Conference Paper

EFFECT OF *Polygonum minus* (KNOTWEED) LEAVES EXTRACT ON THE HISTOPATHOLOGICAL CHANGES OF KIDNEY IN MICE (*Mus musculus*) INDUCED BY MERCURIC CHLORIDE

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Abstract

This research was conducted to investigate the protective effect *Polygonum minus* leaves extract on the histopathological changes of kidney induced by mercuric chloride in mice (*Mus musculus*). Thirty male mice were divided into five groups and were administered via intragastric gavage with different treatments for 21 days. The treatment were C- (CMC Na 0.5% solution + aquadest), C+ (CMC Na 0.5% solution + 8 mg/kg bw of mercuric chloride), T1, T2, and T3 (200, 400, and 800 mg/kg bw of *Polygonum minus* leaves extract respectively + 8 mg/kg bw of mercuric chloride). The histopathological changes of kidney were examined by using Arshad Scoring method. Then the data was analysed using Kruskal Wallis and continued with Mann-Whitney test. The result showed *Polygonum minus* leaves extract could protect mice kidney from the damage effect of mercuric chloride. The best dose of *Polygonum minus* on this research was 400 mg/kg bw.

Keywords: *Polygonum minus*, mercuric chloride, *Mus musculus*, kidney.

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1. Introduction

Environmental pollutant has become the major problems since the industrialization rapidly increased without adequate residue and waste control in many country especially in the developing country (Xianghua, 2000). It is impact to the water, air, and soil. One of the heavy metals that act as the environmental pollution is mercury. Mercury is usually used in the industrial processes such as in dry battery, paint production, plastic, soda, gold mining, agriculture fungicide, disinfectant and topical antiseptic (Patrick, 2002; Nabi, 2014).

The levels mercury pollution in Indonesia has serious implication. In Kapuas River, West Borneo, and in Cikaniki River in West Java the mercury levels in water was about 0.002 ppm (Triana, 2012), while the level standard based on government in PP. No. 82/2001 is 0.001 ppm. Handajani and Budiono (2000) in Rahayu (2002) found that the mercury concentration in shellfish from Kenjeran beach was about 0.6418 ppm that is higher than the maximum rate of mercury in food based on WHO and FAO that is 0.5 ppm.

Mercuric chloride is one of the inorganic mercury that is mostly found in water (Junita, 2013). Mercuric chloride has been reported can cause acute renal, corrosive in the gastrointestinal tract, hepatomegaly, and the death (Triunfante *et al*., 2009; Verma *et al*., 2010; Dias *et al*., 2015). The primary target organ of exposure to mercuric chloride is kidney (Zalups and Lash 1994). Within the kidney, mercuric chloride will induce the formation of ROS such as hydrogen peroxide. It is also decreasing the activity of antioxidant enzyme like superoxide dismutase, catalase, and GSH (Glutahione Sulfhydryl) (Zalups, 2010).

The common medication for mercury intoxication is DMSA (Dimercaptosuccinic Acid) that is given orally. It increase urinary mercury excretion but result some adverse effects such as diarrhea, bloating, rashes, vomiting and GI discomfort (Crinnion, 2000). From that adverse effects, it is needed natural product which has low side effect. Herb plants are well known to be associated with many medicinal properties (Hassim *et al*., 2014) which has low side effects and can be as an alternative treatment (Desai, *et al*., 2003).

Polygonum minus or kesum in Indonesia is traditionally used as food ingredient, hair dandruff, and also indigestion (Ghazali *et al*., 2014). It can be found also in Sumatera and Kalimantan. The high polyphenol content, quercetin, myricetin, suggested to be responsible for the antioxidant activity of *Polygonum minus* (Christapher *et al*., 2015).

Based on the background above due the adverse effect of mercuric chloride especially cause damage in kidney, and the potential of *Polygonum minus* due its high antioxidant that against the free radicals, might be considered as a natural medicine resource to protect the effects of individual and simultaneous exposures to mercuric chloride.

2. Materials and Methods

This research was conducted at the Animals Model Laboratory and Veterinary Pathology Department of Veterinary Medicine Faculty, Faculty of Medicine, Universitas Airlangga. Implementation of this research was carried out from December 2016 - January 2017. The object in this study are thirty healthy male mice (*Mus musculus*) strain BALB/C aged 10 weeks old with an average weight of 25-35 grams, maintained at the same place and were given the same feed.

