The effect of extract guava, bitter melon, broccoli, and shallots on liver damage due to indoor pollutant exposure

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Abstract. Air freshener is a chemical-based product commonly used to freshen the air in the room. However, it usually has a variety of harmful compounds, and if it enters the body it can trigger the formation of free radicals, which in turn can cause cell and tissue damage in vital organs such as liver. One way that can be used to treat cell damage due to free radicals is by consuming foods that contain antioxidants. In this study, mice were used to determine the effectiveness of the antioxidant extracts of guava, bitter melon, broccoli, and shallots. Types of free radicals and levels of liver cells damage of mice due to free radicals were analyzed using quantitative atomic spectroscopy and levels assay of malondialdehyde (MDA). The results showed that the effect of the antioxidants mentioned above could reduce the number of free radicals and cell damage that occurred in the liver of mice exposed to air freshener, and the optimal dose of antioxidants was obtained at 69.5 mg per 20 grams of mice.

1. Introduction
Clean air is the most important requirement for living things, especially humans. Along with the times, pollution is increasing, so need for fresh air is no longer offset by clean air quality [1]. Air pollution can be distinguished based on its place, namely outdoor air pollution and indoor air pollution. Air pollution that is classified as indoors does not only occur in a factory or industrial site, but air pollution can occur in a community's residence. One household product that produces pollution is air freshener, both in liquid and gel form. Chemicals contained in air freshener such as phthalates, acetaldehyde, toluene, chlorbenzene, paradichlorobenzene, formaldehyde, benzyl acetate, benzyl alcohol, ethanol, limonene, linalool, each of these compounds are toxic and when entering the body can trigger emergence of free radicals [2]. Free radicals are atoms, molecules, or compounds that have lost one electron from their lone pair. They are unstable and very reactive which can create oxidative stress in the body [3].

Liver is the largest organ of the body located in abdominal cavity below diaphragm. Most of the blood (about 70%) comes from the portal vein supplied by the hepatic artery to the liver to do important work in the body such as carrying out functions of the endocrine system, exocrine system, synthesizing protein, storing useful metabolic waste, detoxification, transfer of minerals, up to buffering blood glucose. Function of the liver is vital for the body's metabolism, so that if the content of harmful chemical compounds in the air freshener is inhaled or enters body through the blood, it will cause damage to liver cells [4]. Antioxidants are biologically defined as compounds that can delay, slow down and prevent lipid oxidation reactions, while chemically meaning antioxidants are compounds that can give or donate electrons. The way antioxidants work is by donating one electron to an oxidant compound so that the activity of the oxidant compound can be stopped [5]. Sources of antioxidants can be obtained from types
of plant foods or also called phytonutrients which contain many compounds of vitamin C, vitamin E, and flavonoids. Of the various types of plant foods, guava, bitter melon, broccoli, and shallots are foods that contain the most complete antioxidants compared to others [6].

2. Material and Method

2.1. Preparation of mice and antioxidants

This research was divided into several stages starting from preparation of materials and grouping the mice. There were 35 mice divided into 5 mice for control group (K1), 5 mice for group who were exposed to the air freshener without antioxidants (K2), and 5 mice for each group that were treated with variations in antioxidant doses and were exposed to air freshener (K3). Doses of antioxidants given in each treatment group is explained in Table 1

|   |   |
|---|---|
| K1 | Without exposure to air freshener and without giving antioxidants |
| K2 | The air freshener is exposed and without antioxidants |
|   | 1. Exposed 9 sprays of air freshener and given 51 mg of antioxidants |
|   | 2. Exposed 9 sprays of air freshener and given 69.5 mg of antioxidants |
| K3 | 3. Exposed 9 sprays of air freshener and given 88 mg of antioxidants |
|   | 4. Exposed 9 sprays of air freshener and given 106.5 mg of antioxidants |
|   | 5. Exposed 9 sprays of air freshener and given 125 mg of antioxidants |

Antioxidants in form of extract guava, bitter melon, broccoli, and shallots weighed doses using digital scale. After finishing weighing antioxidants are mixed and dissolved with distilled water. Solution that has been made, then given to mice using gastric sonde with volume of 0.3 ml, as shown in Figure 1.

2.2. Exposure and surgery of sample

Process of exposing air freshener was carried out after acclimatization to mice for seven days on chamber. Exposure of mice is done once a day with a given doses of exposure is 9 spray and carried out for 21 days. This exposure is done by inserting air freshener contents into chamber containing mice through a small hole in chamber. Exposure time is 20 minutes. After 20 minutes chamber cover was opened and mice were given a mixture of antioxidants extract guava, bitter melon, broccoli, and shallots using gastric sonde.

