Effect of Prunus Dulcis Extract against Total Cholesterol Level in Mice that Given Monosodium Glutamate

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Abstract. Monosodium glutamate (MSG) is chemical compound that when it is used excessively it can cause various side effects such as hypercholesterolemia. Hypercholesterolemia will result in coronary heart disease (CHD), which is a disease that can cause death. The aim of study was assessing the effect of almond nut / Prunus dulcis extract against total cholesterol level in mice that given MSG. This study was randomized post test controlled group design. The sample consisted of 30 mice (Mus musculus L. Strain DDW) that were divided into three groups: control group, treatment group 1 and treatment group 2. The plasma total cholesterol of mice was measured by the method of CHOD with spectrophotometer whose wave length 500nm. Data obtained was analysed by SPSS program version 16. The mean total cholesterol level in mice that treated with Prunus dulcis extract after 4 weeks experiment was 130.37mg/dl. It were highest compared to the other groups. One way anova showed the significance value was $p=0.44$, means, there was no difference of total cholesterol level between control and treatment groups 1 and 2. There was no effect of Prunus dulcis extract against total cholesterol level in mice that given monosodium glutamate.

1. Introduction
Monosodium glutamate (MSG) is a sodium salt of L-glutamic acid (Glu) also known as glutamate. Nowadays, it is often found in a daily meal with the various concentrations that serve as flavorings [1]. The using of MSG is effective because it can increase the taste of food without having to spend a lot of budget. In the public report to the Food Administration (FDA), 2% of all MSG user experience health problems, among the problem is increased of plasma total cholesterol level (hypercholesterolemia) [2]. Several studies have found that giving MSG to per oral wistar rats can increase total cholesterol level, very low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol when compared to the control group [3,4].

Hypercholesterolemia can cause atherosclerosis, cardiovascular system disorders as a risk factor for coronary disease (CHD) which is a disease that can cause death. In 2008, according to the World Health Organization (WHO), there were 17.3 million deaths worldwide caused by cardiovascular disease and 80% cause of death in low, middle and high economic country such as Indonesia [5].

Currently, many efforts are done to prevent above conditions by using herbal plant. One of them is almond nut / Prunus dulcis. Prunus dulcis are a kind of bean that has benefit in today’s medicine. The use of Prunus dulcis is increasingly widespread in the manufacture of cakes and chocolates, so many parties are trying to do research to assess the efficacy of Prunus dulcis in daily society. Prunus dulcis are rich of monounsaturated fatty acids/ MUFA. It is 30.89 g MUFA in 100g Prunus dulcis. A person who changes saturated fatty acids with unsaturated fatty acids in a daily diet, may decrease the risk of
cardiovascular disease. In addition, Prunus dulcis also contain vitamin E as much as 26.22mg / 100g which also serves as an important antioxidant in controlling free radical production in the body and niacin as much as 3.39mg / 100g that can reduce the production of VLDL cholesterol in the liver [6].

Based on the using of MSG that spread in the society as a flavoring and the factor of Prunus dulcis which can be used in the daily food both as a variation and the effect of Prunus dulcis that can cure various diseases, the researcher is interested to know the effect of Prunus dulcis extract against total cholesterol level in mice that given MSG.

2. Methods
The study was randomized post test controlled group design. The study was conducted after obtaining ethical clearance from the Ethics Committee of Faculty of Medicine, University of Sumatera No. 43 / KOMET / FK USU. The place of study was conducted in Integrated Laboratory, Faculty of Medicine, and Biology Laboratory of Faculty of Mathematics and Natural Sciences (FMIPA), University of Sumatera Utara (USU), Medan Indonesia. Thirty male mice (Mus musculus L. DDW strains) were obtained from the Laboratory of Biology, FMIPA, USU were used as research samples with criteria: 1). Aged 3-4 months; 2). Weight of mice t 20-35 grams; 3). Healthy condition which is active mice and no flaw. The sample is excluded from the study if 1). Weight of mice decreased to less weight than 20gram; 2). Mice infected diarrhea during the research.

2.1 Procedure
All samples of this study was male mice, were caged separately in the FMIPA, Biology Laboratory and maintained at room temperature 25 ± 5 ° C. Male mice were used because they can provide more stable research result because they are not affected by menstrual cycles and pregnancies like female mice. Moreover, male mice also have a faster metabolic rate and more stable biological conditions than female mice [7].