The equipment used in this study include animal, balance, water container, spuit 1 ml, feeding tube, gloves, surgical instruments, microscope, a series of dehydration apparatus, microtome, water bath and hot plate. Materials used in this study were *Polygonum minus* leaves extract, aquadest, mice feed, *ad libitum* drinking water, Hematoxylin Eosin (HE) stain, and buffer formaline. Chemicals used in histopathological preparation are 70, 80, 90 and 96% alcohol, xylol, paraffin, entellan and Hematoxylin Eosin.

Treatment started after the adaptation period for a week, then the animal models weighed and randomly divided into five groups:

C (-): CMC Na 0.5% solution+ aquadest 0.01 ml/g bw

C (+): CMC Na 0.5% solution + 8 mg/kg bw mercuric chloride (Sheikh *et al.*, 2013).

T (1): 200 mg/kg bw *Polygonum minus* leaves extract (George *et al.*, 2014a) + 8 mg/kg bw mercuric chloride

T (2): 400 mg/kg bw *Polygonum minus* leaves extract (George *et al.*, 2014a) + 8 mg/kg bw mercuric chloride

T (3): 800 mg/kg bw Polygonum minus leaves extract + 8 mg/kg bw mercuric chloride

2.1. Polygonum minus Leaves 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°C with 40 rpm for 4-5 hours to obtain a viscous extract.

Table 1: Data Result ($p<0.05$).

Treatment	Parameters (Mean ± SD)		
	Hydropic Degeneration	Necrotic Tubular Cells	Tubular Cast
C-	$0.27^{a} \pm 0.10$	$0.90^{a} \pm 0.17$	$0.30^{a} \pm 0.11$
C+	$0.90^{b} \pm 0.28$	$2.90^{d} \pm 0.17$	$1.93^{d} \pm 0.41$
T1	$0.83^{b} \pm 0.15$	$2.63^{d} \pm 0.23$	$1.67^{d} \pm 0.16$
T2	$0.80^{b} \pm 0.18$	$1.17^{ab} \pm 0.32$	$0.50^{b} \pm 0.11$
T3	$0.93^{b} \pm 0.27$	$2.23^{c} \pm 0.23$	$1.30^{c} \pm 0.28$

2.2. Histopathological Preparation Observation

The observation was conducted by using Olympus® CX-21 microscope that connected to Opti Lab Viewer and Image Raster 3 software with 100x and 400x magnification. Then the examination was done by calculating the percentage of the lesion per five times from random fields of view area using Arsad scoring (Arsad *et al.*, 2014).

3. Data Analysis

Data for each group were analysed statistically using Kruskal Wallis Test followed by Mann-Whitney Test to compare the treatment effect of each group. Statistical analysis for this experiment is using SPSS 20.0.

4. Result

The examination results obtained from each treatment groups of C-, C+, T1, T2 and T3, then processed with Statistical Product and Service Solutions (SPSS) program using Kruskal-Wallis test. Results Obtained by Kruskal-Wallis test showed $p= 0.004$ ($p<0.05$) for hydropic degeneration of tubules, $p= 0.000$ ($p<0.05$) for necrotic tubular cells and tubular cast, which are all of those parameters are having the significant difference. After that, continued with Mann-Whitney U test to see the differences between each group. The data result could be seen in Table 1 below.

The data results and histophatological features of kidney tubules on mice were also shown on the Figure 1 and Figure 2 below.

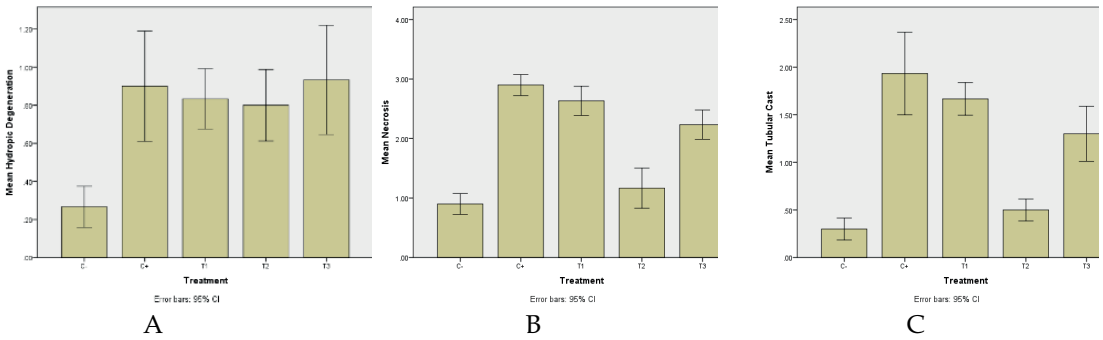

Figure 1: Data result of hydropic degeneration (A), necrotic tubular cells (B), and tubular cast (C) of mice kidney.

