Effects of ethanolic extract of arrowroot tubers (maranta arundinacea l.) on the level of MDA, SGPT and SGOT in ethanol induced rats

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ORIGINAL ARTICLE

ABSTRACT

**Background:** The exposure of free radicals can induce oxidative stress. In the liver, this process will cause impaired liver function. Oxidative stress can be inhibited by antioxidants. Arrowroot tubers (Maranta arundinacea) contains phenolic, flavonoid, alkaloids and saponin compound that are potential as antioxidants.

**Objective:** Determine the effect of ethanolic extract of arrowroot tubers (Maranta arundinacea) against oxidative stress using the parameter of MDA, SGPT and SGOT level in ethanol-induced rats.

**Methods:** Animal models were divided into 5 groups, in which each group contained 6 rats. Group I (normal) and group II (control) was induced with CMC Na 0,5%. Group III, IV and V were given ethanolic extract of arrowroot tubers in the dosage of 125, 250 and 500 mg/Kg/day respectively. The extract was administered orally for 14 days. Induction of ethanol 5 gram/KgBW was administered orally 1 hour after the last administration on day 14th except for group I. On day 15th, the animal blood was drawn to measure the levels of SGPT and SGOT, then the animals were sacrificed and their organs were analyzed to measure the levels of MDA in the liver. Data obtained in the form of MDA, SGPT and SGOT levels were statistically analyzed using ANOVA.

**Results:** There is statistically significant difference between the utilization of ethanolic extract of arrowroot tubers (Maranta arundinacea) group with the control group in reducing the concentration of MDA (p<0,05), SGPT, and SGOT (p<0,05).

**Conclusions:** The ethanolic extract of arrowroot tubers (Maranta arundinacea) is able to reduce the concentration of MDA, SGPT and SGOT in ethanol-induced rats.

**Latar Belakang:** Paparan radikal bebas akan menginduksi stress oksidatif. Pada organ hepar proses ini akan mengakibatan terjadinya gangguan fungsi hati. Umbi garut mengandung senyawa fenol, flavonoid, alkaloid dan saponin yang berkhasiat sebagai antioksidan.

**Tujuan:** Untuk mengetahui efek ekstrak etanol umbi garut terhadap stress oksidatif melalui parameter kadar MDA, SGPT dan SGOT.

**Metode:** Hewan uji dibagi menjadi 5 kelompok, tiap kelompok terdiri dari 6 ekor tikus. Kelompok I (normal), kelompok II (kontrol) diberi CMC Na 0,5% + induksi etanol, kelompok III, IV dan V diberikan ekstrak etanol umbi garut dengan dosis masing-masing 125, 250 dan 500 mg/KgBB/hari. Ekstrak diberikan secara oral selama 14 hari. Induksi etanol 5 gram/KgBB diberikan secara oral 1 jam setelah pemberian terakhir pada...
INTRODUCTION

More than 240 billion people are suffering from chronic liver infections, approximately 600,000 deaths occurred every year due to acute or chronic hepatitis. Liver damage caused by infection, drugs or viruses would cause inflammation (hepatitis) or death of liver cells (necrosis), which would result in permanent damage of the liver cells. Chronic liver function disorder can be caused by oxidative stress. Exposure to free radicals in the body would cause lipid peroxidation, destruction of fat molecules (phospholipids) in the cell membranes, damage to DNA, and protein oxidation. Oxidative stress is a condition where the production of free radicals is higher than the amount of antioxidants in the body. Oxidative stress can be inhibited by antioxidants.

Arrowroot tubers (Maranta arundinacea L.) is an example of local tuber that is widely available in Indonesia, but is still very rarely used. Generally, arrowroot tubers is consumed only as a source of carbohydrate. Arrowroot tubers had been found to have antioxidant activity when tested in vitro. Thus, in vivo study about the potential of arrowroot tubers as hepatoprotective antioxidant is conducted. Arrowroot contains phenolic compounds. The examples of chemical compounds included in the class of phenolic compounds are: phenols, lignin, anthraquinone, flavonoids, tannins, and phenols propanoid. Research had shown that phenol could function as an antioxidant due to its ability to scavenge radicals in the form of DPPH, hydroxyl radicals, and superoxide radicals. In addition to phenol, arrowroot tubers also contains flavonoids, alkaloids, tannins and saponins. Flavonoids are known to have hepatoprotective activity. Hepatoprotector is a type of drug which compounds are able to protect the liver from any damages caused by toxins, drugs, and any other possible causes.

