Effect of Celery Extract Administration on 8-OHdG and Number of Foam Cell in Wistar Strain Rats With A High-Fat Diet

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ABSTRACT

BACKGROUND: A high fat diet, accompanied by high frequency eating, results in hyperlipidemia. One prevention for hyperlipidemia is administration of celery extract. OBJECTIVE: to explore the effect of celery extract on the level of 8-OHdG (8'-Hydroxy-2'-Deoxy Guanosine) and the number of foam cell.

METHODS: Experimental studies with post-test only control group design used Wistar rats, administered with adrenaline and high-fat diet for hyperlipidemic induction. Total of 35 rats were divided into 5 groups: negative controls (Neg-G); positive control treated with simvastatin (Nor-G); ES25-G, ES50-G, and ES75-G group were treated with 25 mg/200 g BW, 50 mg/200 g BW, and 75 mg/200 g BW celery extract, respectively. The treatment was given for 30 days. The 8-OHdG level was determined by ELISA, whereas the number of foam cells was determined by histopathologic preparations with HE staining. Data analysis was conducted by Kruskal-Wallis and Mann-Whitney.

RESULTS: Mann-Whitney analysis showed levels of 8-OHdG in ES-25-G 12.13±0.21, ES-50-G 7.23±0.25, ES-75-G 4.41±0.45 significantly lower than the Nor-G 14.30±0.66, respectively p<0.001. The number of foam cells in the ES-25-G was 7.57±0.53, ES-50-G 6.57±0.79, ES-75-G 3.43±0.53, significantly lower than the Neg-G 13.57±1.27, respectively p<0.001.

CONCLUSION: Celery extract capable of decreasing levels of 8-OHdG and the number of foam cells in Wistar rats induced with adrenaline and high-fat diet.

Keywords: celery, 8-OHdG, foam cell

INTRODUCTION

The increased incidency of cardiovascular diseases due to hyperlipidemia in developed and developing countries raises concern in the field of medicine. Hyperlipidemia is a lipid metabolic disorder characterized by elevated levels of triglycerides and cholesterol in the blood. Hyperlipidemia increase the production of oxygen radicals that can cause lipid peroxidation in the cell membranes. Lipid peroxidation occurs due to the fatty acid reaction of the phospholipid cell membrane with Reactive Oxygen Species (ROS). Increased levels of ROS and other free radicals that far exceed antioxidant levels generate a condition called oxidative stress. The occurrence of oxidative stress in the body can be detected from the presence of oxidative stress marker compounds, such as 8-OHdG, which is produced from the oxidation of guanine DNA nucleotides by ROS. 8-OHdG can be used as a marker of DNA damage through urine, serum, and saliva (Valavanidis et al., 2013). The Indonesian government is promoting the use of herbal remedies to reduce levels of hyperlipidaemia by using ingredients from herbal plants. Celery (Apium graveolens L) is a herbal plant contains high flavonoid. Flavonoid content of celery decrease cholesterol levels in the blood, thereby reducing the risk of stacking or depositing cholesterol on artery walls that often leads to atherosclerosis (Dalimartha, 2011). But it is still not known whether the provision of celery can lower levels of 8'-Hydroxy-2'-Deoxy Guanosine (8-OHdG) and the number of foam cells that are predisposing factors of atherosclerosis events.

Hyperlipidemia is an early stage of cardiovascular disease that caused for about 18% of cerebrovascular disease, and about 56% of ischemic heart disease worldwide (Angraeni et al., 2016). The American Heart Association estimated the prevalence of coronary heart disease (CHD) in the United States in 2004 around 13.2 million (Lloyd-Jones & Adams, 2009). Around the world, there were 50 million deaths each year due to CHD and 39 million of which are found in developing countries. While the prevalence of heart disease in Indonesia is 7.2% of the entire national population (Riskesdas, 2010).

Previous studies showed that celery extract
at the dose of 4 mg/200 g BW and 8 mg/200 g BW, decreased arterial blood pressure in proportion to normal group (Siska&Farida, 2010). Component of 3-n-butylphthalide in celery contribute to lower blood pressure by relaxing the smooth muscles in blood vessels (Fitria, 2016). Other studies showed that the effect of celery tannin extract is significantly decreased the total cholesterol level but showed no significant effect in triglycerides and HDL levels on hypercholesterolemic rats (Umarudin & Susanti, 2012). Furthermore, the administration of celery ethanol extract dose 250mg/kg BW/day in rats effectively decreased the cholesterol level and increased the HDL level (Anggraeni et al., 2016). Referring to the results of these studies, celery is expected to have the potential to decrease levels of 8-OHdG (8-Hydroxy-2’-Deoxy Guanosine) and number of foam cell.