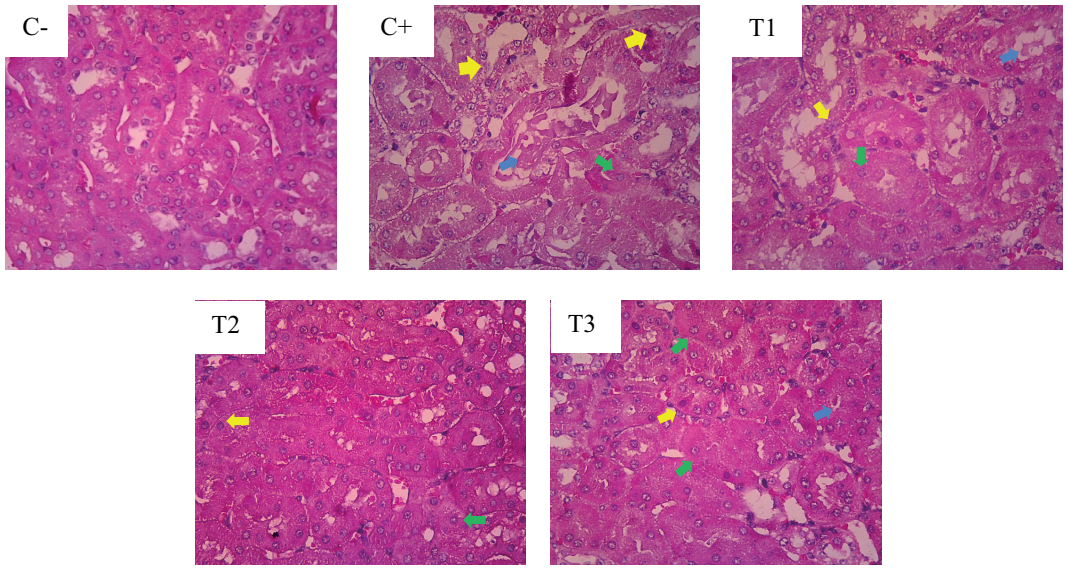

Figure 2: Histophatological features of kidney tubules on mice (400x magnification, H&E) necrosis (yellow arrow), hydropic degeneration (green arrow) and tubular cast (blue arrow). C- (negative control group), C+ (positive control group), T1(200 mg/kgBW of *Polygonum minus*), T2 (400 mg/kgBW of *Polygonum minus*), T3 (800 mg/kgBW of *Polygonum minus*).

4.0.1. Examination of Hydropic Degeneration

Table 1 represents the effect of *Polygonum minus* leaves extract on histopathological changes of kidney. Treatment group of C+ that were administered with mercuric chloride showed significant difference compared to C- ($p<0.05$) but did not show significant difference with the group T1, T2, and T3 that were administered with *Polygonum minus* leaves extract doses of 200, 400, and 800 mg/kgbw.

4.0.2. Examination of Necrotic Tubular Cells

The necrotic tubular cells was significantly increased after mercuric chloride administration, while the administration of 200 mg/kg bw of *Polygonum minus* leaves extract

(T1) showed the same result with mercuric chloride administration. *Polygonum minus* leaves extract administration at the dose of 400 mg/kg bw (T2) significantly decreased the necrotic tubular cells. Meanwhile the dose 800 mg/kg bw of *Polygonum minus* leaves extract (T3) showed significant different with mercuric chloride administration but not as same as group of 400 mg/kg bw of *Polygonum minus* leaves extract (T2).

4.0.3. Examination of Tubular Cast

Mercuric chloride administration significantly increased the tubular cast, while the administration of 200 mg/kg bw of *Polygonum minus* leaves extract (T1) showed the same result with mercuric chloride administration. *Polygonum minus* leaves extract administration at the dose of 400 mg/kg bw (T2) significantly decreased the tubular cast. Whereas the dose 800 mg/kg bw of *Polygonum minus* leaves extract (T3) showed significant different with mercuric chloride administration but not as same as group of 400 mg/kg bw of *Polygonum minus* leaves extract (T2).