Arrowroot tubers is also able to inhibit free radicals such as H₂O₂ and NO, because it contains polyphenolic compound, such as flavonoids. Alcohol (ethanol) is used as a model inducer for liver damage. Alcohol can increase the production of free radicals in the microsomal cytochrome P450. Free radicals can increase oxidative stress that would cause damage to the liver cells. Free radicals could cause lipid peroxidation in poly unsaturated fatty acid (PUFA), which is a component of cell membranes, thus causing the production of malondialdehyde (MDA) as its end product. MDA is a good indicator to be used in measuring the velocity of lipid peroxidation. Liver cell damage is normally expressed by the increase in concentration of Serum Glutamic Pyruvic Transaminase (SGPT) or Alanine AminoTransferase (ALT) and Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate Amino Transferase (AST). Liver cell damage would increase the concentration of these enzymes in the blood.

The purpose of this study is to determine the effect of ethanolic extract of arrowroot tubers (Maranta arundinacea L.) on the parameters of liver damage caused by the increased of free radicals that cause oxidative stress from the induction of alcohol (ethanol) in large quantities. The parameters of liver damage used in this study were MDA levels as an indicator of lipid peroxidation, as well as the levels of SGPT and SGOT as enzyme indicators for liver damage in ethanol-induced rats.

METHODS
Materials
The materials used in this study was white female wistar rats weight 150-200 gram, aged...
1.5-2 months in healthy condition obtained from Gadjah Mada University in Yogyakarta. Arrowroot tubers (**Maranta arundinacea**) obtained from Bringhardjo market Yogyakarta. Solvent used for extraction was 96% ethanol, and the anesthetic substance used was chloroform. Solvent used to dissolve the extract was CMC Na 0.5%, obtained from the Laboratory of Pharmacy University of Ahmad Dahlan, and the feed used for rat models was AD 2. Material used to measure the levels of SGPT and SGOT was obtained from (Diasys) with the component: SGPT reagent (reagent I) TRIS, L-aspartic, malate dehydrogenase (MDH), lactate dehydrogenase (LDH); SGOT reagent (reagent II) 2-oxoglutarate, nicotinamide adenine dinucleotide hydrogen (NADH); ALT reagent (reagent I) TRIS, L-alanine, LDH; ALT reagent (reagent II) 2-oxoglutarate, NADH. Materials used in order to measure MDA levels was trichloroacetic acid (TCA), thiobarbituric acid (TBA) and tetraethoxyprophan (TEP).

**Plant Determination**

The determination of arrowroot tubers (**Maranta arundinacea**) was conducted at the Laboratory of Biology, University of Ahmad Dahlan Yogyakarta.

**Ethanolic Extract of Arrowroot Tubers**

Powder of Arrowroot tubers 250 gram was macerated in 500 ml of ethanol 96% for 3x24 hours in room temperature and the maceration was repeated 3 times. The filtrat (extract) obtained was collected in a container. Then the extract was evaporated in 80 °C temperature. Then the ethanol 96% at dose 5 g/KgBW was administered orally on day 14th, one hour after the last administration (for group II, III, IV, and V). On day-15th, the blood of animal models was drawn through orbital sinus using a capillary tube in order to measure the levels of SGPT and SGOT. Then the rats were sacrificed in order to took the liver to measure the levels of MDA.

