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Extract of Purple Sweet Potato Against Heat-Shock Protein 70 Expression in White Male Rat of Atherosclerosis Model

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ABSTRACT

Shock protein 70 (Hsp-70) molecule is pro-inflammatory mediator cytokine that trigger atherosclerosis. The purple sweet potato has many natural antioxidants such as flavonoids (anthocyanins) and is valuable for reducing Hsp-70 expression due to its antioxidant content.This study aims to determine the effect of purple sweet potato (*Ipoema batatas* L.) extract in reducing the Hsp-70 levels in the white male rat atherosclerosis model.This study was a posttest-only control group design with normality, homogeneity, ANOVA, post hoc test, correlation, and regression tests. There were five groups in this study. Groups 1 (negative control) and 2 (positive control) were given 2 ml/day of high-cholesterol diet for eight weeks, and the other three groups were given purple sweet potato extract at 24, 48, and 96 mg/kg doses weight per day for eight weeks. Results: The purple sweet potato extract affected the Hsp-70 levels in Wistar strain rats ($p < 0.05$). There was a significant difference in the Hsp-70 level between the positive control group and the group with purple sweet potato extract. The highest Hsp-70 level reduction was seen in the group with a 96 mg/day dose of purple sweet potato extract. In conclusion, theadministration of purple sweet potato extract (*I. batatas* L.) reduced the Hsp-70 levels. The dose of 96 mg/kg BW/day had the highest effect on decreasing Hsp-70 levels in the male rat atherosclerosis model.
Keywords: Atherosclerosis; Purple sweet potatoes extract; Heat shock protein 70

INTRODUCTION

Cardiovascular disease is the leading cause of death globally, and in 2004, it is estimated that 17.1 million of the world's population died due to this disease (Mc Namara *et al.* 2019; Pagidipati *et al.* 2013; WHO, 2009). According to the Ministry of Health, Republic of Indonesia, in 2013, coronary heart disease was the leading cause of death in Indonesia, with around 883,447 people.

Atherosclerosis is a chronic inflammatory process that involves lipids, thrombosis, immune cells, and endothelial dysfunction (Badimon and Vilahur, 2014; Conti and Shaik-Dasthagirisaeb, 2015; Whayne, 2011). Oxidized low-density lipoprotein (Ox-LDL) increases reactive oxygen species (ROS). It is a cytotoxic and a chemotaxis factor for monocytes (Collins *et al.* 2001). In addition, Ox-LDL causes endothelial dysfunction and induces the emergence of heat-shock protein (Hsp), a protein that stimulates innate immune responses, including the production of pro-inflammatory cytokines, macrophages, and adhesion molecules in endothelial cells (Hu *et al.* 2002; MsCully, 2009).

Hsp-70 is the most common heat-shock protein in the human body. Increased Hsp-70 levels are found in people with endothelial damage (Bernardini *et al.,* 2011). Hsp70 levels are related to the thickness of the tunica intima-media. Expression of Hsp70 is also associated with pro-inflammatory states and plays a role in the induction and progression of atherosclerosis so that it may be used as an early marker of cardiovascular disease (Pockley, 2000). Elevated Hsp70 levels are associated with an increased risk of coronary heart disease, and Hsp60 expression is increased in acute myocardial infarction (Zhang, 2008).

When stressed (Ox-LDL), HSP 70 will stimulate the emergence of pro-inflammatory cytokines (TNFα, IL 1), adhesion molecules (ICAM-1 and VCAM-2), and increase monocyte and T cell aggregation (Pockley *et al*. al. 2008). Monocytes will enter the tunica intima and turn into macrophages. These macrophages will digest and oxidize LDL piles so foam cells will form (foamy cells). Then these foam cells will form a fatty streak (Brown, 2005). Pro-inflammatory cytokines (TNFα, IL1) will stimulate/invite leukocytes to the lesion site and make fibrous tissue proliferation to join the fatty streak, that Hsp 70 also raises adhesion molecules (ICAM-1 and VCAM-2) so fatty streaks further develop and become atherosclerotic plaques (De Maio, 2013).

