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THE EFFECT OF CORIANDER LEAF EXTRACT TOWARDS KIDNEY HISTOPATHOLOGICAL FEATURES ON WISTAR RAT INDUCED BY ORALLY ADMINISTERED MERCURY

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ABSTRACT

Background: Mercury's a toxic heavy metal that damages the kidney through generation of Reactive Oxygen Species (ROS). Previous study has established that coriander leaves contained high level of antioxidants. However, there hasn't been any experiment that examined renoprotective effect of coriander leaf extract toward the kidney of Wistar rat induced with orally administered mercury. **Objective:** To examine the nephroprotective activity of coriander leaf extract towards Wistar rat's proximal tubules induced with orally administered mercury **Methods:** : Experimental study with post test only control group design using 20 male Wistar rats divided randomly into 4 groups as samples. Group K0 without treatment, Group K(-) was administered 10 mg/ kgBW mercury chloride orally, Group P1 was administered 10 mg/kgBW mercury chloride and 100 mg/kgBW coriander leaf extract orally, and Group P2 was administered 10 mg/kgBW mercury chloride and 200 mg/kgBW coriander leaf extract orally. The study was carried for 14 days after which the kidneys were examined microscopically. **Results:** The mean values for damaged proximal tubules were as follows: group K0 2,44±1,19; group K(-) 4,76±3,77; group P1 4,52±2,18; group P2 2,60±1,38. Mann-Whitney test showed significant differences between group K(-)>K0 (p=0,009); group P1>K0 (p=0,001); group P2<K(-) (p=0,015); group P1>P2 (p=0,001). Insignificant differences were found between group P2> K0 (p=0,936) and group P1<K(-) (p=0,579). **Conclusion:** Coriander leaf extract could reduce the number of damaged proximal tubules from mercury ingestion, with dose of 200 mg/kgBW showing better result than 100 mg/kgBW.

Keywords: *antioxidants, coriander leaf, kidney, mercury*

INTRODUCTION

Mercury is a toxic heavy metal that can cause serious health issues in different organ systems based on its type, dose, and frequency. There are three forms of mercury, namely elemental mercury, inorganic mercury, and organic mercury. Differences in chemical stuctures affect their pharmacokinetics and clinical manifestation [1].

Mercury is placed third on the *US Government Agency for Toxic Substances and Disease Registry* (ATSDR) priority list of toxic substances that frequently contaminated water, soil, air, and food. According to World Health Organization (WHO), mercury is one of ten chemical substances that cause major public health issue [2]. Globally, ASGM (Artisanal and Small-scale Gold Mining) is the biggest source of mercury contamination by human (37,7%), followed by coal combustion (21%), non-ferrous metal industry (15%) and cement production (11%) [3]. In Indonesia, ASGM provided livelihood for more than one million people from 27 provinces [4]. An experiment conducted in Cisarua village, Bogor, on 2014 found that 60% of samples experience mercury poisoning with concentration over 2 ppm [5].

Mercury exposure to human in present time can be found in the use of dental amalgam, sphygmomanometer, barometer, fuel emmisions, battery, or industrial waste [6]. Every type of mercury is toxic to digestive system, nervous system, and the kidney. The cause for oxidative damage is mercury incitement towards formation of ROS (Reactive Oxygen Species) [7]. Until now, the main therapy for heavy metal intoxication is chelating agent. British Anti-Lewisite (BAL) or other chelating agent like DMSA (meso 2,3-dimercaptosuccinic acid) and DMPS (sodium 2,3-dimercaptopropane 1-sulfonate) with fewer side effects was also introduced as therapy for mercury intoxication. Despite its use, chelating therapy generally cause toxicity and adverse side effects such as nausea, diarrhea, rash, even death [8,9].

Coriander (Coriandrum sativum L.) is a spice plant that is widely used as food seasoning. Coriander leaves have antioxidant properties because it containts vitamin A, vitamin C, alkaloid

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compunds, flavonoid, terpeneoids, tannins, and phenol [10,11]. There has not yet been any experiment that examine the renoprotective effect of coriander leaves towards the kidney of Wistar rat that is given orally administered mercury. Therefore, research for alternative herbal therapy using coriander leaves as means to treat mercury intoxication become important to accomplish. The aim of this study was to prove that administration of coriander leaf extract could protect the kidney proximal tubules of Wistar rats that were induced by orally administered mercury.

METHOD

The research model was experimental with post test only control group design. The samples used in this experiment were 20 Wistar rats provided by Animal Care, Universitas Negeri Semarang, which fullfilled following inclusion criterias: male, age 2-3 months, weigh 100-200 grams, healthy and active, without anatomical abnormalities. Exclusion criterias include rats that got sick or died during the experiment. The independent variable was the dose of coriander leaf extract and the dependent variable was the number of damaged proximal tubules in Wistar rat's kidney.