Celery is a popular plant in Indonesia. Celery has the active substances of the flavonoid, which is one of the natural antioxidant food ingredients. The content of flavonoids in celery include luteolin and apin (Dalimartha, 2011), can decrease cholesterol levels in the blood thereby reducing the risk of stacking or sedimentation of cholesterol on artery walls that often leads to atherosclerosis (Dalimartha, 2011). Hyperlipidemia leads to excessive fat infiltration in the arterial intima causing atherosclerosis. One of the negative effects of hyperlipidemia is the lipid peroxidation caused by free radicals in the body. Hyperlipidemia can increase the production of oxygen radicals that can cause lipid peroxidation in cell membranes. Lipid peroxidation occurs due to the fatty acid reaction of the phospholipid cell membrane with ROS. Increased levels of ROS leads to oxidative stress, where there is an imbalance between pro-oxidants and antioxidants, and causes organic damage. Macromolecules such as lipids, carbohydrates, proteins, and DNA are the oxidation targets of ROS. Production of free radicals that exceed the antioxidant cellular defense system causes oxidative damage by inducing oxidation of polyunsaturated fatty acids in biological systems, resulting in cell membrane damage that would disrupt the balance of organelle cell work and continue to disrupt the normal production of DNA (genotoxicity) (Dwiet et al., 2015). DNA damage became a predisposing factor for the emergence of various degenerative diseases by showing an increase in the rate of oxidative DNA lesions (8-OHdG/8-Hydroxy-2’-Deoxy Guanosine). High levels of 8-OHdG are associated with high aggregation of hydroxyl radicals or low antioxidant adequacy (Kohen&Nyska, 2002). High flavonoid content in celery acts as an antioxidant and serves to neutralize free radicals, so as to reduce levels of 8-OHdG (8-Hydroxy-2’-Deoxy Guanosine). On the illustration on the number of foam cells, it is expected that there will be occurrence of loss or decrease in the number of foam cells in the tunica intima and tunica media. The purpose of this study is to prove that celery extract have an effect on the level of 8-OHdG and the number of foam cell in adrenaline-induced rats and high-fat diets.

METHODS

The experimental research with post test only control group design was conducted in the laboratory of Inter-University Center (PAU), Center for Food and Nutrition Studies of Gajah Mada University (UGM) and Sultan Agung Islamic Semarang Hospital. The population in this study was male wistar rats (Rattus norvegicus) (8-12 weeks old and 200-250 g body weigh) treated adrenaline and a high-fat diet for hyperlipidemic induction. A total of 35 Wistar rats were divided into 5 groups: negative controls (Neg-G); positive control treated with simvastatin (Nor-G); ES25-G, ES50-G, and ES75-G group were treated with 25 mg/200 g BW, 50 mg/200 g BW, and 75 mg/200 g BW celery extract, respectively. Treatment was given for 30 days. On day 31 the 8-OHdG level was measured using the OxiSelect TM Oxidative DNA Damage ELISA Kit and number of foam cells was measured using HE staining histological preparations.

Preparation of Celery Extract

Celery extract made with maceration method, from fresh celery with ethanol solvent. Celery and ethanol 65% were put into a closed erlenmeyer tube with a ratio of 1:4, then celery and solvent ethanol 65% were shaken and left for 3 days. After 3 days, the mixture was filtered using a Buchner funnel and a vacuum pump into the erlenmeyer tube. Then the filter results are poured into a round bottom flask and then processed in a vacuum rotary evaporator. Celery extract was administrated with gavage for 30 days.

Induction of Hyperlipidemia Rats

Thirty five rats were induced by adrenaline on the first day, then divided into five treatment groups and acclimatized in the UGM PAU Biology Laboratory. Administration of high-fat diet in the form of egg yolk diet began to be conducted on the second day. Administration of a high fat diet was given through gavage, every day for 30 days.

Measurement of 8-OHdG Levels

Blood was taken by puncturing the branch of
opthalmicus vein of the animal as much as 2ml, then the blood was put into eppendorf tube, and wait 1 hour until clot. The serum was separated from the blood by centrifugation for 15 minutes at 12,000 rpm speed. OxiSelect TM Oxidative DNA Damage ELISA Kit is required to measure 8-OHdG levels, using a UV-Vis spectrophotometer to measure the absorption of standard solutions and experimental animal samples. The concentration of the standard solution will obtained calibration curve which is used for measuring result of 8-OHdG of the sample solution.

