Effect of purple sweet potato (*Ipomoea batatas* L) extract on malondialdehyde levels of male white rat (*Rattus norvegicus* Wistar strain) model of atherosclerosis

Meddy Setiawan, Fakih Nadhil

Faculty of Medicine, University of Muhammadiyah Malang, Indonesia

Abstract

Cardiovascular disease is a disease caused by impaired functions of heart and blood vessel, one of those is coronary heart disease. This study aimed to identify the effect of purple sweet potato (Ipomoea batatas L) extract on MDA (Malondialdehyde) levels of male white rat (Rattus norvegicus Wistar strain) model of atherosclerosis. This research was an experimental study using Post-Test Only Control Group Design method, used 5 groups (positive control group, negative control, treatment 1, treatment 2, and treatment 3), each group contained 5 rats plus 2 reserve rats. ANOVA one way test obtained a significance of 0.00 ($p < 0.05$). The regression test results showed the coefficient of determination $R^2 = 0.71$, which means a decrease in serum MDA levels of experimental animals is influenced by the dose of purple sweet potato extract administration as much as 71% while the remaining 29% can be influenced by factors outside the study. It can be concluded that purple sweet potato extract can affect serum MDA levels of experimental animals.

Introduction

Globally, cardiovascular disease is the leading cause of death every year. Cardiovascular disease is a disease caused by impaired functions of heart and blood vessel, one of those is coronary heart disease. To date, Coronary Heart Disease (CHD) is the main cause of death among citizens in Indonesia.[1]

In 2008 an estimated of 17.3 million deaths were caused by cardiovascular disease. More than 3 million deaths occurred before 60 years of age and should be prevented. Deaths caused by heart disease occur around 4% in high-income countries and up to 42% in low-income countries.[2]

Atherosclerosis formation in blood vessels is preceded with endothelial dysfunction. Fat plaque in the endothelial region will accumulate until obtrusive atheroma is formed and makes the endothelial lumen narrower. Disorders that occur in the endothelium are much influenced by oxidative stress where there is an imbalance in the body's antioxidant system with other super oxides.[3]

Papua has coronary heart disease prevalence as much as 1.2% that is the lowest in Indonesia.[4] Papuans mostly consume purple sweet potato as staple food every day, but they also consume fats that are cholesterol-rich. However, the incidence of coronary heart disease is the lowest in Indonesia compared to other provinces. The active ingredients (anthocyanin, beta glucan, ascorbic acid) contained in purple sweet potato (Ipomoea batatatas) are very likely to play an important role in the prevention of atherosclerosis in Coronary Heart Disease.

The pathogenesis of atherosclerosis is a complex interaction process. Lipids and inflammation have shown to play an important role in endothelial dysfunction that play a role in the pathogenesis of atherosclerosis. Biochemical or mechanical stimuli can cause endothelial injury thus resulting in abnormalities of endothelial physiological function (endothelial dysfunction). Endothelial dysfunction causes increased vascular permeability, adhesion, and infiltration of monocytes and T cells, and also increased activity of growth factors.[5]

Arterial lesions in endothelial dysfunction are triggered by exposure to inflammatory trigger agents, such as oxidized LDL particles, free radicals, reactive oxygen species (ROS), increased plasma homocysteine (metabolic abnormalities), local genetic mutations, and chronic systemic infections (herpes virus, Chlamydia pneumoniae, Helicobacter pylori).[6,7]

The mechanism of atherosclerotic plaque formation is initiated by oxidation of LDL (ox-LDL) which is chemotactic against monocytes. Monocytes will enter endothelial cells and differentiate into macrophages that will phagocyte ox-LDL. These ox-LDL-containing macrophages are called foam cells that then followed by fatty streak formation. Next, lipid core (fat core) which is covered in smooth muscle cells will be formed. A further phase is the formation of fibrous plaques which are fat nuclei surrounded by fibrotic stamps.[6]

LOX-1 is an endothelial ox-LDL receptor. Ox-LDL will be bound to LOX-1 to enter the cell and induce ROS formation. Endothelial damage by ox-LDL via signaling reactive oxygen species (ROS). ROS such as superoxide anions, hydroxyl radical, hydrogen peroxide (H2O2) can cause serious damage to DNA, proteins, and lipids.[8,9]

Small oxidized lipids which are components of Ox-LDL, such as oxysterols, oxidized fatty acids, and aldehydes, are potential inducers of ROS production. ROS interferes normal redox balance and eventually makes cells in a state of oxidative stress. At the cellular level, ROS acts as a second messenger on various transduction signals, and ROS is formed in response to proliferation, differentiation, cell aging, and cell death. ROS will activate a large number of NF-ϰB main signaling pathways.[10]

Intracellular-formed ROS causes pro-

Correspondence: Meddy Setiawan, Faculty of Medicine, University of Muhammadiyah Malang, Jl. Bendungan Sutami 188A Malang Jawa Timur 65145, Indonesia.
Tel.: +62.341.55149 - Fax: +62.341.582060
E-mail: mohmaroef514@gmail.com

Key words: purple sweet potato extract, malondialdehyde, Rattus norvegicus Wistar strain, atherosclerosis.

