The effect of tiwai onion extract drink on the malondialdehyde levels in mice (*Mus musculus* L.)

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**Abstract.** Tiwai onion contains secondary metabolic compounds of phenol, alkaloid, terpenoid, and has antioxidant and antimicrobial bioactivity. The antioxidant of tiwai onion can be used for supplement drinks. The purpose of this study was to obtain the best treatment of tiwai onion extract drinks on malondialdehyde levels in mice (*Mus musculus* L.). This study used a completely randomized design with one factor consisting of 8 levels of treatments: 1) new oil (mb) as the control, used cooking oil (mj) as the control, sugarless tiwai onion extract drink (ebt) with the doses of 3) 2% ebt, 4) 4% ebt, and 5) 8% ebt, and tiwai onion extract with sugar (meb) with the doses of 6) 2% meb, 7) 4% meb, and 8) 8% meb. The data obtained were analyzed using the analysis of variance and Duncan’s Multiple Range Test. The malondialdehyde level in mice was a test parameter. The results showed that the best treatment was obtained at a 2% dose for each of sugarless tiwai onion extract drink and tiwai onion extract drink with sugar with an average of malondialdehyde levels in the mice of 26.380% and 20.215%, respectively.

1. **Introduction**

Nowadays, most humans tend to consume fast food but seldom to have some exercises and to consume foods containing a lot of polyunsaturated fatty acids (atherogenic). This can cause hypercholesterolemia indicating by the increase in low density of the lipoprotein levels or Low-Density Lipoprotein (LDL), triglycerides, and malondialdehyde. The final product of the lipid oxidation process is malondialdehyde, a membrane precursor, which plays a role in a process for the degradation of polyunsaturated fatty acids (arachidonic acid).

Malondialdehyde (MDA) was a lipid peroxidation biomarker that contributes to the oxidative damage to DNA, namely oxidative stress. High levels of free radicals can cause lipid damage [1]. There are two types of free radicals: hydroxyl and hydroperoxide. Hydroperoxide is the main product of lipid peroxidation including malondialdehyde (MDA), propanol, hexanal and 4-hydroxynonenal (4HNE). MDA is the most mutagenic lipid peroxidation product and included as a lipid peroxidation product [2]. DNA changes induced by MDA can cause cancer and other generative diseases. In other words, MDA in the body can cause cancer, diabetes, liver, cardiovascular, Parkinson's and Alzheimer's disease [3].

The use of cooking oil repeatedly can produce free radicals absorbed in the fried food. If eaten, the radicals will enter the body. Free radicals can damage lipids to form lipid peroxidation and to produce malondialdehyde (MDA). Nevertheless, antioxidants can reduce the malondialdehyde level in the blood
serum. Antioxidants in the form of supplements of vitamin C, vitamin E and multimineral, bioflavonoids, and tomatoes can significantly reduce the plasma MDA levels [4,5]. Several types of secondary metabolic compounds have been proven to have antioxidant properties, such as lycopene, polyphenols, flavonoids, chlorophyll, and some vitamins C and E. Tiwai onion \((Eleutherine americana\) Merr.) is a plant that has some secondary metabolic properties i.e. alkaloids, polyphenol, and flavonoids that are useful as the antioxidant. Based on these facts, this current study is carried out to investigate the effect of tiwai onion extract drinks on the malondialdehyde levels in mice \((Mus musculus\) L.)

2. Materials and methods

2.1. Materials
The materials used in this study included mice \((Mus musculus\) L.), some tubers of tiwai onion \((Eleutherine americana\) Merr.), white sugar, water, filter cloth, cooking oil, fried oil (used), filter paper, styrofoam, aluminium sheets, alcohol, anesthetics, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA) Hydrochlorid Acid, Tetraetoksipropan (TEP).

2.2. Equipment
The required equipment consisted of a blender, gas stove, digital scale, and other cooking utensils, syringes, oral syringes, Sensi-branded gloves, vacutainer, tourniquet, cooler box, centrifuge, micropipettes, microtubes, hot plate magnetic stirrers, and spectrophotometers.