Coriander leaves were acquired from a plantation in West Bandung Regency. Fresh coriander leaves were sun dried and grinded into powder. The powder was macerated in 70% alcohol for three days and filtered. The filtrate was concentrated using rotatory evaporator to gain condensed extract [12]. In this experiment 900 grams of dried coriander leaves produce 37 grams of condensed extract (extraction yield 4.1%).

The mercury used was mercury (II) chloride ($HgCl_2$) manufactured by Pudak Scientific. Mercury stock solution was made by dissolving mercury chloride in water using stirrer.

The rats were acclimated for 7 days before the experiment and were given standard diet and ad libitum water intake. The rats were divided randomly into four groups, each groups composed of five rats, following guidelines from *Institutional Animal Care And Use Comitee Guidebook* and *World Health Organization* on sample size of 5 per group on 3R principle (*Replacement, Reduction, and Refinement*) [13,14], specifically normal control group (K0) that was not given mercury chloride solution nor coriander leaf extract, negative control group (K(-)) that was given mercury chloride solution of 10 mg/kgBW only, experimental group 1 (P1) that was given mercury chloride solution of 10 mg/kgBW and coriander leaf extract of 100 mg/kgBW, and experimental group 2 (P2) that was given mercury chloride solution of 10 mg/kgBW and coriander leaf extract of 200 mg/kgBW.

The experiment was carried out for 14 days. On the fifteenth day, the rats were terminated by cervical dislocation, precedingly given ketamin intramuscular injection as anasthesia. After that, the kidneys were harvested and made into histopathology slides with Hematoxillin-Eosin

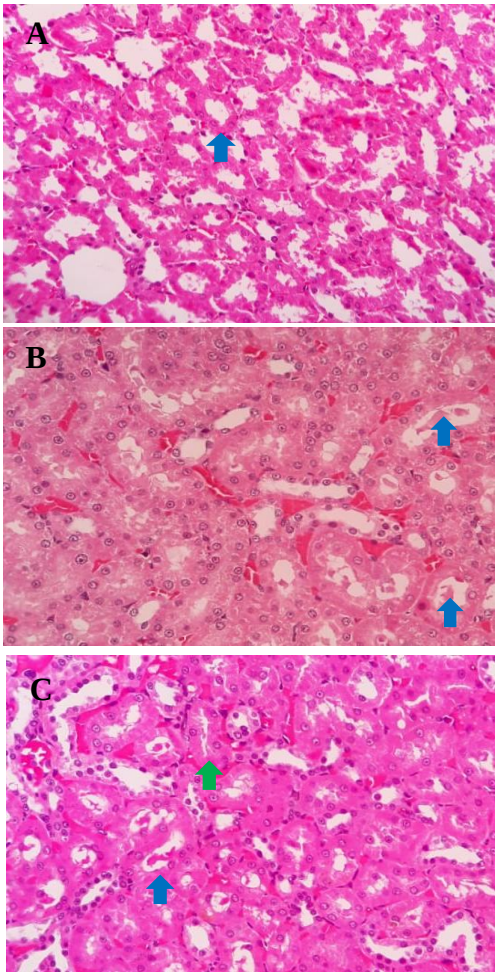

Figure 1. Histopathological features of Wistar rat kidney with HE staining, 400x magnification. A. Normal kidney proximal tubule, open and empty lumen (arrow). B. Damaged kidney proximal tubule, open lumen containing protein cast (arrows). C. Damaged kidney proximal tubule, open lumen (blue arrow) and closed lumen (green arrow) containing protein cast.

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staining. The slides were examined under 400x magnification on 5 microscopic field of view. The collected primary data were the number of damaged renal proximal tubules, ranging from albumin degeneration/ narrowing of proximal tubular lumen, formation of protein cast in proximal tubular lumen, and necrosis of proximal tubular epithelial cell.

Histopathological abnormalities found on microscopic examination were shown in Figure 1. Shapiro-Wilk analysis showed abnormal data distribution, therefore non parametric Kruskal-Wallis test was done, followed by Mann-Whitney test with p value <0.05.

RESULTS

Data in Table 1. shows mean scores, standard deviation, minimum scores, and maximum scores for the number of damaged proximal tubules in Wistar rat's kidney found from five different microscopic field of view for each group.