**Measurement of MDA Levels**

Measurement of MDA levels in this study used the Thiobarbituric Acid Reactive substance (TBARs) method. Measurements was conducted at the Laboratory of the University of Gajah Mada. Liver (1 gram) was homogenized using a 9,0 ml solution of 1,15% KCl by Teflon Potter-Elvehjem homogenizer. A total of 0,2 ml of liver was homogenized, added with 0,2 mL of 8,1% sodium dodecyl sulphate and 1,5 ml of 20% acetic acid solution until the pH become 3,5 using NaOH and 1.5 ml TBA 0,8%. The mixture was added with water until the volume was 4,0 ml, then heated at 95 °C for 60 minutes, and then the mixture was cooled using water. The mixture was added with 1,0 ml water and 5,0 ml N-butanol : Pyridine (15:1, v/v) mixture, and then shaken. Furthermore, the mixture was centrifuged in the velocity of 4000 rpm for 10 minutes, the organic layer was taken and absorbance was measured in 532 nm wavelength using visible spectrophotometer. Standard solution used was 1,1,3,3 Tetraethoxyprophan (TEP). Datas were statistically analyzed using ANOVA with 95% Confidence Interval.

**Measurement Levels of SGPT and SGOT**

Measurements of the level of SGPT and SGOT were conducted in the Laboratory of Pharmacology, Ahmad Dahlan University. Measurement of the activity of SGPT was done using reagent I (TRIS, L-alanine, LDH) and reagent II (2-oxoglutarate, NADH). SGOT measurements was done using reagent I (TRIS, L-aspartic, MDH, LDH) and reagent II (2-oxoglutarate, NADH). Reagent was used as a mixture of reagent I and reagent II in 4:1 ratio. Serum 50 ml + 500 ml reagent kit. Sample solution was incubated in
room temperature for 1 minute and then read with spectrophotometer Caretium NB-201 with the wavelength of 340 nm at 37°C. Data were statistically analyzed using ANOVA with 95% confidence interval.

RESULTS
Measurement of MDA Levels
Measurement of MDA levels was done using the TBARs (Thiobarbituric Acid Reactive substance) method. Measurement was made on the basis of the reaction between 1 molecule of MDA with 2 molecules of TBA, forming a pink-colored MDA-TBA2 complex. The MDA-TBA2 complex absorbance would be measured at the wavelength of 532 nm using a spectrophotometer. The reaction between MDA and TBA occurred in the presence of nucleophilic attack involving Carbon-5 of TBA and Carbon-1 of the MDA, followed by dehydration reaction, in which the same reaction would occur with the second molecule of TBA, forming a MDA-TBA-2 complex.

MDA is one of the indicators most commonly used for lipid peroxidation. MDA is an end product of lipid peroxidation which is used as a biological biomarker of lipid peroxidation and is able to describe the degree of oxidative stress. The results of MDA measurement can be seen in Table 1.

| Groups                      | Doses (mg/Kg BW) | MDA Levels (nmol/gram) Mean & SD |
|-----------------------------|------------------|----------------------------------|
| Normal                      | -                | 2.94 ± 0.38*                     |
| Control                     | -                | 4.07 ± 0.44                      |
| Arrowroot Tubers Extract    | 125              | 3.34 ± 0.35*                     |
|                             | 250              | 3.36 ± 0.51*                     |
|                             | 500              | 2.86 ± 0.40*                     |

Data in the form of mean ± SD; * p< 0.05 = significantly different with control group; Normal Group: Only given food and drink; Control Group: Given CMC Na 0.5% + 95% ethanol; Extract Groups: Given the ethanolic extract of arrowroot tubers + 95% ethanol

Measurement Levels of SGPT and SGOT
SGPT and SGOT are intracellular enzymes that could be found in the heart, liver and skeletal muscle tissues. The release of these intracellular enzymes into the blood could be caused by the necrosis of liver cells or acute liver damage, and would cause their concentration to increase. SGPT enzyme catalyzes the transfer process of L-alanine amino group to 2-oxoglutarate, causing the formation of pyruvate and glutamate. This pyruvate would be reduced along with the oxidation of NADH to NAD+, which is catalyzed by MDH enzyme. SGPT and SGOT would reflect the integrity or integration of liver cells. The increase of liver enzymes concentration would reflect the level of damage occurred in the liver cells. If the concentration of SGPT and SGOT enzymes increase, then the degree of damage occurred in the liver cells might be higher as well. Cell membrane damage would cause the SGOT enzyme to exit from the cytoplasm, thus its number in the blood would increase. Hence, it could be used as an indicator of liver damage. Measurement of SGPT and SGOT concentration can be seen in Table 2.