Mice that have been treated for 21 days, then dissected to take the liver. Separation of mice liver from body must be careful to avoid tearing of blood vessels or lysis. Mice liver that have been obtained then cleaned with PBS solution until clean from the blood. One of the measurement instruments used in this study is Electron Spin Resonance (ESR) as a free radical detector with type Leybold-Heraeus that works at frequency 13 MHz - 130 MHz with maximum current 2 mA. Measurement and detection of free radicals is done by adjusting frequency (f) and current (I), so resonance occurs from two waves, namely radio frequency waves and deactivation waves due to presence of a magnetic field outside Helmholtz coil. Then magnetic field (B) and Lande factor (g) will be obtained. Value of magnetic field can be determined by equation 1,
\[ B = \mu_0 \left( \frac{4}{\pi} \right)^{\frac{1}{2}} \frac{n}{r} I \]  

(1)

Where \( \mu_0 \) is permeability of free space \((1.257 \times 10^{-6} \text{ H/m})\); \( n \) is number of Helmholtz coil windings \((n = 320)\); \( r \) is radius of Helmholtz coil \((r = 6.8 \text{ cm})\); and \( I \) is current on Helmholtz coils \((A)\). Then, magnitude of magnetic field \((B)\) can be used to find value of the Lande factor \((g)\) with equation 2,

\[ g = \frac{hf}{\mu_0 B} \]  

(2)

Where \( \mu_B \) is Bohr magneton \((9.274 \times 10^{-24} \text{ Am}^2)\); \( h \) is Plank constant \((6.625 \times 10^{-34} \text{ Ws}^2)\); \( f \) is resonant frequency \((\text{Hz})\); and \( B \) is magnetic field \((\text{T})\).

Second test is analysis of MDA levels in liver cells. Malondialdehyde (MDA) is one indicator that can be used to describe activity of free radicals in cells, so that it becomes a clue to know oxidative stress due to free radicals. Liver sample is cut and weighed by 100 mg then put into a plastic clip. After that sample is put into a cold mortar and crushed until smooth. After sample smooth is carried out centrifugation and homogenation. Finally, sample is heated and the absorbance value is measured. Measurement of MDA concentration was carried out using a spectrophotometer with an excitation wavelength of 515 nm and emissions of 553 nm.

3. Result And Discussion

In this study, sample used was liver of mice aged 2-3 months. Samples were divided into seven treatments to be tested, namely control samples, samples that only received exposure to air freshener, and samples that received exposure of air freshener with given five doses variation of antioxidants. Table 2 shows that test of control samples using ESR did not show presence of free radical content, although it has been tracked at various frequencies. Whereas test of samples that were exposed to air freshener only, and samples with given five doses variation of antioxidants shown free radical content of superoxide anion type or \( O_2^- \). Superoxide anion free radicals belong to the Reactive Oxygen Species (ROS) group. Compounds contained in air freshener certainly do not contain free radicals, but when entered and interact in body will cause a variety of chain events that trigger occurrence of respiration chain disruption resulting in \( O_2 \) reduction becomes \( O_2^- \) [7].

Free radicals identified in mice liver are type \( O_2^- \). \( O_2^- \) free radicals will be very dangerous if present together with hydrogen peroxide, because they will produce hydroxyl radicals. These radicals can be suppressed by giving antioxidants guava, bitter melon, broccoli, and shallots. This damping reaction can occur due to presence of electron donors and two hydrogen atoms, so that hydrogen peroxide and radical antioxidants are produced. Then \( H_2O_2 \) will react again with antioxidants that form \( H_2O \), hydroxyl radicals \((OH^-)\), antioxidant radicals caused by electron donors, and one hydrogen atom. Hydroxyl radical \((OH^-)\) becomes stable when it receives an electron donor and one hydrogen atom, resulting \( H_2O \) and antioxidant radicals [5].

| Treatment | Frequency (Hz) | I (A) | Factor-g | Factor-g [8] | Radical type |
|-----------|----------------|------|----------|--------------|-------------|
| Control   | -              | 0,199 | -        | 1,501-1,75   | -           |
| AA        | 18700000      | 0,199 | 1,59     | 1,501-1,75   | O_2^-       |
| AO 1      | 18800000      | 0,199 | 1,59     | 1,501-1,75   | O_2^-       |
| AO 2      | 18900000      | 0,199 | 1,60     | 1,501-1,75   | O_2^-       |
| AO 3      | 18600000      | 0,199 | 1,58     | 1,501-1,75   | O_2^-       |
| AO 4      | 20000000      | 0,199 | 1,70     | 1,501-1,75   | O_2^-       |
| AO 5      | 19300000      | 0,199 | 1,64     | 1,501-1,75   | O_2^-       |
Figure 2. Vitamin E reaction with $O_2^-$.  

Figure 3. Vitamin C reaction with $O_2^-$.  

Figure 4. Carotenoids reaction with $O_2^-$.  

Figure 5. Flavonoids reaction with $O_2^-$.  