2.3. Methods

2.3.1. The making of tiwai onion extract. The making of tiwai onion extract was carried out in the laboratory of Samarinda Research and Industry Standardization Center and made by the following process. First, 500 grams of clean tiwai onion was put in a blender then blended with 1 liter of water until the form was mush. Then the tiwai onion mush was heated at a temperature of 50°C for 10 minutes. After that, the mush was filtered using a filter cloth so that the extract of tiwai onion was obtained. Next, the 400 grams of onion extract filtrate was placed on the stove and cooked with a small flame. The 600 grams of white sugar was added while stirring the extract until completely dissolved and the heat reached a temperature of 50°C for 10 minutes. The sugarless tiwai onion extract filtrate and the tiwai onion extract filtrate with sugar were the ingredients of the research.

2.3.2. Experimental design. The experimental design in this study applied a completely randomized design with one factor consisting of 8 eight levels of treatments. Level 1 used new cooking oil (labelled mb) while used cooking oil (labelled mj) was for Level 2. The next levels of treatments used sugarless tiwai onion extract drinks (labelled ebt) with the concentrations of 2% (Level 3), 4% (Level 4), and 8% (Level 5). The other levels of treatments used tiwai onion extract with sugar (labelled meb) with the concentrations of 2% (Level 6); 4% (Level 7), and 8% (Level 8).

Each treatment level was conducted as follows. First, in each treatment 3 groups or cages were made so that there were a total of 24 groups or cages of mice for all the treatments. Each group or cage consisted of 6 mice so that there were a total of 144 mice. Second, all the treatment ingredients were administered to the mice as the experimental animals orally (in vivo) by giving them 0.5 ml/20 g body weight (bw) of mice. Third, the treatment ingredients were given by the following doses: 1) \(mb =\) new cooking oil of 0.5 ml/20 g bw; 2) \(mj =\) used cooking of 0.5 ml/20 g bw; 3) \(mj + ebt = 0.5\) of used cooking oil and 0.5 ml of ebt 2%/20 g bw (c1); 4) \(mj + ebt = 0.5\) ml of used cooking oil and 0.5 ml of ebt 4%/20 g bw (c2); 5) \(mj + ebt = 0.5\) ml of used cooking oil and 0.5 ml of ebt 8%/20 g bw (c3); 6) \(mj + meb = 0.5\) ml of used cooking oil and 0.5 ml of
meb 2%/20 g bw (d1); mj + meb = 0.5 ml of used cooking oil and 0.5 ml of meb 4%/20 g bw (d2); and 8) j + meb = 0.5 ml of used cooking oil and 0.5 ml of meb 8%/20 g bw (d3).

2.3.3. In vivo test. The mice were acclimated for one week after being grouped in each group/cage. They were given a BR-branded pellets feed and clean water for drinking. They were placed at the Pharmacology Laboratory of the Faculty of Medicine, Mulawarman University. The treatments were given every day in accordance with the predetermined doses for three weeks. The observation was also carried out during the 3 weeks. The data taken from the observation were analyzed using one way ANOVA to see the comparison of the malondialdehyde levels in the blood serum of mice from each group of cooking oil (mb), used cooking oil (mj), sugarless tiwai onion extract (c-c2-c3), and tiwai onion extract with sugar (d1, d2, d3).

2.3.4. Data analysis. The observation data were analyzed using one way ANOVA to determine the comparison of the malondialdehyde levels in the mice blood serum from each group of cooking oil (mb), used cooking oil (mj), onion extract without sugar (2% ebt, 4% ebt, 8% ebt), and tiwai onion extract with sugar (2% meb, 4% meb, and 8% meb).

