EFFECT OF Polygonum minus (KESUM) LEAVES ETHANOLIC EXTRACT ON HISTOPATHOLOGICAL CHANGES ON THE WALL AORTA OF MICE (Mus musculus) INDUCED BY CADMIUM CHLORIDE ANTIOXIDANT

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ABSTRACT

This study was conducted to investigate the protective effect Polygonum minus leaves extract on the histopathological changes on aorta wall of mice (Mus musculus) induced by cadmium chloride. Thirty male mice were divided into five groups and were administered via intragastric gavage with different treatments for 21 days. The treatment were Negative Control (CMC Na 0.5% solution + aquadest), Positive Control (CMC Na0.5% solution + 8 mg/kg bw of mercuric chloride), Treatment 1, 2, and 3 (200, 400, and 800 mg/kg bw of Polygonum minus leaves extract respectively + 8 mg/kg bw of cadmium chloride). The microscope examine followed analyzed by One Way ANOVA continued with Duncan test for counting the amount of foam cell and the histopathological changes of wall aorta were examined by using Ifora Scoring method was analysed using Kruskal Wallis and continued with Mann-Whitney test. The result showed Polygonum minus leaves extract could protect wall aorta of mice from the damage effect of cadmium chloride. The best dose of Polygonum minus on this research was 400 mg/kg bw.

Key words: Polygonum minus, cadmium chloride, Mus musculus, aorta

INTRODUCTION

The amount of heavy metals in industrials waste are use in a few amount, such as mercury, lead, copper, alumunium and cadmium. Those heavy metals that contributed to contaminate of water environment (Istarani and Pandebesie, 2014). Cadmium (Cd) is one of the heavy metal group II B, it has white bluish colour, soft, and non-soluble base (Erliyanti, 2015). The cadmium exposure through ingestion of contaminated water, food, and maybe from air polution through inhalation with the primarily affects the kidneys, liver and intestine, also mentioned in other result of a study that it can cause damaging cardiovascular system by promote endothelial damage that might in turn contribute to inflammation, vascular injury the development of atherosclerosis (Almenara, 2013).

Has been reported that cadmium spesifically will be accumulated in the wall of aorta (Hayeh, 2001). Cadmium stimulate reactive oxygen species (ROS) production in cell. While, ROS, lead to cellular damage in this condition contributes oxidation of low density lipid (LDL) into oxidized LDL (ox-LDL), endothelial dysfunction, migration and proliferation of vascular smooth muscle cell, adhesion and migration monocytes with the development of foam cells those are the indication of atherosclerotic lesion (Boharun et al., 2006).

Atherosclerosis is the major risk of coronary artery disease (CAD), which has become a leading cause of death in developed countries and antioxidant enzymes are considered as the first line of cellular defense to prevents cellular components from oxidative damages
Putri Anggraheni. dkk. Effect of *Polygonum minus* (Kesum) Leaves Ethanolic Extract

(Lei, 2011). This condition initiated by lipid retention, oxidation, and modification, which provoke chronic inflammation, ultimately causing thrombosis or stenosis (Insull, 2009).

Some studies reported that there is no specific antidote for cadmium intoxication. Giving dimercaprol as one of the chelating agent is used to do, but it just attenuate its toxicity by converting them in less toxic compounds (Crisponi and Nurchi, 2016). Most of the chelators have the disadvantages of numerous adverse effects, it non-specific binding and administration is inconvenience (Flora and Pachauri, 2010).

Herbs plants are well known to be associated with many medicinal properties (Hassim et al., 2003) which has low side effects and can be as an alternative treatment (Desai et al., 2003). *Polygonum minus* or “kesum” in Indonesian are used as food ingredient, hair dandruff and also indigestion (Ghazali et al., 2014) and easily find in Sumatra and Kalimantan. The high contents of polyphenol, vitamin C and β carotene suggested to be responsible for the antioxidant activity of *Polygonum minus*. Furthermore, this plant has flavonoid named quercetin that connected to the antioxidant activity of this plant (Christapher et al., 2015). Previous histological observation of the aorta for any changes or modifications indicated that the extract of *Polygonum minus* also known as *Persicaria minor* is quite safely even at higher doses and had no acute toxicity (Muhammad et al., 2013).