Table 2. Mean of SGPT and SGOT Levels of Normal Group, Control Group, and Arrowroot Tubers Extract Groups in the Dosage of 125, 250, and 500 mg/KgBW respectively

| Groups                      | Doses (mg/Kg BW) | SGPT (U/L) Mean & SD | SGOT (U/L) Mean & SD |
|-----------------------------|------------------|----------------------|----------------------|
| Normal                      | -                | 2.94 ± 0.38*         | 3.34 ± 0.35*         |
| Control                     | -                | 4.07 ± 0.44          | 3.36 ± 0.51*         |
| Arrowroot Tubers Extract    | 125              | 3.34 ± 0.35*         | 3.36 ± 0.51*         |
|                             | 250              | 3.36 ± 0.51*         | 3.36 ± 0.51*         |
|                             | 500              | 2.86 ± 0.40*         | 3.36 ± 0.51*         |

Data in the form of mean ± SD; * p< 0.05 = significantly different with control group; Normal Group: Only given food and drink; Control Group: Given CMC Na 0.5% + 95% ethanol; Extract Groups: Given the ethanolic extract of arrowroot tubers + 95% ethanol
Table 2. Mean Levels of SGPT and SGOT in Normal Group, Control Group and Arrowroot Tubers Extract Group in the Dose of 125, 250, and 500 mg/KgBW

| Groups                     | Doses (mg/Kg BW) | SGPT and SGOT Levels (U/L) |
|----------------------------|------------------|----------------------------|
|                            |                  | SGPT                       | SGOT                       |
| Normal                     | -                | 31.83 ± 5.49               | 1.13 ± 0.76                |
| Control                    | -                | 46.00 ± 6.60*              | 2.30 ± 0.31*               |
| Arrowroot Tubers Extract   | 125              | 27.00 ± 3.22*              | 1.05 ± 0.49*               |
|                            | 250              | 26.67 ± 5.16*              | 0.97 ± 0.30*               |
|                            | 500              | 21.67 ± 5.16*              | 0.62 ± 0.23*               |

Data in the form of mean ± SD; *p< 0.05 = significantly different with control group; Normal Group: Only given food and drink; Control Group: Given CMC Na 0.5% + 95% ethanol; Extract Groups: Given the ethanolic extract of arrowroot tubers + 95% ethanol.

DISCUSSION

Flavonoid as antioxidant would act as a radical scavenger. It would also interact with free radicals using a variety of enzyme systems. Flavonoid is a phenolic compound which could capture free radicals that enter the body. Flavonoids act by bonding or forming a chelate that would change free radicals into non-toxic compounds, thus causing the free radicals to not be able to damage the liver. Flavonoids could also inhibit the activity of cytochrome P450 enzyme and its metabolites. Flavonoids that is contained in the arrowroot tubers would be able to prevent oxidative stress that occurs in the liver. Flavonoids will bind the free radicals that occur from the metabolism of alcohol, prevent lipid peroxidation (release of MDA), and prevent liver damage (release of enzymes SGPT and SGOT).

Metabolism of alcohol at microsomal pathway will produce free radicals, like superoxide and hydroperoxide, that would lead to oxidative stress. Oxidative stress can cause lipid peroxidation that could be detected by the increase of MDA concentration. The result of alcohol metabolism through Alcohol Dehydrogenase (ADH) enzyme system is acetaldehyde. Acetaldehyde would be metabolized further by Aldehyde dehydrogenase (ALDH) into acetate. Acetaldehyde is a reactive metabolites that can cause various injuries through various pathways. Alcohol metabolism through the peroxisomes would lead to increased production of free radicals by various mechanisms, causing oxidative stress which would damage liver tissues. SGOT and SGPT could be used as a parameter to detect liver damage.

According to Table 1, it could be seen that the 96% ethanol (alcohol) was not effective in increasing the level of MDA in the liver of rat models. It could be seen by comparing normal with control group. The mean of MDA concentration in control group was 4.07 ± 0.44, which was higher than normal group (2.94 ± 0.38). SPSS comparison of the two groups showed p value < 0.05 which means that there were statistically significant difference between the two groups, meaning that the induction of alcohol could increase the levels of MDA significantly.