3.  Results and Discussion
The results of this study was to determine the malondialdehyde (MDA) levels in the mice (Mus musculus L.) blood serum. Malondialdehyde (MDA) is the compound resulted from lipid peroxidation which is generally used as the indicator of oxidative stress. The analysis results of the MDA levels in the mice blood serum from the treatments using tiwai onion extract drinks are presented in table 1.

| Treatment                        | Week/average value |
|----------------------------------|--------------------|
|                                 | I      | II      | III     |
| cooking oil(mb)                 | 10.727 | 20.847  | 25.767  |
| used cooking oil(mj)            | 40.021 | 54.072  | 60.767  |
| Tiwai onion extract (ebt)       |        |         |         |
| mj-ebt 2%                       | 29.066 | 23.453  | 26.380  |
| mj-ebt 4%                       | 25.606 | 27.362  | 35.276  |
| mj-ebt 8%                       | 27.606 | 27.362  | 35.273  |
| Tiwai onion extract drink       |        |         |         |
| mj-meb 2%                       | 19.377 | 18.893  | 20.215  |
| mj-meb 4%                       | 14.533 | 17.264  | 25.767  |
| mj-meb 8%                       | 16.609 | 26.384  | 34.356  |

Table 1 shows the malondialdehyde (MDA) levels in the blood serum of the mice that got new and used cooking oil increased until the third week. The malondialdehyde levels in the blood serum of the mice that got used cooking oil were higher with a range of 40.021 to 60.767 compared to that of the mice given new oils with a range of 10.727 to 25.767. This indicates that the lipid peroxidation or MDA continued to increase in the mice blood serum after being given the new oil (mb) and the used cooking oil (mj). The high peroxidation values also indicate that oil has been oxidized that was marked with rancid taste and smell [6,7,8]. Fat or oil at high temperatures (200-250°C) will become toxic in the body and causes various diseases. Peroxide levels increase due to oxidized cooking oil after being heated at 200-250°C [9,10].

The malondialdehyde (MDA) levels in the blood serum of the mice after getting sugarless tiwai onion extract drink (mj + ebt) and tiwai onion extract drink with sugar (mj + meb) in Week I of observation
decreased with a range values of 25.606 to 29.066 and 14.533 to 19.377, respectively. These values were much lower compared to the MDA levels in the blood serum of the mice that got used cooking oil (mj) with a value of 40.021. Similarly, MDA levels in the mice blood serum decreased in Week II in the range of values of 23.453 to 27.362 for mj + ebt and 17.264 to 26.384 for mj + meb when compared to that of used cooking oil (mj). Also, there was a decrease in the MDA levels of the blood serum in Week III with a range of 26.380 to 35.276 from the mice with mj + ebt and 20.215 to 34.356 from the mice with mj + meb, compared to that of the mice with used cooking oil (mj).

Lipid peroxidation can damage the cells in the membrane which is caused by the increased ROS production. Henderomartono [11] showed that an increase in ROS in the cell membrane could increase the formation of MDA. The product from lipid oxidation caused by free radicals in the body is malondialdehyde (MDA) [12]. MDA is an indicator to determine the oxidative stress in the body and as a biomarker of oxidative stress. ROS production that exceeds the cell’s antioxidant capacity can cause an increase in the oxidative stress along with the occurrence of lipid peroxidation in cell membranes. Therefore, the increased MDA due to lipid peroxidation in oxidative stress is in line with the lipid peroxidation in cell membranes. Thus, the increased MDA is the result of lipid peroxidation [13,14]. These results prove that the malondialdehyde levels in the mice blood serum decrease by giving tiwai onion extract. It shows the active role of tiwai onion extract as an antioxidant in preventing lipid peroxidation in cell membranes.