The aqueous extract of *Polygonum minus* has significant values (p<0.005) cytoprotective properties in rats (Wasman et al., 2010). There are several methods have been used to assess the antioxidant activity of *Polygonum minus* such as total phenolic compound (TPC), 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) and ferric reducing antioxidant power (FRAP) (Huda-Faujan et al., 2010; Sumazian et al., 2010). The presence of phenolic compounds in high antioxidant of *Polygonum minus* (Scalzo J et al., 2005; Shela G et al., 2010). It also mentioned about vitamin that kesum has such as carotene, retinol equivalent, vitamin C, vitamin E, and also contains several minerals like calcium, copper, zinc, phosphorous, iron, sodium, potassium, and magnesium (Ching et al., 2001). The use of vitamin C to protect celluler components from free radical-induced damage (Dasgupta and Klein, 2014).

**RESEARCH MATERIALS AND METHOD**

**Material and Equipment**

The experimental units that used in this research are 30 healthy male mice (Mus musculus) strain BALB/C with the average weight of 25-35 grams, 10 weeks old from Pusat Veterinaria Farma (PUSVETMA).

Materials that used in this research are included of cadmium in the form of Cadmium(II)chloride with chemical formula (CdCl2) from PT. Multi Eka Chemicalindo, sterile aquadest, *Polygonum minus* leaves, ethanol 96 %, broiler feed, 0.5 % Na CMC, and Neutral Buffered Formalin 10 % for tissue fixation. Chemicals that were used in histopathological preparation are 70, 80, 90 and 96 % alcohol, xylol, paraffin, Hematoxylin, Eosin and entellan.

The equipment that used in this research include of: scale to measure the weight of mice, five units of mice cage in the form of rectangular plastic tubs covered with wire, container for feed and drink, rotavapor, analytical scale, intubation needle for mice, 1 ml tuberculin syringes, surgical scissor, forceps, scalpel, plastic pots, object glass, cover glass, microscope and camera for research documentation. For histopathological preparation, the equipment that used were a series of dehydration apparatus, microtome, water bath and hot plate.
Mice were divided randomly into five groups, C (-), C (+), T (1), T (2) and T (3). Each treatment consists of six mice. Then all groups of mice were adapted for one week, provided balance and water ad libitum daily.

Research Plan

This research using Completely Randomized Design. In this design there is only one source of variability, that is the random effect of treatment on the mice, so the different result of the treatment only caused by the treatment’s effect and random effect. Then this research using five groups and six replicates for each group.

Polygonum minus Extract Preparation

Fresh leaves of Polygonum minus were collected from Singkawang, West Borneo, shade dried and pounded into powder before extraction. Polygonum minus leaves powder (2 kg) soaked in 8 liter of 96 % ethanol for 12 days. Filtration was done to separate the dregs from the solution. Then it was evaporated using a rotavapor at 50 0C with 40 rpm for 4-5 hours to obtain a viscous extract.

Treatments

Mice (Mus musculus) were captured in mice cage placed in Laboratory of Experimental Animal at the Faculty of Veterinary Medicine Universitas Airlangga, randomized by lottery and were divided into five groups. Then were adapted to the environment for one week. After the adaptation period, the treatment group was administered with P. minus leaves ethanol extract for 3 days.

Experimental animal were administered with P. minus leaves ethanol extract and cadmium chloride by intragastric gavage using 1 ml disposable tuberculin for 21 days. The treatment explained as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (-)</td>
<td>CMC Na 0.5% solution + aquadest</td>
</tr>
<tr>
<td>C (+)</td>
<td>CMC Na 0.5% solution + 12 mg/kg bw cadmium chloride</td>
</tr>
<tr>
<td>T (1)</td>
<td>200 mg/kg bw Polygonum minus leaves ethanol extract + 12 mg/kg bw of cadmium chloride</td>
</tr>
<tr>
<td>T (2)</td>
<td>400 mg/kg bw Polygonum minus leaves ethanol extract + 12 mg/kg bw cadmium chloride</td>
</tr>
<tr>
<td>T (3)</td>
<td>800 mg/kg bw Polygonum minus leaves ethanol extract + 12 mg/kg bw cadmium chloride</td>
</tr>
</tbody>
</table>

Each group are administered the treatment in the same time every day for 21 days. The experiment was done in 31 days.

After 24 hours from the last treatment, treatment groups of C (-), C (+), T (1), T (2) and T (3) are sacrificed by cervical dislocation method and the testes of mice collected.

Microscopic Examination

Microscopic observation of mice aorta used a microscope with 100 times magnification and continued with 400 times magnification. The examination was done by counting the amount of foam cell per five random fields of view, also grading the histopathological changes per five random fields of view of aorta structure using Ifora scoring (Ifora et al., 2016).