These results were similar with previous study conducted by Wang et al., which stated that the induction of alcohol 5 g/KgBW was able to induce oxidative stress and increased the levels of MDA in the liver. However, this study was using mice instead of rats as animal models.

MDA is one of the end-product of lipid peroxidation and is able to be used as a tool to describe the degree of oxidative stress. Oxidative stress can cause lipid peroxidation. Lipid peroxidation is responsible for tissue damage. MDA could be formed when
hydroxyl free radicals react with the fatty acid component of the cell membrane, causing a chain reaction known as lipid peroxidation. The lipid peroxidation chain would induce the breakdown of fatty acids into a variety of toxic compounds and cause damages to the cell membrane.\textsuperscript{27}

The ethanolic extract of arrowroot tubers can reduce MDA concentration. It can be determined by comparing the control group (group II) with the extract groups in the dosage of 125 mg/KgBW (group III), 250 mg/KgBW (group IV), and 500 mg/KgBW (group V). SPSS analysis between those groups showed p value < 0.05, which means that there is statistically significant difference. The most effective dose of ethanolic extract of arrowroot tubers to reduce MDA concentration was 500 mg/KgBW. In this study, the decrease of MDA concentration caused by the administration by ethanolic extract of arrowroot tubers when compared to the normal group, for the dosage of 125 mg/KgBW was obtained $3.34 \pm 0.35 (p>0.05)$, for the dosage of 250 mg/KgBW was obtained $3.36 \pm 0.51 (p>0.05)$, and for the dosage of 500 mg/KgBW was obtained $2.86 \pm 0.40 (p>0.05)$. Statistical analysis showed that there were no statistically significant difference, proving that the ethanolic extract of arrowroot tubers were effective in reducing MDA concentration into similar concentration with normal group. For the group who was given 500 mg/KgBW of extract, the MDA concentration was even lower than the concentration of normal group. This might occur because the concentration of MDA in the normal group was already high, probably due to certain infection, such as parasitic infection, that already occurred prior to the intervention, thus causing the concentration of MDA to be higher.\textsuperscript{28}

According to Table II, 96% ethanol (alcohol) was effective to increase the level of SGPT and SGOT. It could be determined by comparing normal group with control group. The mean levels of SGPT in control group was 46.00 ± 6.60, which was higher when compared to normal group ($31.83 \pm 5.49$); and the mean levels of SGOT in control group was 2.30 ± 0.31, which was higher when compared to normal group ($1.13 \pm 0.76$). SPSS comparison of the two groups showed p value < 0.05 which means that there was statistically significant difference between the two groups, meaning that the induction of alcohol could increase the level of SGPT and SGOT significantly.

These results was similar with previous study conducted by Wang et al. which stated that the induction of alcohol 5 g/kgBW could induce an increase of SGPT and SGOT concentration.\textsuperscript{24} The concentration of SGPT and SGOT was found smaller compare to the use other inducers of liver damage, such as CCl$_4$ or paracetamol, which means that the degree of oxidative stress that occurred was probably small as well. The dose of ethanol used as a liver-damage inducer could be increased for the acute liver damage model or the induction could be changed into chronic ethanol administration to provide a more effective results of liver damage. Further histopathological analysis is needed to provide broader perspective of liver damage. The activities of SGPT and SGOT would increase when cell damages occur, such as in acute hepatocellular necrosis as in impaired liver and biliary tract function, heart and blood vessel diseases, and impaired renal and pancreatic function.\textsuperscript{15}

In this study, it can be seen that the ethanolic extract of arrowroot tubers was able to reduce the concentration of SGPT and SGOT. It could be seen by comparing the control group (group II) with the extract groups in the dose of 125 mg/KgBW (group III), 250 mg/KgBW (group IV), and 500 mg/KgBW (group V), in which p value < 0.05 was obtained. The most effective dose of the ethanolic extract of arrowroot tubers to reduce the concentration of SGPT and SGOT was 500 mg/kgBW. The decreased in SGPT concentration of the extract groups when compared with the normal group for the dosage of 125mg/KgBW was $27.00 \pm 3.22 (p>0.05)$; and for the dosage of 250 mg/KgBW was $26.67 \pm 5.16 (p>0.05)$. From the statistical analysis, the difference was found not significant when compared with normal group, proving that the ethanolic extract of arrowroot tubers was effective to reduce SGPT concentration, until it
reached similar concentration to normal group. For the extract group that used 500 mg/KgBW, decreased in SGPT concentration was even lower than the normal group, which was 21.67 ± 5.16 (p<0.05). This might be because the normal group had higher SGPT concentration, which is probable because this study did not measure SGPT concentration prior to intervention. Possibly, the rat models used in this study was already suffering from infection or other disorders, such as parasitic infestations, prior to intervention.