The process of adaptation (acclimatization) is done on the mice during 1 week before being treated. During 1 week, 30 mencit were given standard food and beverage, with 12 hours light and 12 hours dark cycle. After 1 week of adaptation, 30 mencit were divided into three group randomly [7]. The number of each group based on the sampling formula was 7 mice by taking into account the risk of drop out, then 3 mice were added in each group [8], is 10 mice normal group (K), 10 mice for treatment group 1 (P1) and 10 mice for treatment group 2 (P2). In group K mice were given 0.9% NaCl solution as much as 0.2 ml. In given P1 group, referred to previous research the dosage of given MSG in wistar rat was 4 g/KgBW – 4, then based on the conversion factor 0.14 from comparison table of body surface area of experimental animals given MSG solution as much as 5.6 mg/gBW orally [9]. Group P2 will be given a solution of MSG 5.6 mg/gBW weight and Prunus dulcis extract 3.25 mg/gBW divided into 3-4 times fed per day during 4 weeks. The dosage of Prunus dulcis extract in mice follow the conversion of Jamshed et al [10] study by taking the absolute dose in humans, was 10 g taking 0.0026 conversion factor from the comparison table of animal body surface area [9]. All groups were kept fed and drank with appropriate standard. After 4 weeks, the mice were determinate by neck dislocation, after anesthetized with ether. The blood of the mice was taken from the heart using 1 ml syringe

2.2 Plasma total cholesterol analysis
The procedure of examining total cholesterol of mice was performed in Integrated Laboratory, Faculty of Medicine, using CHOD-PAP cholesterol control kits. The instrument used is a spectrophotometer with a wavelength of 500 nm. Blood is taken from the heart of the mice that have been fasted first for 12 hours, as many as 1 ml were accommodated by using centrifuge tubes. Afterwards, the blood is centrifuged for 10 minutes at a rate of 3000 rpm. The reagent solution and blood plasma solution obtained was incubated for 20 minutes at a temperature of 20-25 ° C or 10 minutes at 37 ° C. The sample was read based on the comparison of standard and blank.

1. Mentions of institutions and contributors (if any) are included.
2. The text is properly formatted and aligned.
3. All necessary steps and conditions for the experiment are outlined clearly.
4. The methodology is described in a logical and coherent manner.
3. Results and discussion

Along with the study there were 8 mice that drop out, so that at the end of the study there were 22 mice with the total number of 8 mice in group K and each 7 mice in group P1 and P2. Table 1 showed the weight of mice in grams each week before the treatment started, so that 4 weeks after treatment. From this data we can see that the weight of mice in the control group (K) increased every week, until to the end of the study when the group of mice given MSG solution without Prunus dulcis decreased (P1). However, it happened weight loss in the MSG-given group and Prunus dulcis extract (P2) at 1 week after treatment subsequently increased again 3rd and 4th week study.

**Table 1. Body weight of mice before and after given MSG**

| Groups    | Weight of mice before given MSG (g) | Weight of mice after given MSG (g) |
|-----------|-------------------------------------|-----------------------------------|
| K(N=7)    | 25.76 ± 3.02                        | 27.43±3.18                        |
| P1(N=7)   | 29.08 ± 2.91                        | 28.91±2.78                        |
| P2(N=7)   | 28.56 ± 3.18                        | 25.26±2.43                        |
|           | Week 1                               | Week 2                            |
| K(N=7)    | 27.43±3.18                          | 28.58±3.23                        |
| P1(N=7)   | 28.91±2.78                          | 28.46±2.68                        |
| P2(N=7)   | 25.26±2.43                          | 25.85±2.72                        |
|           | Week 3                               | Week 4                            |
| K(N=7)    | 28.58±3.23                          | 29.27±4.88                        |
| P1(N=7)   | 27.58±3.63                          | 27.59±3.65                        |
| P2(N=7)   | 25.85±2.72                          | 27.50±3.65                        |

Using the SPSS statistical program version 16, based on the Shapiro-Wilk test (n <30), it was found that the distribution of weight data for each group of mice in each week (a week before treatment and after 4 weeks treatment) was homogeneous or normally distributed p> 0.05. One Way Anova test in each group, showed the significance value of no difference in body weight of mice with each p value in each week of 0.08; 0.05, 0.12; 0.62; 0.37 (p> 0.05)
In this study was not found association between body weight and givenMSG in the treatment group mice compared to control. These results were different from previous studies that given MSG will increase the body weight and even the risk of metabolic syndrome in the consumption [11]. The different results in this study may be caused by the treatment method of feeding the mice treatment group for the MSG giventhat can induce stress. The feeding process was not carried out in the control group. Standard feeding and drinking in control group given (and also in mice treatment groups) by letting food and drink in mice cage.