Acknowledgments: We gratefully thank to University of Muhammadiyah Malang for their helps and support to this work. Authors are also thankful to the respondents of the study for providing valuable information.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Clinical trials: This research has been approved by research ethics committee of Faculty of Medicine, University of Muhammadiyah Malang.

Conference presentation: part of this paper was presented at the 3rd International Symposium of Public Health, 2018 October 31 - November 1, Universitas Airlangga, Surabaya, Indonesia.

Dedication: the article is dedicated to Faculty of Medicine, University of Muhammadiyah Malang.

Received for publication: 28 July 2019.
Revision received: 9 September 2019.
Accepted for publication: 15 October 2019.

Journal of Public Health in Africa 2019; 10(s1):1187
doi:10.4081/jphia.2019.1187

tein kinase activation and NF-κB transcription factors (Wang, 2010). The ox-LDL bond to LOX-1 initiates NF-kB activation, which in turn stimulates all of the target genes, especially genes associated with inflammation.[11,12]

Oxidative stress is a condition where there is an imbalance between free radicals (prooxidants) and antioxidants. If free radicals are higher in levels and meet unsaturated fatty acids, they trigger the occurrence of lipid peroxidation which can produce aldehyde compounds, one of which is MDA. (Malondialdehyde). MDA can be used as a biological biomarker as a marker of lipid peroxidation occurrence and can describe the degree of oxidative.[13] Purple sweet potatoes contain flavonoids which are colored phenolic substances and are found in many high-level plants. Flavonoids are the main source of red, blue, and yellow pigments in flowers and fruits, except carotenoids. The highest concentration of flavonoids is found in the colored outer tissues like fruit peels. Most flavonoids have a basic structure of 1,4-benzopyrone. Flavonoids are divided into 12 subgroups according to their chemical structure, namely flavines, falvonols, flavanonols, isoflavones, anthocyanins, anthosianidins, leucoanthosyanins, chalcones, dihydrochalcones, aurones, and catechins.[14]

Flavonoids have various effects, namely antitumor effect, anti-HIV, immunostimulant, antioxidant, analgesic, anti-inflammatory, antivirus, antifungal, anti-diarrhea, anti-hepatotoxic, anti-hyperglycemic, and as a vasodilator. The mechanism of flavonoids as antioxidants can be divided into two ways based on their chemical differences, first, to prevent the formation of free radicals as a metal ion binding agent (chelator) and reduce hydroperoxide to be less reactive hydroxides; and secondly, as free radical collectors through the formation of less reactive "radical antioxidants" by dismutase, recombination, or reduction also catalyze the form changes into non-radical.[15] The aim of this study was to identify the effect of purple sweet potato (Ipomoea batatas L) extract on MDA (Malondialdehyde) levels of male white rat (Rattus norvegicus Wistar strain) model of atherosclerosis.

Materials and Methods

This research was an experimental study using Post-Test Only Control Group Design method, which was only measured at the end of the study by using the control groups which were positive control and negative control. This study used a population of white male rats selected using a random sampling method with inclusion criteria that were 150-200 grams of weight and aged 2-3 months old with a healthy condition characterized by active movement and clear eyes.

This study used 5 groups (positive control group, negative control, treatment 1, treatment 2, and treatment 3), each group contained 5 rats plus 2 reserve rats. The research procedure began with adaptation process as long as 7 days by giving standard BR-1 feed and drink ad libitum. Furthermore, treatment was given to each positive control group, treatment 1, treatment 2 and treatment 3 with a hypercholesterol diet plus administration of purple sweet potato extract in treatment group 1, treatment 2 and treatment 3 each dose of 120 mg/kgBW/day, 240 mg/kgBW/day, and 480 mg/kgBW/day, respectively. After 2 months, surgery was performed on these rats and then the MDA level was measured through TBARS test using spectrophotometric method. After that, data analysis was carried out using one way ANOVA test. It is said that the extract has an effect if the results obtained from the test are $p < 0.05$.

Results and Discussion

The results showed MDA levels for each group and graph of average of experimental animal MDA level for each group.

Table 1 and Figure 1 represent the MDA levels of experimental animals in each group.