In managing coronary heart disease, chemical drugs are needed for a long time. However, the use of chemical drugs often causes side effects (Kuhn *et al.,* 2016). Therefore, many treatments and prevention of coronary heart

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disease have been developed using natural ingredients (herbs). It is relatively safer for long-term use and has a minimum of side effects.

Many studies have been performed that the high incidence of coronary heart disease is an alternative to atherosclerosis prevention (Pothineni *et al.,* 2017). Purple sweet potato contains many antioxidants, such as flavonoids (Phuoc, 2020). Anthocyanin is a type of flavonoid in purple sweet potato (Ginting *et al.,* 2011). Flavonoids (anthocyanins) are bioactive substances that have antioxidant activity and play a role in inhibiting inflammation (Meyer and Schmitt, 2000; Ranzio, 2002). Anthocyanins are natural antioxidants that capture free radicals by providing electrons to inhibit oxidant compound activity (Kilua *et al.,* 2019; Zulaikhah, 2017).

As a pro-inflammatory mediator, Hsp can release necrotic and non-necrotic substances into the extracellular environment, activating various immune and inflammatory responses, including activating several immune system effectors releasing cytokines, and inhibiting antioxidant activity. Hsp also plays a role in many other cellular processes that occur during and after exposure to oxidative stress. For example, under oxidative stress conditions, there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive substances. This imbalance can result in excessive ROS production as in conditions of ischemia or conditions where antioxidant enzymes are decreased, resulting in a decrease in intracellular conditions leading to protein and DNA aggregation and ultimately failure of normal cell function (Erwindoraldo, 2014).

The study on the effect of purple sweet potato extract on coronary heart disease is limited. Therefore, we investigated the effects of purple sweet potato extract for the prevention of coronary heart disease on Hsp-70 expression in the male rat atherosclerosis model.

METHODOLOGY

Study Design

This study was an experimental study using the posttest-only control group design and simple random sampling. A total of thirty-five healthy male *Rattus norvegicus* strain Wistar were used according to inclusion criteria, weighing 200–250 grams, and aged 2–3 months. Rats were obtained from the Biomedical Laboratory of the Faculty of Medicine, University of Muhammadiyah Malang.

There were positive control, negative control, and three treatment groups. First, a standard BR-1 diet of 20 mg/day/rat and drinking *ad libitum* was given for 8 weeks. BR-1 is a standard feed consisting of 675 comfeed PAR-s, 33% flour, and sufficient water. The negative control group was given a standard BR-1 feed of 20 mg/day/rat for eight weeks, then the positive control was given a high-cholesterol feed of 2 ml/200 g BW/day for eight weeks; and treatment groups were given purple sweet potato extract (*Ipoema batatas* L.) at doses of 24, 48, and 96 mg/kg BW/day, respectively with a high-cholesterol diet. The high-cholesterol feed included comfeed PAR-S (50%), wheat flour (25%), duck egg yolk (5%), goat fat (10%), coconut oil (1%), and cholic acid (0.1%) (Romadhoni, Murwani, Oktavianie, 2014). The Research Ethics Committee approved the experimental protocols of Brawijaya University (Ethics committee approval number: 591-KEP-UB).

Preparation of Purple Sweet Potato Extract

The purple sweet potato extract was obtained from the Biomedical Laboratory of the Faculty of Medicine, University of Muhammadiyah Malang. The study dose was converted to purple sweet potato extract: bilberry extract contains 300 mg/100 g of anthocyanin extract. Therefore, its effects as a hypo cholesterol agent at a dose of 480 mg/kg BW. The dosage was calculated based on the average conversion calculation, and the resulting dose was 238.6 ~48 mg/kg/day. Therefore, the dosage variations of formula 1/2n, n, 2n were 24, 48, and 96 mg/kg weight per day.

Statistical Analysis

Data were analyzed using the normality, homogeneity, ANOVA, post hoc, correlation, and regression tests using SPSS 12.

