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Detoxification of Cadmium on The Levels of Urea and Creatinine on *Rattus Norvegicus* with Tea Mistletoe (*Scurrula Atropurpurea*)

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Abstract. The present of cadmium in the body is nephrotoxic due to its effect in increasing urea and creatinine. Tea mistletoe (*Scurrula atropurpurea*) has quercetin compound that able to become cadmium chelator. This study aims to know the effect and effective dose tea mistletoe as natural chelator in detoxifying cadmium towards urea and creatinine levels of male white rats (*Rattus norvegicus*). The study result obtained that all doses of tea mistletoe extract decreased urea and creatinine levels of rat that induced by cadmium. Effective dose of tea mistletoe extract that decreased urea and creatinine levels was 400 mg.kg⁻¹ WB.

INTRODUCTION

Pollutants in the environment in the form of heavy metals, one of which is cadmium. Source of cadmium pollution comes from the paint, mining and ceramic industries [1]. The source of cadmium pollution comes from the use of fertilizers with high phosphate levels and household waste deposits. Cadmium pollution can also be caused from various industries, including plastic industry, battery batteries, metal coating or welding[2]. Cadmium in the body is toxic to the kidneys, especially the glomerulus and tubules which can cause a decrease in kidney function[3].

Cadmium can accumulate in the kidneys and cause a decrease in glomerular filtration rate and reabsorption in the tubules. Substances that should be excreted from the body in the filtration process will be disrupted by cadmium. Filtering useless substances such as urea, creatinine and ammonia will be inhibited. This situation causes the third level of substances to increase in the blood [4]. Cadmium enters the body and binds to Metallothionein (MT), forming complex Cd-MT bonds. This bond will cause an increase in free radicals in the body, especially in the form of Reactive Oxygen Species (ROS) [5]. Free radicals formed do not have electron pairs in their outer orbitals, so they are reactive and will take free electrons from other molecules. The existence of free radicals under normal conditions can be neutralized by endogenous antioxidants in the form of enzymes such as superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GPx) [6]. If there are too many free radicals, antioxidants from outside the body are needed to neutralize [7].

Actually the human body has the glutathione S-transferase (GST) enzyme which will chelate cadmium in the body but the role of GST will decrease if too many free radicals [8]. Cadmium poisoning generally uses a chemical kelator namely British Antelewisite (BAL). The administration of BAL as cadmium chelating has the disadvantage of not being able to remove complex Cd-MT bonds. British Antelewisite causes side effects in the form of hypertension[9].

The existence of these side effects is needed as an alternative to exogenous antioxidant compounds from flavonoid groups such as quercetin [10]. One of the plants containing antioxidants in the flavonoid group is quercetin of mistletoe. Mistletoe plants are parasitic plants that are traditionally used for treatment [11]. The role of mistletoe is diverse, especially in preventing oxidative stress. The quercetin content of mistletoe leaves is equal to 9.6 mg.g⁻¹ [12]. Quercetin compounds have benefits as antiviral, anticancer, antidiabetic, and antioxidants [13]. Quercetin has the ability to chelate heavy metals in the body, so it can detoxify cadmium [14]. Quercetin compounds have 5 hydroxyl groups, so they can donate more hydrogen ions to neutralize free radicals [15]. The aim of the study was to determine the effect of natural chelator of mistletoe extract to reduce cadmium-induced levels of rat urea and creatinine, and determine the effective dose extract of mistletoe plants in reducing cadmium-induced levels of urea and creatinine in rats.

RESEARCH METHODS

The study was conducted at the Laboratory of Ecotoxicology at the Faculty of Biology, Jenderal Soedirman University. The study was conducted for 3 months, from February to April 2018. The study used an experimental method with Completely Randomized Design (CRD) consisting of 6 treatments and 4 replications, namely as follows: P0: Negative control (without cadmium and mistletoe extract.), P1: Positive control (cadmium induced), P2: induced cadmium and mistletoe extract 100 mg. Kg⁻¹ BW., P3: induced cadmium and mistletoe extract 200 mg. Kg⁻¹ BW., P4: induced cadmium and mistletoe extract 300 mg. Kg⁻¹ BW and P5: induced cadmium and mistletoe extract 400 mg.kg⁻¹ BW. Mistletoe samples were collected from the Kemuning Tea Garden, Karanganyar, Central Java and determined at the Laboratory of Plant Taxonomy at the Faculty of Biology, Jenderal Soedirman University. Mistletoe was extracted by maceration method using 96% ethanol. The test animals used were male white rats from the Wistar strain. The average weight of the rat is 200-300 g, which is acclimatized for 7 days by being put into a cage based on the treatment group. CdSO₄ induction is carried out orally, at a dose of 25% LD₅₀.