Data Analysis

Data for each group was analysed statistically One-way ANOVA then followed by Duncan test for the amount of foam cell and used Kruskal Wallis test then followed by Mann-Whitney for analysed the pathological change of wall aorta. The test to compare of the treatment effect of each group. Statistical analysis for this experiment is using SPSS 20.0 for Windows software.
RESEARCH RESULT

The examination of histopathological preparation were using Hematoxylin Eosin (H&E) staining. Examination on 5 fields of view randomly per areas, using microscope with 100x times magnification, then continued with 400x times magnification. Histopathological changes in aorta that have been observed use Ifora Scoring (2016) are endothelial cell rupture, amount of foam cell, smooth muscle cell proliferation using SPSS for Windows 20 program using one way ANOVA test then and plaque performing. The scoring result were averaged and then analyzed followed by Duncan test for the amount of foam cell. The result of One Way ANOVA test showed (p<0,05) and Kruskal-Wallis test showed (p<0,05) then it followed by Mann-Whitney test to show the difference between each group.

The results of examination of histopathological changes and amount of foam cell formation of wall aorta of mice could be seen in table 4.1 below:

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Amount of Foam Cell (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Negative control</td>
<td>3,33d ± 5,04</td>
</tr>
<tr>
<td>2.</td>
<td>Positive Control</td>
<td>197,66a ± 41,32</td>
</tr>
<tr>
<td>3.</td>
<td>Treatment 1</td>
<td>120,83b ± 36,91</td>
</tr>
<tr>
<td>4.</td>
<td>Treatment 2</td>
<td>65,33c ± 9,91</td>
</tr>
<tr>
<td>5.</td>
<td>Treatment 3</td>
<td>52,16c ± 2,63</td>
</tr>
</tbody>
</table>

Table 4.2 Atherosclerotic Lesions Grades on mice’s (Mus musculus) wall aorta

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Figure 4.2 Amount of foam cell on wall aorta of mice (*Mus musculus*).

Figure 4.3 Histopathological changes of wall aorta, foam cell formation (black arrow), aorta lumen (bold A), endothelial cell (yellow arrow), endothelial cell rupture (blue arrow), plaque performing (red arrow), smooth muscle cell proliferation (grey arrow) (400x magnifying, H&E).
DISCUSSION

Based on statistical analysis result from Table 4.1, it can be seen treatment group of Negative Control showed significant difference with treatment group Positive Control, Treatment 1, Treatment 2, and Treatment 3. Positive Control group showed significant difference with Negative Control, Treatment 1, Treatment 2, and Treatment 3. Treatment 1 group has significant difference with Negative Control, Positive control, Treatment 2 and Treatment 3. Treatment 2 Has significant difference with Negative Control, Positive Control and Treatment 1, but not significant difference with Treatment 3. Data of amount of foam cell was also shown on Figure 4.2.

The effect of Polygonum minus with 200 mg/kg dose still not giving differences yet to foam cell formation, it can be seen between Positive Control group and Treatment 1 has no significant difference to amount of foam cell formation. But with 400 mg/kg and 800 mg/kg doses it has significant difference with Positive Control group and Treatment 1. It can be implied, Polygonum minus leaves extract has ability to foam cell formation on wall aorta.

Statistical analysis result from Table 4.2, it can be seen Negative Control group showed significant difference with treatment group Positive Control, Treatment 1, Treatment 2, and Treatment 3. Positive Control group showed significant difference with Negative Control, Treatment 2, and Treatment 3 but not significant with Treatment 1. Treatment 1 group has no significant with Positive control, but significant difference with Negative Control, Treatment 2 and Treatment 3. Treatment 2 Has significant difference with Negative Control, Positive Control and Treatment 1, but not significant difference with Treatment 3.

Cadmium toxicity is normally involved with the depletion of cellular GSH and protein bound sulhydryl groups, resulting in disturbance of the cellular redox balance which leads to enhancement production of ROS (Casalino et al., 1997, Waisberg et al., 2003, Wang et al., 2004). Moreover increasing of ROS formation provoke lipid, protein, and DNA oxidation (Oda and Ibrahim, 2012). Those ox-LDL would phagocytosed by macrophage which developed from macrophage colony-stimulating factor (MCSF) and its turn into foam cell (Shworak et al., 2013; Crowther et al., 2005).

(Figure 4.1 C-): Negative control group showing a normal appearance of spermatogenic cells with no histopathological changes. The condition of endothelial cell has no damages, foam cel (Figure 4.1 C+): Sections of histopathological changes in
wall aorta of mice showing endothelial rupture, foam cell formation, irregular elastica membrane interna, and smooth muscle cell proliferation.