5. Discussion

Mercuric chloride that has entered to the body will bind with sulfhydryl group to form GSH-Hg-GSH that will be cleavage to Cys-Hg-Cys by GGT in kidney. Moreover, binding of mercuric ions with sulfhydryl group may cause decrease glutathione levels, superoxide dismutase, catalase and GSSG. It lead to increase in levels of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl (Zalups, 2010). Those ROS formation provoke lipid, protein, and DNA oxidation (Oda and Ibrahim, 2012).

According to McGavin and Zachary, (2007) lipid peroxidation can destroy plasma membrane that will cause increasing the permeability of Na^+, H_2O, and Ca^{2+} and disturb Na^+-K^+ ion pumps. Those will move into the cells and lead to cell swelling or hydropic degeneration. On this study, C+ that were only received mercuric chloride showed no significant different with group T1, T2, and T3 that were received *Polygonum minus* leaves extract.

Those insignificant difference on C+ might be caused the cell injury had passed hydropic degeneration stage because hydropic degeneration is early stage of cell injury and on this research mercuric chloride was continuously given for 21 days resulting the severe changes and become necrosis. Therefore, in the group of C+ had predominant necrotic tubular cells with less of hydropic degeneration.

According to Zalme *et al* (1976) in Zalups 2010 during early stages of nephropathy induced by mercury, tubular cells undergo number of degenerative changes. When tubular injury becomes severe (either the high dose or prolong exposure) the necrosis of tubular cell will appear.

Furthermore, those lipid peroxidation will also damage some organelles membranes such as mitochondria and lysosome. The damage of mitochondrial membrane induce Ca^{2+} efflux to the cytosol, depletion of oxidative phosphorylation and ATP. Increasing intracellular Ca^{2+} activates number of enzymes such as ATPases that also resulting depletion of ATP, proteases that will cause cell membrane damage and endonuclease that will degrade the chromatin cells. Injury to lysosome membrane results in leakage of lysosome enzymes into the cytoplasm, leading to enzymatic digestion of protein, RNA, DNA, glycogen, and the cells die by necrosis (Kumar *et al.,* 2014).

Those necrosis tubular cells will fulfill the tubular kidney as cellular cast (Ringsrud, 2001), which also will impact upon debris formation to do the removal. Those rest of removal will present on the tubules as granular cast (Lindquist and Peter, 2016). According to Berlin *et al* (2015) mercuric chloride can damage tubules and glomerulus, so it diminish tubules and glomerulus function on filtration and reabsorption. Those resulted protein cast on tubules lumen. This mechanism is parallel with the results of C+ that were only administered by mercuric chloride showed the highest on necrotic tubular cells and tubular cast compared to other groups.

Polygonum minus leaves contain of flavonoids such as myricetin, quercetin, gallic acid and coumaric acid (Imelda *et al.,* 2014; Qader *et al*., 2012). Myricetin has been reported can inhibit lipid peroxidation (Hasan *et al*., 2017), and DNA peroxidation (Abalea *et al.,* 1999).

In the parallel of this, quercetin can also prevents lipid peroxidation that helps to preserve membrane integrity, donate electrons, and increase the production of antioxidant enzyme such as superoxide dismutase, catalase, glutathione reductase, and glutathione-peroxidase (Banjarnahor and Nina, 2014). The other compound in *Polygonum minus* leaves extract that play important 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 mercuric chloride can be reduced so histopathological changes such as hidropic degeneration, necrosis and tubular cast were also reduced.

On the present study treatment group of T1 with dose 200mg/kg bw of *Polygonum minus* showed significant difference with C- and no significant difference with C+,

indicates the antioxidant activity from *Polygonum minus* on that dose was not sufficient to against ROS formation.

Whereas treatment group of T2 with dose 400mg/kg bw of *Polygonum minus* showed significant different with C+ and no significant difference with C-. This indicate there was the sufficient protection mechanism from *Polygonum minus* leaves extract.

Treatment group of T3 with 800mg/kg bw dose of *Polygonum minus* leaves extract showed significant difference with C- and C+, indicates there was the reduction of antioxidant activity. 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.

6. Conclusion

Based on this research, it could be concluded *Polygonum minus* leaves extract could reduce the damage of histopathological changes in mice kidney (*Mus musculus*) with mercuric chloride induction. The best dose of *Polygonum minus* on this research was 400 mg/kg bw.

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