These results indicate that the highest MDA level is found in positive control, while the lowest MDA level is in negative control. These results indicate that MDA levels of experimental animals in the treatment groups, namely treatment 1, treatment 2, and treatment 3, have decreasing levels. The analysis was started by using Normality test to see whether the serum MDA level distribution of experimental animal was normal or not. In the analysis results, it was obtained a result of shapiro-wilk test with a significance of 2.63 ($p> 0.05$). It could be concluded that the serum MDA level distribution of experimental animal was normal. Homogeneity test was then conducted to determine whether the variant of experimental animal serum MDA level was homogeneous or not. Analysis of the data shows that Levene test obtained a significance of 1.04 ($p> 0.05$). It can be concluded that variants of experimental animal serum MDA data were homogeneous.

ANOVA one way test was applied to see whether purple sweet potato extract affects serum MDA levels of experimental animals. From the data analysis obtained a significance of 0.00 ($p <0.05$). It can be concluded that purple sweet potato extract can affect serum MDA levels of experimental animals. Then, to find out the degree of influence of the extract was by using Post Hoc Tukey test. From the Post Hoc test it was found that treatment 1 and treatment 2

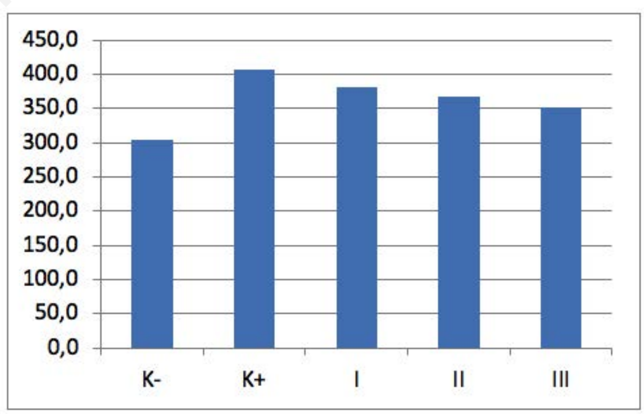

Figure 1. Average of experimental animal MDA level.

Table 1. MDA levels for each group.

Group	Repetition					R
	1	2	3	4	5	
C(-)	356.5	271.5	261.5	264.0	296.5	290.0
C(+)	441.5	379.0	381.5	434.0	416.5	410.5
T1	374.0	371.5	361.5	396.5	399.0	380.5
T2	391.5	369	354	361.5	364	368.0
T3	309.0	321.5	331.5	344.0	346.5	330.5

were significant to negative control. However, when compared with negative controls, treatment 3 showed insignificant difference. This means that the MDA level in treatment 3 were close to the level of the negative control as the normal control in the study. It can be concluded that extract administration in treatment 3 with a dose of 480 mg/KgBW/day has an effect to prevent an increase in serum MDA levels of the experimental animal close to normal levels.

Correlation test was used to see how strong the effect of purple sweet potato extracts on serum MDA levels in rats. From the data analysis, it can be seen that the strength of the correlation (Pearson Correlation) = - 0.843 and the value of Sig. (2-tailed) = 0.00 < p (0.05) indicates that there is a strong correlation that is inversely proportional and meaningful between an increase in the dosage of purple sweet potato extract and a decrease in MDA levels of experimental animals. The regression test results shows the coefficient of determination $R^2 = 0.71$, which means a decrease in serum MDA levels of experimental animals is influenced by the dose of purple sweet potato extract administration as much as 71% while the remaining 29% can be influenced by factors outside the study.

Purple sweet potato (Ipomoea batatas) contains active ingredients of anthocyanin, beta-glucan and ascorbic acid. Those three active ingredients function as anti-oxidants which can reduce oxidative stress. The antioxidant activity in purple sweet potatoes has a considerable influence to prevent the increase of free radicals. Free radicals can cause damage from various cells, one of which is blood vessel endothelial cells. Endothelial cells that have lesions or damaged will express various kinds of proinflammatory cytokines (VCAM, ICAM, MCP, TNFα, IL6) which make it easier for monocytes to attach to the arterial wall and LDL cholesterol. This process will initiate a series of inflammatory processes that form atherosclerotic plaques. Atherosclerosis plaque will manifest coronary heart disease if it ruptures and blocks the coronary arteries.[16,17]

Conclusions

There is a significant effect, a very strong correlation, and inversely proportional of the administration of purple sweet potato (Ipomoea batatas) extract to decreasing MDA level in atherosclerotic rats. Purple sweet potato (Ipomoea batatas) extract can reduce malondialdehyde (MDA) levels of white male rats (Rattus norvegicus Wistar strain) model of atherosclerotic.

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