(Figure 4.1 T1): Treatment 1 showing endothelial cell still good but foam cell formation are increasing, also there was found plaque formation (Figure 4.1 T2): Treatment 2 showing endothelial cell still good, foam cell formation decrease and smooth muscle cell decrease (Figure 4.1 T3): Treatment 3 showing endothelial rupture, foam cell formation decreases but the smooth muscle cell are increase.

Other pathological changes that happened on wall aorta such as endothelial rupture, elastica membrane interna being irregular, smooth muscle proliferation and accumulation of foam cell on sub-endothelial became plaque those were leading to atherosclerosis lesions (Ifora, et al., 2016).

The earliest changes that precedes the formation of lesions of atherosclerosis take place in the endothelium, with resultant EC dysfunction. The initial response of EC to injury can result in decreased production of nitric oxide (NO), increased permeability to lipoprotein and other plasma constituents (Ross, 1999), leukocyte adhesion, and thrombotic potential (Schoen, 2005). Ifora, et al. (2016) also mentioned atherosclerotic lesion, such as endothelial rupture, foam cell formation, irregular membrane elastica interna, smooth muscle cell proliferation and plaque performing. Furthermore, activated macrofag released chemoattractant substance and cytokine (monocyte chemoattractant protein-1, tumor necrosis factor α, IL-1, IL-6, CD40 dan C-reactive protein), the substances caused migration smooth muscle cell from tunica media to tunica intima (Kumar et al., 2007; Kumar et al., 2009).

In addition, tunica intima was separated from tunica media by an elastical membrane interna, this membrane was elastin fibers, has function to nourish cells of wall blood through fenestra which allows substance diffusion (Mescher, 2011).

After intimal injury, different cell types, including endothelial cell, platelets, and inflammatory cells release mediators, such as growth factors and cytokines that induce multiple effects. This growth factors and cytokines will promote the change of vascular smooth muscle cells from the quiescent contractile state to the active synthetic state, extracellular matrix protein deposition, vascular smooth muscle cell migration and proliferation from the media in to the intima (Willis et al., 2004).

Afterward the cycles of accumulation of mononuclear cells, proliferation of vascular smooth muscle cell, and formation of fibrous tissue leads to further enlargement and advanced complicated lesion formation (Rudijanto, 2007). Changes in arterial wall histopathology are the end stage of atherosclerosis process, which can be evaluated (Kabo, 2008)

Result in this study about atherosclerosis lesions showed that Treatment 1 with dose 200mg/kg bw of Polygonum minus has significant difference with Negative Control, and no significant difference with Positive Control. It indicates that the antioxidant activity from Polygonum minus leaves extract on that dose was not sufficient to against ROS formation.

Treatment 2, dose 400 mg/kg bw of Polygonum minus, was showing a significant difference with Positive Control and Negative Control. Even though amount of foam cell showed no significant different with Treatment 3, 800 mg/kg bw of Polygonum minus, but this dose show the lowest of atherosclerosis lesions compared to Positive Control and Treatment 1. As it has been explain, Polygonum minus leaves contains of flavonoids such as myricetin, quercetin,
gallic acid and coumaric acid (Imelda et al., 2014; Qader et al., 2012) that play important role as antioxidant.

The other compounds in Polygonum minus leaves extract that play role as antioxidant is gallic acid that can protect peroxidation of lipid by scavenging the free radical and lipid peroxidation inhibitory activity (Badhani et al., 2015). Through this mechanism, ROS production by cadmium chloride can be reduced so the atherosclerosis lesion can be reduced. Also the polyphenol content that known as anti-atherosclerotic and antioxidant messenger, suppress Scavenger Receptor – A1 (SR-A1) expresion by inhibiting peroxisome proliferator-activated receptor-γ (PPAR-γ), a transcriptional regulator that controls lipid uptake, fatty acid storage, and glucose metabolism (Zhao et al., 2012).

Treatment 3, 800mg/kg bw of dose Polygonum minus leaves extract showed significant difference with Negative Control and Positive Control. It was implicated the reduction antioxidant activity on this dose, resulted from the excessive of flavonoid. Skibola and Martyn (2000) reported that excessive intake of flavonoid especially quercetin can act as pro-oxidants that generate free radicals, as mutagens, and as inhibitors of key enzymes involved in hormone metabolism. Therefore the atherosclerosis lesion still happened after given Treatment 3.

CONCLUSION

Based on this research, it could be concluded Polygonum minus leaves ethanol extract could reduce the histopathological changes on wall aorta of mice (Mus musculus) due to cadmium chloride exposure. The best protective dose of Polygonum minus ethanol extract is 400 mg/kg bw.

REFERENCES


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