Table 1. Number of damaged proximal tubules between groups

Groups	Mean±SD	Maximum value	Minimum Value
Normal control group / K0 (Not given mercury chloride solution nor coriander leaf extract.)	2,44±1,19	0	4
Negative control group / K(-) (Given mercury chloride solution of 10 mg/kgBW only)	4,76±3,77	1	16
Experimental group 1 / P1 (Given mercury chloride solution of 10 mg/kgBW and coriander leaf extract of 100 mg/kgBW)	4,52±2,18	2	10
Experimental group 2 / P2 (Given mercury chloride solution of 10 mg/kgBW and coriander leaf extract of 200 mg/kgBW)	2,60±1,38	1	6

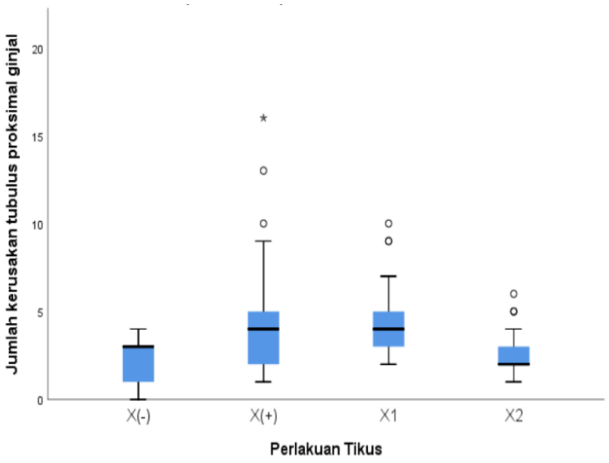

Figure 2. Boxplot for number of damaged proximal tubules
Circular symbol (o) and star symbol (*) shows outliers data for the number of damaged renal proximal tubules, consecutively representing number of damaged tubules of more than 1.5 IQ and more than 3.0 IQ

According to Table 1. It was found that normal control group had the lowest mean for number of damaged proximal tubules meanwhile the highest mean value was found in negative control group. Experimental group 1 had higher mean for number of damaged proximal tubules compared to experimental group 2. The range for number of damaged proximal tubules in each groups is shown in Figure 2.

Data normality for Wistar rats' number of damaged renal proximal tubules was tested using Shapiro-Wilk normality test and the result acquired shows p value <0.05, which means that the data were not normally distributed. Therefore, data analysis was carried on using Kruskal-Wallis non parametric hypothesis test. Kruskal-Wallis test shows p value of less than 0.05, thus further analysis was done using Mann-Whitney test. The result for Mann-Whitney distribution test is shown in Table 2.

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Tabel 2. *Mann-Whitney* analysis result

Kelompok	K0	K(-)	P1	P2
K0	-	0,009*	0,001*	0,936
K(-)	-	-	0,579	0,015*
P1	-	-	-	0,001*
P2	-	-	-	-

**Mann-Whitney* result is significant if p value <0.05
K0 : without oral mercuric chloride nor coriander leaf extract administration
K(-) : only oral mercuric chloride administration
P1 : oral mercuric chloride and coriander leaf extract of dose 100 mg/kgBW
P2 : oral mercuric chloride and coriander leaf extract of dose 200 mg/kgBW

Based on Mann-Whitney test, it was found that the p value between group K(-) and K0, P1 and K0, P2 and K(-), also between P1 and P2 consecutively are: p=0.009, p=0.001, p=0.015, p=0.001. Therefore, it could be inferred that there were significant differences between those groups. Otherwise, the p value between group P2 and K0 as well as between group P1 and K(-) consecutively are p=0.936 and p=0.579, inferring that there were no significant differences between those groups.

DISCUSSION

Kidney is an organ that has vital function in elimination of toxin and metabolic waste product from the body. Kidney elimination function cause kidney to be particularly susceptible to damage, one of which is caused by exposure to nephrotoxic substances. Acute damage to kidney tubule is also known as Acute Tubular Necrosis (ATN). One of nephrotoxic substance that cause damage to the kidney is heavy metal, such as mercury. Inorganic mercury is the most nephrotoxic form of mercury. Kidney damage from inorganic mercury is a result of kidney's capacity to reabsorp and concentrate divalent ion. High reabsoptive capacity of proximal tubule upon drugs or toxins through tubular apical and basolateral membrane makes renal proximal tubule prone to damage caused by mercury [15–17].