Table 1. Levels of 8-OHdG and Number of Foam Cell

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Groups</th>
<th>p-value (Kruskal Wallis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg-G (n=7)</td>
<td>Nor-G (n=7)</td>
<td>ES-25-G (n=7)</td>
</tr>
<tr>
<td>8-OHdG Levels</td>
<td>14.30 ± 0.66</td>
<td>2.29 ± 0.34</td>
<td>12.13 ± 0.21</td>
</tr>
<tr>
<td>Number of Foam Cells</td>
<td>13.57 ± 1.27</td>
<td>2.57 ± 0.53</td>
<td>7.57 ± 0.53</td>
</tr>
</tbody>
</table>

^ = Kruskal Wallis test

Preparation of Foam Cell Preparations

On day 31 the rats were decapitated, then part of the aortic bifurcation was removed from the rats and fixed with 10% formalin for 24 hours. The fixed tissues preparation were removed by placing it into 70% alcohol 1x, 80% alcohol 1x, and 2x absolute alcohol. The net preparations were casted with liquid paraffin and then sliced with a rotary micotome with a thickness of 4-6 microns. The tissue band was transferred into the waterbath using spatel, if it has been exposed then adhered it slowly to the glass object.

Figure 1. The number of Foam Cell in Aorta’s Rats stained with HE method.
(a) Neg-G, (b) Nor-G, (c) ES-25-G, (d) ES-50-G, dan (e) ES-75-G. (400 times magnification)
that has been coated by Mayer’s egg albumin, so that the ribbon is not folded. Samples were then dried in an oven at 60°C for 15 minutes. Furthermore, the samples were stained and mounted using Hematoxylin eosin (HE) method. The observations were performed under a microscope with 400 times magnification and quantitatively calculated.

STATISTICAL ANALYSIS
The statistical analysis used Kruskal-Wallis and followed by Mann-Whitney. The analysis result is considered normal if the p value is < 0.05. This study was approved by Bioethics Commission for Research in Medical/Health, Faculty of Medicine Sultan Agung Islamic University Semarang.

RESULTS
After celery extracts administration for 30 days, the results of 8-OHdG and number of foam cells shown in Table 1 and Figure 1.

Table 1 showed that the highest mean of 8-OHdG levels was found in Neg-G group, followed by ES-25-G group, ES-50-G group, ES-75-G group and the lowest in the group Nor-G. Similarly, the average number of foam cells. Given the data is not normal and not homogeneous, further analysis used Kruskal-Wallis test. Kruskal-Wallis analysis showed that levels OHdG and the number of foam cells between groups was significantly different, p < 0.001.

8-OHdG levels
Statistical analysis of Mann-Whitney test showed that levels of 8OHdG in ES-25-G, ES-50-G, and ES-75-G groups were significantly lower than Neg-G, p <0.05. The levels of 8OHdG in the ES-75-G group were significantly lower than ES-25-G and ES-50-G, p <0.05. However, compared with the Nor-G group, the levels of 8OHdG in the ES-75-G group were still significantly higher or not comparable, p <0.05 (figure 2).

Number of Foam Cell
The result of Mann - Whitney test showed that levels of 8OHdG in ES25-G, ES50-G, and ES75-G groups were significantly lower than Neg-G, p <0.05. The number of foam cells in the ES75-G group was significantly lower than ES25-G and ES50-G, p <0.05. However, compared with the Nor-G group, 8 OHdG levels in the ES75-G group were still significantly higher or not comparable, p <0.05 (figure 2).

DISCUSSION
The results of this study indicated that levels of 8-OHdG and the number of foam cells in groups treated with various dose celery extracts (25 mg/200 g BW, 50 mg/200 g BW, and 75 mg/200 g BW) were significantly lower than in the negative control group. This illustrated that administration of celery extracts can repair DNA damage and reduce the formation of foam cells. Administration of celery extract at the dose of 75 mg/200 g BW has the highest effect in decreasing 8OHdG levels and the number of foam cells. Decreased levels of 8OHdG and reduction in the number of foam cells due to the extract of celery were appeared to be dose dependent. However, the administration of celery extract at the dose of 75 mg/200 g BW for 30 days is still not equivalent to the positive control group (simvastatin). Based on the result that the decrease in the levels of 8OHdG and the number of foam cells appear to have an inverse relationship, with increasing doses of celery extract to 100 mg/200 g BW/day it is expected to decrease the levels of 8OHdG and the number of foam cells, but requires further study.