Some research has been done to investigate about tiwai onion and its ability to reduce lipid peroxidation. For instance, H.S. and Sampepana [15] reported that water and methanol extracts of tiwai onion contain secondary and primary metabolic aldehydes-ketones, phenols, glycosides, tannins, flavonoids and carboxylic acids, carbohydrates, and proteins. Tiwai onion also contains flavonoids, alkaloids, tannins, sesteroids, and triterpenoids [16], and water and methanol extracts of tiwai onion contain antioxidant activity [17]. Besides, Saputra [18] believes that tiwai onion extract as an antioxidant can inhibit the speed of the occurrence of lipid peroxidation in cooking oil from coconut until the shelf life of 3 months which still meets the requirements of SNI: 3741: 1995. The quality standard [19] showed that the cooking oil is maximum 2 mg/kg. Further, Saputra [20] confirms that water and ethanol extracts have antioxidant activity, free radical scavenging capacity, good reducing power, and reactive oxygen species. Percent of antioxidant activity of tiwai onion syrup, when stored for 2 days, is 64.47% [21]. Another study reveals that ethanol extract of tiwai onion has antioxidant activity as much as IC50 25,339 µg/ml [22]. Indeed, tiwai onion has several benefits as an herbal remedy for breast cancer, hypertension, diabetes, cholesterol, stroke, and anti-acne [23]. Winarsi [24] also found that the treatment of vitamin E decreases MDA level in the blood serum. Primary antioxidants, mostly phenolic compounds, are a group of compounds that stop the formation of free radicals in lipid oxidation including tocopherol, buthylated hydroxytoluene (BHT), buthylated hydroxyanisole (BHA), and tertiary-buthyl hydroquinone (TBHQ) [25]. Some natural compounds that have antioxidant effects are phenols, polyphenols and flavonoids, consisting of flavonols, isoflavones, flavones, catechins, and flavonoids as well as polyfunctional organic acids. Phenolic compounds are bioactive components that are widely found in plants [26]. Tiwai onion (E.americana Merr.) syrup has phenolic compounds, flavonoids, and antioxidant activity [21]. It is estimated that the decrease in MDA levels in blood serum is due to the primary antioxidant effect which acts as a one-atom free radical scavenger and, together with the SOD enzyme, is able to minimize and prevent lipid peroxidation reactions so that the formation of malondialdehyde products can be suppressed. The provision of vitamins can reduce MDA levels in the blood serum of the white mice with diabetes mellitus. If MDA in the body is formed as a result of the oxidative stress due to an imbalance between the formation of ROS and antioxidants, free radical level will be higher [27].

High MDA level can cause high level of damage to cell membranes and lipoproteins. According to a study by Makaryani et al. [28], consuming green grass jelly can reduce the MDA levels in the students of
IPB who consumed fried food. Last, it has been revealed that garlic extract can reduce MDA levels in the objects after getting exposure to cigarette smoke [29].

4. Conclusion
The results of this study revealed that both of tiwai onion extract drinks with and without sugar could reduce the malondialdehyde (MDA) levels in the mice blood serum. The best dosage for reducing MDA levels up to the third week was the 2% for each of sugarless tiwai onion extract drink and tiwai onion extract drink with sugar.

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References
[1] Wahyuni S. 2012 Malondialdehyde Monograph Precursor to Oxidative Stress (Bali: Udayana University Press)
[2] Esterbauer H, Eckl P and Ortner A 1990 Possible mutagens derived from lipids and lipid precursors Mutation Research 238(3) 223–33
[3] Ayala A, Munos M F and Aguuelles 2014 Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal Hindawi Publishing Corporation Oxidative medicine and cellular longevity 360438 1-31
[4] Ramatina, Amalia L, Ekayanti I 2014 Effect of antioxidant supplements on malondialdehyde plasma level among college students of IPB Journal Nutrition and Food 9(1) 35-42.
[5] Wahyuni, Asj’ari S R, Sadewa A 2008 Study of the ability of tomato juice to inhibit increased levels of plaque malondialdehyde after high impact aerobic exercise Journal Health 1(2) 123-32.
[6] Ketaren S 1986 Introduction to Food Oil and Fat Technology (Jakarta:UI Press)
[7] Siti N W, Tri Dewanti W, Kuntanti 2001 A study of the level of damage and food safety of used cooking oil (a study of the different types of cooking oil and fried foodstuffs) Research Report (Malang: Faculty of Agricultural Technology Brawijaya university)
[8] Nawar W W 1985 Lipids Food Chemistry 2nded, ed O R Fennema (New York, Basel: Marcel Dekker Inc.)
[9] Nurhayati H, Supriningrum R and Caesariana N 2015 Determination of levels of free fatty acids and peroxide numbers in cooking oil used by fried food traders on the street A.W. Sjahranie samarinda. Journal Scientific Manuntung 1(1) 25-30
[10] Suroso A S 2013 Studies of consumable cooking oil in terms of peroxide numbers, acid numbers and water content Journal Indonesia Pharmacy 3(2) 77-88
[11] Henderomartono 2000 The world health report 2000 World Health Organization 1-10
[12] Jeyabalan A and Caritis S N 2006 Antioxidant the prevention of pre-eclampsia unresolved issues. The England Journal of Medicine 354(17) 1841-3
[13] Maslachah L, Sugihartuti R and Kurniasanti R 2008 Inhibition of production of reactive oxygen species superoxide radicals (O2-) by antioxidant vitamin E (-tocopherol) in white rats (Rattus norvegicus) receiving electric shock stressors Veterinary Media 24(1) 21-6
[14] Soviana E, Rachmawati B and Nyoman W 2014 The effect of β-carotene supplement on blood glucose levels and malondialdehyde levels in Streptozotocin-induced Spague Dawley rats. Indonesian Nutrition Journal 2(20) 41-6
[15] Saputra H S and Sampepana E 2007 Chemical content analysis and utilization of tiwai onion for industrial raw materials *Journal Industrial Technology Research* **1**(1) 25-33

