried out using the Catalase, SOD and GR Activity Assay Kit spectrophotometer method from Abcam – Cambridge, UK. Plasma was collected after surgery on day 15 by centrifugation at a speed of 6000 RPM, then stored at -20° C in a dark condition until the time of measure.

# Study limitations

The disadvantage of this study is that we did not measure oxidative stress such as lipid peroxidation, total antioxidant capacity, total oxidant, oxidative stress index and GPx.

# Ethical considerations

All experiments were performed following guidelines from the "Principles of Laboratory Animals Care". All efforts were made to minimize animal suffering and reduce the number of animals used.

### Results

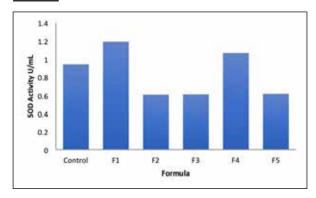


Fig 1. SOD Activity. F1, MSG; F2, Curcuma 100 mg+MSG; F3, Curcuma 200 mg+MSG; F4, Vit C 250 mg+MSG; and F5, Vit C 500 mg + MSG. Figure 1 showed that the average value of SOD in the group of white turmeric and vitamin C lower than the control group and MSG.

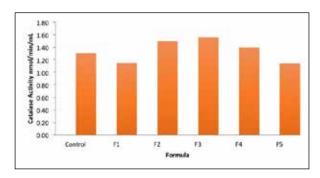


Fig 2. Catalase activity. F1, MSG; F2, Curcuma 100 mg+MSG; F3, Curcuma 200 mg+MSG; F4, Vit C 250 mg+MSG; and F5, Vit C 500 mg + MSG. Figure 2 showed that the average value of Catalase activity in the group of white turmeric and vitamin C higher than the control group and MSG.

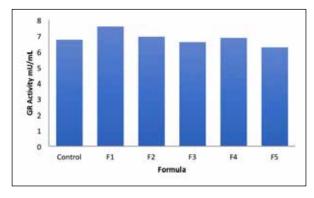


Fig 3. GR-activity. F1, MSG; F2, Curcuma 100 mg+MSG; F3, Curcuma 200 mg+MSG; F4, Vit C 250 mg+MSG; and F5, Vit C 500 mg + MSG. Figure 3 showed that the average value of GR-Activity in the group of white turmeric and vitamin C lower than MSG group.

# **Discussion and Conclusion**

The oxidative stress can cause damage to various organs. However, the body has of defense systems to reduce or protect the effect of free radical attacks namely antioxidants. The antioxidants are divided into two types, endogenous and exogenous antioxidants. Endogenous antioxidants include enzyme systems such as the enzyme Superoxide Dismutase (SOD) found in mitochondria and cytosols, Glutathione Peroxidase (GPx), Glutathione Reductase (GRx), and catalase (CAT). While exogenous antioxidants that can not be produced by the body and are found in fruits, vegetables, nuts, seeds and some meat. These foods contain β-carotene, vitamin C and vitamin E (4,9). Saefudin et al., 2014 explained that white turmeric (Curcuma zedoaria) extract contains essential oils, polysaccharides and substances that can have a function as antioxidants (6). Monosodium glutamat (MSG) could produce reactive oxigen spesies (ROS) as free radicals such as superoxide anion (O2 and will neutralized by endogenous antioxidants, such as SOD, GPx, GRx dan CAT. High doses of MSG will produce high concentration of ROS and finally will affect the work of antioxidants enzyme (1-6). To help work of endogenous antioxidant mechanisms, we need exogenous antioxidants. In our experiment, we used white tumeric and vitamin C as exogenous antioxidant.