A high-fat diet given to rats for 30 days may lead to hypercholesterolaemia or dyslipidemia, then trigger oxidative stress in rats endothelial cells. The oxidative stress results in increased lipid oxidation,
especially LDL (ox-LDL), which is then regarded as an unknown component or toxic substance by the body, which triggers the receptor scavenger macrophages to capture the ox-LDL. This is consistent with the results of studies stating that the full oxidation of LDL (ox-LDL) is the initial stage of the formation of foam cells, because LDL can be recognized by the macrophage scavenger receptor if its in the ox-LDL form (Maramis & Kaseke, 2014). The more ox-LDL scavenged, caused the macrophages to develop into foam cells.

The results of this study indicated that a significant increase in the number of foam cells was found in the negative control group, atherosclerosis occurs in rats after a high-fat diet for 30 days (Loho & Lintong, 2015). The large number of foam cells is an early sign and pathognomonic of an atherosclerotic state. Atherosclerosis as a risk factor for cardiovascular disease closely associated with blood lipid disorders state, i.e. the levels of total cholesterol, LDL cholesterol, high triglycerides, and low HDL levels. This situation triggers oxidative stress, endothelial dysfunction and the progression of atherosclerosis (Nur et al., 2012). This finding supports those of previous study that hyperlipidemia is closely related to cardiovascular events such as oxidative stress, endothelial dysfunction, blood brain barrier disorder, and vascular inflammation (Anggraeni et al., 2016). Oxidative stress is characterized by a high increase of free radicals, exceeding the capacity of antioxidant enzymes in cells, thus destroying proteins, DNA, and lipid cells (Susantiningsih, 2015). Free radicals and ROS attack at atomic C number 8 on nucleotide bases produce 8OHdG, higher amount of ROS cause higher number of guanosin oxidized into 8OHdG (Dwi et al., 2015). The inhibition mechanism of elevation of 8-OhdG level mediated by flavonoid (luteolin and apiin) contained in celery extract. Its work by capturing free radicals against hydroxyl radicals (OH•). Flavonoids can regenerate antioxidants to donate hydrogen atoms and act as a chelating agent as well as binding the redox active metals including Fe2+ and Cu2+ involved in the formation of free radicals, thereby reducing the formation of OH• radicals (Ifalah et al., 2016). Referring to the above description, the decrease of 8OHdG and the reduction of the number of foam cells due to the extract of celery is mediated by luteolin and apiin contained in celery (Dalimartaha, 2011). Repair mechanism of single strand DNA damage due to oxidative stress contributes to 8-OhdG excretion, which involves Base Excision Repair, which is to repair one nucleotide damage, excised by glycosylase and replaced by DNA repair synthesis by ligase enzyme (Dwi et al., 2015).

The role of antioxidants in flavonoids of celery extract is to improve the state of oxidative stress by reducing the oxidation process especially on the components of lipoproteins and dampening the reactivity of lipid peroxides that have behaved as free radicals. This caused by the ability of flavonoid extract celery to neutralize oxidants and free radicals due to double bonds and hydroxyl groups in the flavonoid compound (Umarudin & Susanti, 2012).

Decreased levels of 8-OHdG and the number of foam cells with the administrated of celery extract in this study, indicated the ability of celery flavonoids in inhibiting LDL oxidation rate by ROS (Dwi et al., 2015). 8-OHdG as the end product of the lipid oxidation process is a marker that showing the complete lipid oxidation event to form ox-LDL. When LDL is lightly oxidized it is inadequate to be captured by macrophages, but it is capable of causing endothelial dysfunction. The advanced oxidative stress stage that results in complete LDL oxidation can be recognized by the macrophage scavenger receptor in the intima tunica which further forms the foam cell (Nur et al., 2012).

The results of this study showed that 8-OHdG and the numbers of foam cell in the administration of celery extract at the dose of 75 mg/200 g BW were not equivalent to the administration of simvastatin. It is thought to be associated with a relatively low dose. Therefore, further research is needed to obtain the optimal dose celery extract to decrease levels of 8-OHdG and the number of foam cells so its equal with the administration of simvastatin.

CONCLUSION
Administration of celery extract decreased the level of 8-OHdG and the number of foam cell. There was a significant difference of 8-OHdG levels and foam cell number (p < 0.05) between the negative control group (Negr-G) and other groups (Nor-G, ES-25-G, ES-50-G and ES-75-G).

REFERENCES
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