Vitamin E from the antioxidant mixture will reduce and capture free radicals. Tocopherol isomer in vitamin E is an antioxidant that breaks bonds. Antioxidative activity of tocopherol donates electrons to free radicals, so that free radicals become stable [7]. Radical $O_2^-$ can be suppressed by antioxidants derived from guava, bitter melon, broccoli, and shallots extracts because they contain high vitamin E active compounds. Reaction to reduce free radicals of type $O_2^-$ by vitamin E can be shown in Figure 2. From the mixture of guava, bitter melon, broccoli, and shallots also found vitamin C for lipid and protein antioxidants. Vitamin C, also called ascorbic acid, is a carbon-carbon compound which can dissolve in water. Vitamin C compounds will react with oxygen, so there will be no reaction between oxygen and lipids. This process will prevent the formation of lipid hydroperoxide. According to Böhm (2012), superoxide anion is a type of oxygen radical that can be reduced by vitamin C with a reducing reaction which can be seen in Figure 3.

Carotenoids from mixture of guava, bitter melon, broccoli, and shallots can reduce type free radical singlet oxygen and peroxide radicals. Energy of oxygen singlet molecule will move to carotenoid molecule, then obtained ground state of oxygen and carotenoid excitation triplet [9]. Double bond in hexane structure of beta carotene compound will tend to release its electron pair so that it will interact with free electrons in free radical $O_2^-$. The release of these electrons will make free radical $O_2^-$ turn into $O_2$ and stick to beta carotene group [6]. Figure 4 shows the interaction of beta carotene with free radicals of type $O_2^-$. Flavonoids are polyphenol compounds in plants that contain 15 carbon atoms in their basic nuclei. Flavonoids also have two solubility properties, polar and non-polar. Flavonoids have ability as an antioxidant because they are able to donate an electron to free radical compound [5]. Flavonoids are one of ingredients found in antioxidants of guava, bitter melon, broccoli, and shallots. Structure of
flavonoids which have several hydrogen atoms can reduce amount of free radical $O_2^-$ [10]. Reaction of free radical scavenging type $O_2^-$ with flavonoids can be shown in Figure 5.

After knowing type of free radicals in liver of mice, then magnitude of amplitude of resonance curve was calculated for each treatment from control samples, samples that only received exposure to air freshener, and samples that received exposure of air freshener with given five doses variation of antioxidants. So we get the results as shown in Figure 6.

![Antioxidant Doses and Squared Amplitude of ESR Curve Resonance](image1)

![Antioxidant Doses and Malondialdehyde Levels](image2)

**Figure 6.** Graphs of relationship between antioxidant doses and squared amplitude of ESR curve resonance.

**Figure 7.** Graph of relationship between antioxidant doses and MDA levels.

The graph in Figure 6 explains squared amplitude of ESR curve resonance for control treatment is almost invisible or equal to zero. This value is constant in control treatment because mice did not receive air freshener or antioxidant. The highest value is shown by air freshener treatment only and changes when starting to be added antioxidant. Most significant decrease occurred in second doses. This proves addition of antioxidants affect intensity of free radicals in mice liver that exposed to air freshener, because guava, bitter melon, broccoli, and shallots contain active compounds such as beta carotene, flavonoid, vitamin C, and vitamin E. From second doses analysis is also explained that even most optimal doses of antioxidants still cannot cure and suppress liver damage due to free radicals caused by air freshener to zero or equal to control. Whereas for third to fifth doses, intensity of free radicals in sample increases again, explaining that antioxidant already overdose and become toxic.

Malondialdehyde (MDA) is a compound produced by body when interacting with free radicals, therefore MDA can be used as an indicator presence of free radicals in body [11]. Based on this research, relationship between antioxidant treatment and MDA levels is shown in Figure 7. Graph in Figure 7 explains that for control treatment lowest MDA level is $70 \pm 10 \text{ ng} / \text{mL}$, then for air freshener treatment but does not receive antioxidants, highest value is $510 \pm 20 \text{ ng} / \text{mL}$. In treatment of mice that received first and second doses showed a significant decrease in MDA levels, but for treatment of third doses to five, MDA values instead increased again. Increased levels of MDA that occur indicate amount of free radical content in mice liver increases again. This is due to antioxidants from guava, bitter melon, broccoli, and shallots that enter body too much and re-formed electron chain is out of balance. Excess number of electrons causes re-formation of free radicals. MDA test results obtained were highly correlated and appropriate with squared amplitude of ESR curve resonance.

**4. Conclusion**

Treatment of antioxidants of guava, bitter melon, broccoli, and shallots that is right and optimal can reduce number of free radicals and liver damage due to air freshener exposure. The reduction in liver cell damage is correspond with decreased free radical intensity as shown by the ESR curve and MDA levels. Damage healing could not be 100% like a healthy mice liver, but the effect was seen to be quite
significant in reducing the damage. If the types of antioxidants extract of guava, bitter melon, broccoli, and shallots are given incorrectly and overdosed on, the antioxidants will not heal but become toxic.

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