The microscopic features of kidney damage may manifest as degenerative changes, accumulation of intracellular substances, or reversible and irriversible damage like formation of proteinaceous cast and necrosis of tubular cells. Albumin degeneration is the first manifestation of cellular damage that is characterized by cellular swelling, causing cell to appear pale with granullar cytoplasm and narrowing of tubular lumen. Tubular cast formation is caused by weakening of proximal tubule brush border, causing the epithelial surface to slough off and get carried into the urine, forming cast in tubular lumen. Necrosis is a form of cell death indicated by loss of membrane integrity, leading to leakage of cellular content. Nucleus of necrotic cell might shrink, become fragmented, or disappear [18–20]. Mercury cause damage on proximal tubule by formation of Reactive Oxygen Species (ROS). Accumulation of mercury ion in renal proximal tubule cause formation of free radical that result in cellular damage from rising oxidative stress level, depletion of internal antioxidant, and mitochondrial dysfunction. Depletion of internal antioxidant is caused by mercury's high affinity to thiol, an intracellular antioxidant. Depletion of intracellular thiol and inactivation of enzyme that contain thiol group, disruption of renal tight junction from occludin phosporylation, increasing N-acetyl β-glucosaminidase (NAG), myeloperoxidase (MPO) and malondialdehyde (MDA) concentration, and apoptosis of renal proximal tubular cells from activation of NF-κB contribute to proximal tubular damage. In addition, mitochondrial dysfunction also contribute to tubular damage by causing disturbance in ATP intracellular gluthation (GSH) production and renal redox status [15,17,21].

According to statistical analysis, there was a significantly larger difference in number of damaged proximal tubules in negative control group (group K(-)) compared to normal control group (group K0). Therefore, the result of this experiment supported the previous study that stated mercuric chloride exposure at dose of 10 mg/kgBB within 14 days cause microscopic damage to renal proximal tubules [22].

Coriander leaf is a herb that has potential as natural source of antioxidant. The antioxidant potency is found in quercetin, a type of flavonoid that can neutralize free radical by transfer of hydrogen atom or transfer of single electron [23,24]. The experiment result showed that the number of proximal tubular damage in experimental group 2 (group P2) was significantly smaller compared to negative control group (group K(-)) and insignificantly larger compared to normal control group (group K0). This result showed that coriander leaf extract at dose 200 mg/kgBW could reduce the number of mercury-induced renal proximal tubule damage so that it had an equivalent features to

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proximal tubules in normal rat. This result supported previous study about renoprotective effect of corriander leaf at dose 200 mg/kgBW towards gentamicin-induced renal damage and the chelating potency of coriander leaf extract to lead acetate [12,25]. Other study also mentioned similar antioxidant activity in different organs, such as the effect of coriander leaf extract in improving histological feature of liver induced with CCl_4 or its effect in lowering level of oxidative stress of brain tissue induced with pentylenetetrazol [26,27].

Statistical analysis showed that the number of damaged proximal tubules in experimental group 1 (group P1) was insignificantly smaller than negative control group (group K(-)). In addition, statistical analysis also showed that the number of damaged proximal tubules in group P1 was significantly larger compared to both group K0 as well group P2. This showed that there was no apparent improvement to renal tubular damage in group P1 and coriander leaf extract at dose 100 mg/kgBW had not been able to prevent damage to renal proximal tubules caused by mercury chloride. This result was likely to be caused by dose-response effect of external antioxidant content in coriander leaf extract, in which the dose of extract showed proportional relationship toward its antioxidant content [10].

Some limitations to this study included unknown correlation between antioxidant content from flavonoid to the number of damaged proximal tubules because flavonoid content was not measured. There might also be differences between rats that were hard to measure such as genetic or physiological differences that could have affected parenteral absorption and metabolism of flavonoid. Individual difference of proximal tubular cells response toward toxic injury and regenerative ability of kidney tissue could have affected histopathological feature of rat's proximal tubules. In addition, rats's ureum and creatinine levels were not taken before the experiment in order to minimize physical and physiological trauma to rats. Thus, the starting condition of the rats's renal function were not known.

CONCLUSION

Oral administration of mercuric chloride cause damage to renal proximal tubules. Oral administration of coriander leaf extract with dose of 100 mg/kgBW couldn't protect the kidney from damage caused by mercuric chloride. Meanwhile, oral administration of coriander leaf extract with dose of 200 mg/kgBW could protect the kidney and showed similar result to normal control group.

Ethical Approval

This research has gotten ethical approval from The Health Research Ethics Committee, Diponegoro University Medical Faculty / Dr. Kariadi General Hospital Medical Center with certificate reference number 44/EC/H/FK-UNDIP/VI/2020.

Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions

The contributions for each authors were as follows : conceptualization, IKP, MP, and NM; methodology, IKP, MP, and NM; software, MP. and NM.; validation, IKP, NM, and IRED; formal analysis, MP and NM ; investigation, IKP and MP; resources, MP; writing—original draft preparation, MP; writing—review and editing, MP; visualization, MP; supervision, IKP; project administration, MP; funding acquisition, MP.

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