RESULTS AND DISCUSSION

There was a difference in the Hsp-70 levels in each group. The Hsp-70 level in the K+ group was significantly increased compared to the K- group (Figure 1), indicating that the high-cholesterol diet-induced an atherosclerosis rat model, triggers oxidation to increase ROS and endothelial dysfunction. Furthermore, the dose variations of the purple sweet potato extract had a different effect on the Hsp-70 levels in the atherosclerosis rat model. Figure 1 shows the decrease of Hsp-70 levels at a dose of 24 mg/day (P1) compared to the K+ group. Moreover, the Hsp-70 levels were also reduced at a dose of 48 (P2) and 96 mg/day (P3).

The Hsp level data were normally distributed based on the normality test using the Shapiro-Wilk test. The homogeneity test using the Levene test showed significant $p = 0.414$ ($p > 0.05$). Therefore, the data in this study was

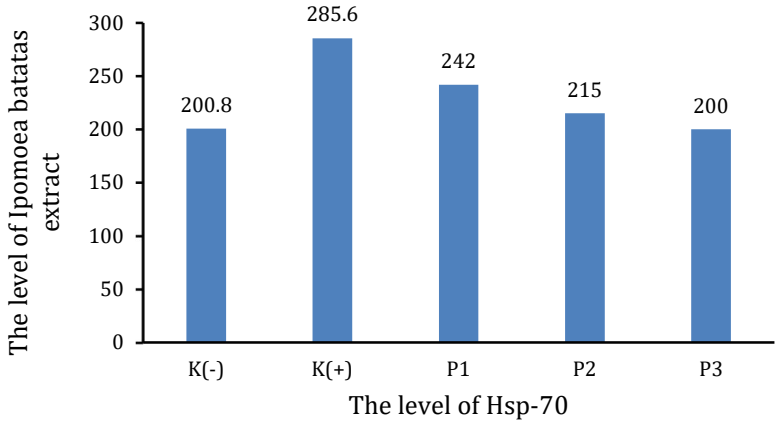

Figure 1. Hsp-70 levels of atherosclerosis rat model

homogeneous. There was a significant effect between the Hsp-70 levels in the treatment group compared to the control group. The Tukey test showed that the Hsp-70 level in the positive control group (K+) were significantly different compared to the treatment groups at a dose of 24 (P1), 48 (P2), and 96 mg/day (P3) and negative control group (K-) ($p < 0.05$). The Hsp-70 levels in the treatment group at 24, 48, and 96 mg/day were significantly different from the positive control group (K+) and negative control (K-). The Pearson correlation test was used to determine the relationship between the dose of the purple sweet potato extract and the Hsp-70 level in the atherosclerosis rat model. The results showed that the sig (two-tailed) value = 0.000 ($p < 0.01$). It indicated that there was a robust correlation between the sweet potato extract and Hsp-70 levels. Moreover, the higher dose of purple sweet potato caused the lower Hsp-70 levels.

Hsp-70 extracellularly circulates after tissue damage. It triggers the activation of the immune system, macrophages, and pro-inflammatory cytokines. Therefore, this molecule is known as an immunomodulator. HSP stimulates the emergence of TNF-α, IL-1, and adhesion molecules (ICAM-1 and VCAM-2). Furthermore, it increases the aggregation of monocytes and T11 cells. Monocytes enter into the tunica intima layer and turn into macrophages. The macrophages digest and oxidize the LDL stack. Therefore, a foamy cell will be formed, causing a fatty streak. Pro-inflammatory cytokines, such as TNF-α and IL-1, stimulate leukocytes to the lesion site and make fibrous tissue proliferation to join the fatty streak. These fatty streaks stimulate atherosclerotic plaques.