Stress that happened in the mice can stimulate the hypothalamus to produce the release of hormones such as corticotropin-releasing hormone (CRH) and growth hormone-releasing hormone (GHRH). Both of these hormones will stimulate adenohypophysis and release adrenocorticotropic hormone (ACTH) and human growth hormone (hGH). Furthermore, ACTH will stimulate the adrenal glands to produce cortisol. Cortisol caused fat breakdown in adipose tissue and increased levels of triglycerides and free fatty acids in the blood [12,13]. The results of in vitro experiment showed that cortisol increased lipoprotein lipase in adipose tissue and some part of visceral fat where lipolysis is activated. In the early stage condition that caused the breakdown of these adipose cells will lead to weight loss. Weight loss was apparent in the P1 group mice during the study week, but there was no in the P2 group.

In the P2 group mice, the mean of mice body weight was decreased in the first week after got treatment and increased again until the end of the study. In this group mice given MSG and also given extract Prunus dulcis. Prunus dulcis is rich with unsaturated fatty acid that has a role in reducing the risk of heart disease. Moreover, Prunus dulcis is also rich with vitamin E (alpha tocopherol) an antioxidant enzyme that can reduce the oxidative stress. Furthermore, Prunus dulcis also rich with thiamine, riboflavin, pantothenic acid, niacin and folate. These compounds have a role as enzyme cofactors in the level of cellular metabolism [14]. With the existence of the enzyme cofactor molecules guaranteed to the process of metabolism intracellular nutrients occur in balance without the effect of stress caused by feeding so that there is no weight loss happened in the P2 group mice.

The total cholesterol level of each group of mice can be seen in table 2. The mean total cholesterol level in the group of mice given by MSG solution and Prunus dulcis extract was the highest compared
to the other groups. Afterwards, followed by the control group having the second highest average. The mean total cholesterol level in mice given by the MSG solution was the lowest among the three groups.

| Groups | Cholesterol Level (mg/dl) | p* |
|--------|---------------------------|----|
| K (N=7) | 119.00 ± 61.85            | 0.449 |
| P1(N=7) | 97.46 ± 22.95             |     |
| P2(N=7) | 123.91 ±67.79             |     |

*one-way ANOVA

From the results of this study was found that the total cholesterol level of mice group that only given MSG (P1) is lower compared to another mice group. This result was different from the results of previous research studies [15,16]. MSG is a sodium salt of L-glutamate acid (Glu) made by the fermentation process of molasses by Brevibacterium lactoflavourum bacteria. From this fermentation was produced glutamic acid. Glutamic acid was then added soda (Sodium carbonate) until formed MSG. The plasma glutamate concentration will increase significantly if the daily MSG dose is 5g and only 4% of it will be eliminated from the body. This glutamate in the body will be converted to glutamine using the glutamine synthase enzyme in the mitochondria. One role of glutamine in the body other than gluconeogenesis and urea cycle is lipogenesis, which acted as a precursor in the process [17]. The process of lipogenesis is what causes the occurrence of hyperlipidemia including the increased cholesterol. A recent study was conducted the fact that MSG can increase of total cholesterol levels on mice only if given in high doses of 6g /KgBW and given by intraperitoneal injection for 68 days [18].

There was no relation between Prunus dulcis and total cholesterol levels in this study. This result was different from previous results by Berryman et al, and Holy et al [19,20]. This prior study was conducted to look at the effects of almond on human blood cholesterol without look at subject MSG consumption, and performed on healthy subject, with an almond intake of 50 to 75 grams per day for 6 weeks. Theoretically, Prunus dulcis are rich in monounsaturated fatty acids (MUFAs), as a good source of polyunsaturated fatty acids (PUFAs) and linoleic acids that play a role in controlling lipid profile in hypercholesterolemia subject. This is because MUFA and PUFA caused an increase in LDL receptors resulting in increased LDL catabolism and decreased cholesterol level in the body. However, the mechanisms are clearly still unknown [21,22].

Total cholesterol is combination of HDL cholesterol, LDL cholesterol and triglycerides. Previous study showed dietary Prunus dulcis increase serum HDL cholesterol [10]. In this study, the HDL cholesterol, LDL cholesterol and triglycerides are not measured. Increased total cholesterol in this study may due an increase of HDL cholesterol not LDL cholesterol, effect of Prunus dulcis

4. Conclusion
In this study, the MSG given in mice did not result in weight gain and hypercholesterolemia and there was no effect of Prunus dulcis extract against total cholesterol level in mice that given monosodium glutamate.

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