[16] Sukrasno, Umemi Sadaruddin, 2006. Microopagation traditional medicine research and the development of tiwai onion/sabrang onion (*Eleutherine americana* L.) as an herbal medicine. ITB. Bandung.

[17] Saputra S H 2007 Bioactive analysis and utilization of tiwai onions (*Eleutherine americana* Merr.) For food additives *Journal of Industry Technology Research* **1**(2) 24-30.

[18] Saputra S H 2012 Tiwai onions (*Eleutherine americana* Merr.) As a preservative, antioxidant and food coloring *Journal of Industrial Technology Research* **6**(12) 102-10

[19] National Standardization Agency 1995 SNI. 375-1995 Cooking oil quality standards

[20] Saputra S H 2010 Tiwai onion extract as an antioxidant in coconut oil *Journal Industrial Technology Research* **4**(8) 14-9

[21] Saputra S H, Sampepana E and Susanty A 2018 The effect of bottle, temperature and circular storage packaging of flash drink tiwai extracts (*Eleutherine americana* Merr) on secondary metabolic and microba *Journal Industrial Technology Research* **12**(2) 156-65

[22] Kuntorini E M and Astuti M D 2010 Determination of the antioxidant activity of Dayak bulb (*eleutherine Americana* Merr.) ethanol extract *Science and Applied Chemistry* **4**(1) 15-22

[23] Galingging RY 2009 Dayak onion (*Eleutherine palmifolia* L. Merr) as a multifungsional medicinal plant. Research and development news *Agricultural research and development agency* **15** 10-6

[24] Winarsi H 2005 Effect of soybean sprout protein extract supplementation on levels of IL-1 beta of type 2 diabetics *Journal Technology and Food Industry* **21**(1) 6-10

[25] Kochhar S P and Rossel, J B 1990 Detection, estimation and evaluation of antioxidant in food sytems *Food Antioxidant* 19-64

[26] Pratt D E and B J F Hudson 1990 Natural antioksidant not exploited commercialy Food Antioksidant ed B J F Hudson (New York:Elsevier Applied Science) pp 171-92

[27] Vera B, Al Azhar D, Karmi T F, Rindy G and Sabri M 2018 The effect of vitamin E to malondialdehyde (MDA) serum level in diabetes mellitus induced white rat (*Rattus norvegicus*) *JIMVET* **2**(1) 70-6

[28] Makaryani I, Amalia L, Ramadhani N R, Pertiwi KI, Aprillia D D 2014 The effect of antioxidant feeding on plasma malondialdehyde levels of fried food students. *Journal Indonesia Nutrion Clinic* **10**(04)169-79

[29] Duwairoh A M, Wirajatmadi B, Adriani M 2018. The Effect of Solo Garlic Extract in Decreasing Malondialdehyde (MDA) Levels due to E-Cigarette Exposure *Journal Medical Scientific Wijaya Kasuma* **7**(2) 149-57