In the present study, the purple sweet potato extract at doses of 24 and 48 mg/day significantly affected Hsp-70 levels in the positive control group. Meanwhile, giving purple sweet potato extract at a dose of 96 mg/day significantly differed in the Hsp-70 levels in the negative control group (Figure 1). The purple sweet potato extract contains anthocyanins, ascorbic acid, and beta-glucans (Thakur and Sharma, 2018). They are known as antioxidants that inhibit free radicals by giving electrons to inhibit the activity of oxidant compounds. Therefore, they can reduce the Hsp-70 expression (Adewole, 2009). Anthocyanin has an essential role in stabilizing ROS by donating hydrogen ions to free radicals and stabilizing free radicals (Dixit *et al.* 2012). Ascorbic acid protects the cell from oxidative stress by increasing endothelial cell proliferation, stimulating collagen synthesis, and degrading LDL oxidation (Mayes, 2013). Beta-glucans prevent high lipid peroxide in the blood (Pengkumsri *et al.* 2016). Antioxidant activity inhibits oxidative stress conditions and a decrease in ROS levels. It is indicated by a decrease in the Hsp-70 levels. Therefore, the purple sweet potato extract affects the Hsp-70 levels.

In the present study, we reveal that the purple sweet potato extract reduces the Hsp-70 levels by 79%, and only 21% is due to endogenous and exogenous factors. Endogenous factors such as superoxide dismutase (SOD), reduced glutathione (GSH), and catalase can inhibit free radicals by inducing the enzyme Nrf2-mediated antioxidant and minimizing the inflammatory mediators of COX-2 and iNOS through the inhibition of NF-kB (Hwang *et al.* 2011). The exogenous factor induces an inflammatory reaction in each rat due to the injection. If an inflammatory reaction occurs, many

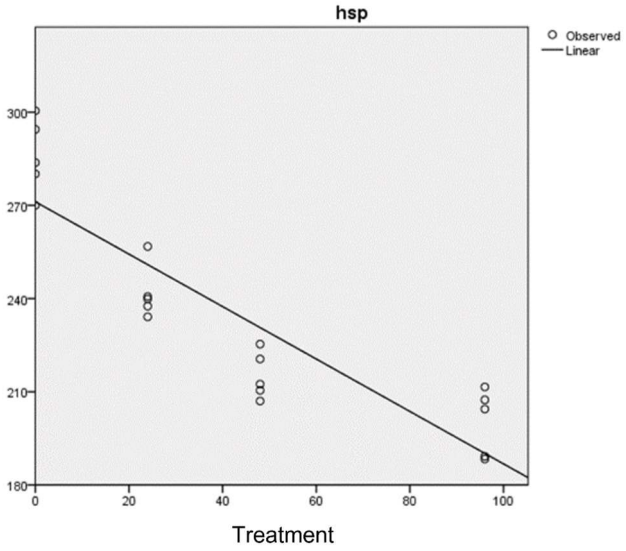

Figure 2. Linearity graph of the purple sweet potato extract with Hsp-70 Levels

pro-inflammatory factors induce the formation of free radicals (ROS). These conditions affect the Hsp-70 levels in the rat.

Based on the linear regression test, the value of R2 (R square) was 0.79. It indicated that the dose of the purple sweet potato extract on Hsp-70 level reduction was 79%, and other factors influenced the remaining 21%. In addition, it showed a significant effect with sig 0.000 ($p < 0.05$) on the provision of purple sweet potato extract on the Hsp-70 level reduction. The linear regression equation was Y = a + bX, where the value of "a" was a constant number and b was the regression coefficient. Therefore, it can be explained as follows:

$$Y = a + bX$$
$$Y = 271.161 + (-0.844)\ (x)$$

Noted: y = Hsp-70 level; x = dose of the purple sweet potato extract (mg/hr)

The regression coefficient (b) in this study was negative. It revealed that the purple sweet potato extract (x) had a negative effect on the Hsp-70 levels in the rat. The regression line between giving purple sweet potato extract with Hsp-70 showed a lower than the positive control group. It revealed that the purple sweet potato extract tends to reduce Hsp-70 levels. Our study has a limitation, particularly not examining the effect of endogenous antioxidants. Therefore, further research is needed to measure the SOD, reduced GSH, and catalase levels.

CONCLUSIONS

The purple sweet potato extract had an effect in reducing the Hsp-70 levels in the atherosclerosis rat model. Purple sweet potato extract at a dose of 96 mg/day had the highest effect in reducing the Hsp-70 levels.

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