Although the extract group of 500 mg/KgBW had SGPT concentration below the normal groups, but the concentration was still within the normal range of SGPT concentration. According to Kaplan (1993), SGPT and SGOT enzymes are normally present in blood serum in the concentration of less than 30-40 IU/L. Normal SGPT concentration for mice is 17.5 to 30.2 IU/L and SGOT concentration should be 45.7 to 80.8 IU/L. The analysis differences between normal rats SGOT and SGPT activities in this research compared to predetermined values, was probably due to several stress factors that could occur through increased peripheral sympathetic nerves activities, difference in the weight of the mice, hemolysis, the state of physiology and macroenzym, the difference in tools and methods of analysis used, and even the difference in kit reagents used, which would also influence the results of the analysis.

Similar to the measurement of SGPT concentration, the decrease in SGOT activities when compared to normal group was obtained as follow: for the 125 mg/KgBW dose was 1.05 ± 0.49 (p> 0.05), for the 250 mg/KgBW dose was 0.97± 0.30 (p> 0.05); and for the 500 mg/ KgBW dose was 0.62 ± 0.23 (p> 0.05). Statistical analysis showed no statistically significant difference, proving that the ethanolic extract of arrowroot tubers are effective in reducing SGOT concentration, until the concentration was similar to normal group.

Antioxidants may inhibit or prevent oxidation process. Antioxidant compounds provided its effect by reacting with a reactive free radicals, stabilizing free radicals, and turn free radicals to a non-reactive compound. Antioxidants would complement the electron of free radicals and inhibit the chain reactions of free radicals formation. Antioxidant compounds that could be found in the arrowroot tubers include flavonoids, alkaloids and saponins. The antioxidant activities of arrowroot tubers could occur due to a synergistic activity of these compounds.

The antioxidant properties of flavonoids derived from its ability to transfer its electron to free radicals, thus making it non-reactive. Flavonoids could also form a complex with metal compound. The second mechanism that makes flavonoids to have several effects, including inhibiting lipid peroxidation, suppress tissue damage by free radicals, and inhibit the activity of some enzymes. In this study, the type of flavonoids contained in the ethanolic extract of arrowroot tubers was not determined because only qualitative test was performed.

Arrowroot tubers also contain phenols. Phenols are compounds with -OH group attached to an aromatic ring carbon. Phenol compounds have the ability to donate its -H atom, so that the free radicals could be reduced into a more stable form. In addition to flavonoids and phenols, arrowroot tubers also contain alkaloids which serves as a protection against attack or disruption that occur all around, as well as antibiotics and antioxidants. A lot of alkaloids are found in polar solvents, because most alkaloids that have the potentials as antioxidants are polar compounds that would be extracted in polar solvents. Alkaloids act as an antioxidant by donating its hydrogen atoms (-H) to free radicals. This mechanism indicates that alkaloids work as primary antioxidants. Alkaloid, especially indole, has the ability to stop the chain reaction of free radicals efficiently. Radical compounds derived from these amine compounds have a very long termination stage. Another alkaloid compound, that has antioxidant properties, is caffeine which could act as a damper of hidroxyl and melatonin radicals, and plays an important role in protecting the cells from radiation and drugs toxicity. However, this study had not yet
determined the type of alkaloid that contribute in antioxidant bioactivity. Arrowroot tubers also contain saponins which also have antioxidant properties by forming hydroperoxide as secondary antioxidants, thus it could inhibit the formation of lipid peroxide.6,32

CONCLUSION
The ethanolic extract of arrowroot tubers (Marantha arundinacea L.) is able to reduce the concentration of MDA, SGPT and SGOT in ethanol-induced rat models.

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