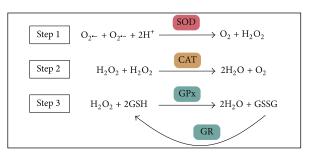


Fig 4. Main enzymatic antioxidant defense system in vivo. SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; and GR, glutathione reductase.<sup>10</sup>

There are two main antioxidant systems, namely enzymatic and non-enzymatic antioxidants, which act to capture and neutralize free radicals. The enzymatic antioxidant system consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Figure 4). This system is the main defense system against ROS exposure in vivo. SOD will catalyze the reaction to decompose superoxide anion radicals to H<sub>2</sub>O<sub>2</sub>, which will then be converted to water and oxygen by CAT

or GPx. CAT is one of the most efficient redox enzymes in catalyzing the conversion of H2O2 to water and oxygen. If not, H2O2 will be converted to hydroxyl radicals, one of the most active and dangerous radicals for living cells. GPx is an enzyme containing selenium, protecting cells and tissues from oxidative damage by removing H2O2 by oxidizing glutathione. On the other hand, GRx can transform oxidized glutathione into a reduced form. Meanwhile, the other is antioxidant non enzymes such as vitamin C, vitamin E etc (10).

Figure 1 showed the affect of tumeric as antioxidant on catalase activity in mice with MSG compared with vitamin C and non tumeric mice with MSG. High concentration of MSG administration in vivo would increased release of free radicals in the blood and would increased the SOD activity on group 1, and pre-treated mice with tumeric and vitamic C will ameliorate of MSG-mediated oxidative stress. SOD consists of two subunits that join disulfide bonds. SOD catalyzes the destruction of O<sub>2</sub> free radicals. Provision of white turmeric and vitamin C in groups of experimental animals after being treated with MSG will protect cells from damage caused by free radicals, helping SOD work in protecting cells from free radicals. This was proved by the result that the group of mice without white turmeric or vitamin C treatment but received MSG treatment showed an increase in SOD activity compared to groups 2-5.

The collaboration between antioxidant enzymes continues with catalase action. In figure 2 it appears that the catalase activity in the MSG group is lower than the other groups. This phenomenon may be due to the routine induction of MSG which will increase superoxide anion in high amounts. The existence of SOD will change the superoxide anion to H<sub>2</sub>O<sub>2</sub>. At a certain amount, catalase will be able to reduce H<sub>2</sub>O<sub>2</sub> to water. However, a high amount of H2O2 will reduce the ability of the catalase to work. This is in accordance with the basic mechanism of action of enzymes where the excess amount of substrate will reduce the speed of the ability of the enzyme to work. The presence of white turmeric and vitamin C will maintain the ability of the rate of catalase work in reducing H,O,.

Glutathione reductase is one of enzymes chains which serves to maintain glutathione in the reduced form. Glutathione plays an important role in maintaining proper function and preventing oxidative stress in cells. It can act as a hydroxyl radical scavenger, singlet oxygen, and various electrophiles. Reduced glutathione reduces

the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a dangerous reactive species in cells. In addition, it plays a key role in xenobiotic metabolism and cleansing, acts as a cofactor in certain detoxification enzymes, participates in transportation, and regenerates antioxidants such as Vitamins E and C to their reactive forms (9,10). Figure 3 shows the increase in GR's work in groups of mice treated only with MSG. This shows the amount of glutathione in the oxidized state (GSSG) which must be restored by the GRx to glutathione in a reduced state (GSH). The process of H<sub>2</sub>O<sub>2</sub> into water is not only catalyzed by catalase, but also by glutathione peroxidase (GPx), as a result, many by-products are GSSG (glutathione in the oxidized state) which must be restored to GSH form by the action of glutathione reductase (GRx). This formed GSH will later be used again to break down H<sub>2</sub>O<sub>2</sub> into water. The high amount of GSSG will cause an increase in GRx activity in the MSG mice group. This phenomenon can also explain why the catalase activity of the F1 group in Figure 2 is lower than in

other groups. High H2O2 makes the rate of action of the enzyme catalase decreases. H2O2 buildup will increase the work of the GPx enzyme and will produce GSSG in large quantities. To restore GSSG to GSH, it is necessary to work the enzyme GRx.

Statistical analysis using Kruskal Wallis for all experiments did not show a significant value. Although it was not statistically significant, the results of this study indicates similarities with other researchers regarding the effect of MSG in producing free radicals, and the effects of turmeric and vitamin C as antioxidants.

**Conclusion** The administration of white turmeric extract (*Curcuma zedoaria*) at doses of 100 and 200 mg/kg/day and vitamin C 250 mg/kg/day can act as antioxidants in suppressing the effects of MSG which can be a free radical producer.

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