The treatment of CdSO₄ was carried out for 14 days as much as 2 mL orally with a sonde tool for treatment P1, P2, P3, P4, and P5. The administration of mistletoe extract was carried out for 28 days after induction of cadmium for treatment of P2, P3, P4, and P5 as much as 2 mL per rat. Blood samples from rat were taken after treatment (post test). Blood is taken through the orbital plexus section using a hematocrite capillary pipette. Blood samples are inserted into a centrifuge to be separated between the natan and the supernatant in the form of serum for 10 minutes at 6,000 rpm. Urea levels were examined by Berthelot method from DiaSys CT FS Urea kit [16]. Examination of creatinine levels using the kinetic Jaffe method from the Creatinine FS DiaSys kit[17]. The results of the obtained urea and creatinine levels were processed using Analysis of Variance (ANOVA) at an error rate of 5% and 1% followed by Duncan's analysis to determine the effective dose.

RESULTS AND DISCUSSION

The average results of examination of urea levels in Figure 4.1, indicate that there are differences in each treatment. The negative control urea level was 31.88 ± 2.87 mg.dL⁻¹ was the lowest and normal level compared to other treatments. This shows that the rat used are healthy rats. According to Derelanko (2000), that the level of normal urea of Wistar rats is around 25.71-38.57 mg.dL⁻¹ [18]. The positive control treatment had the highest urea level of 55.87 ± 4.11 mg.dL⁻¹, indicating kidney damage in rat due to cadmium induction. The urea level in the treatment of the mistletoe extract 100 dose; 200; 300 and 400 mg.dL⁻¹ respectively at 51.08 ± 0.33 ; 48.76 ± 0.74 ; 42.77 ± 2.02 ; and 38.45 ± 1.06 mg.dL⁻¹. This shows a decrease in the mean urea level tends to be normal. The magnitude of the decrease in linear urea levels with the increase in the dosage of induced mistletoe extract. This interpreted that administration of mistletoe extract can reduce urea levels in rat exposed to cadmium.

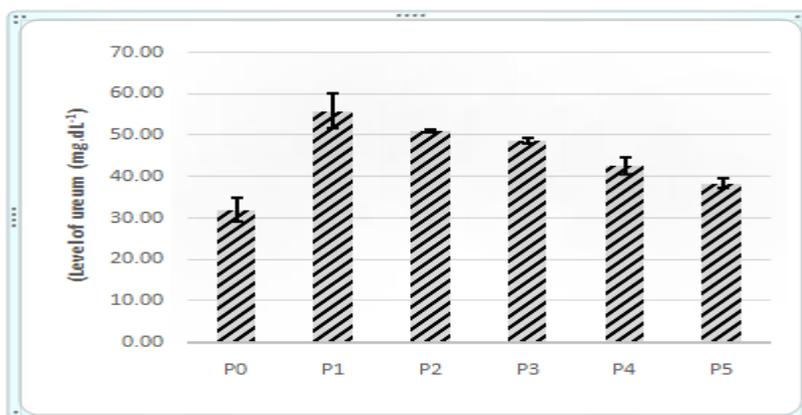


FIGURE 1. Average rat blood urea level. Description: P0: Negative control (without induction of cadmium and mistletoe extract); P1: Positive control (cadmium induced); P2: induced cadmium and 100 mg / kgBW of mistletoe extract); P3: treatment 2 (induced 25% LD50 CdSO4 and 200 mg / kgBW mistletoe extract); P4: treatment 3 (induced 25% LD50 CdSO4 and 300 mg / kgBWW mistletoe extract); P5: treatment 4 (induced 25% LD50 CdSO4 and 400 mg / kgBW mistletoe extract).

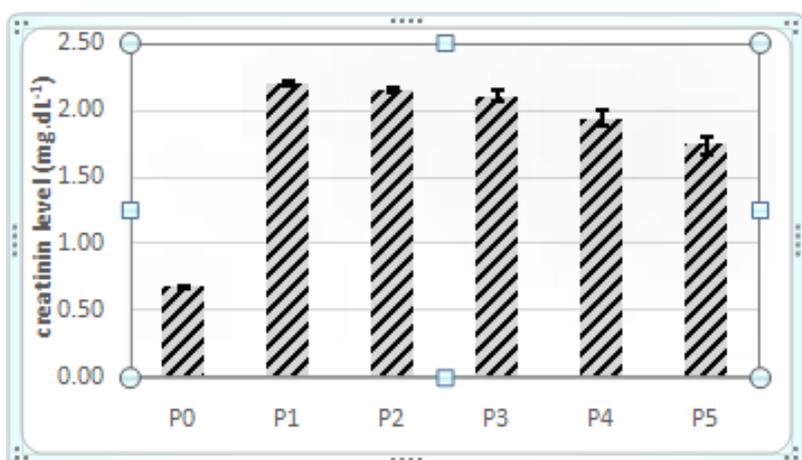


FIGURE 2. Average rat blood creatinine levels. Description: P0: Negative control (without induction of cadmium and mistletoe extract); P1: Positive control (cadmium induced); P2: induced cadmium and 100 mg / kgBW of mistletoe extract); P3: treatment 2 (induced 25% LD50 CdSO4 and 200 mg / kgBW mistletoe extract); P4: treatment 3 (induced 25% LD50 CdSO4 and 300 mg / kgBWW mistletoe extract); P5: treatment 4 (induced 25% LD50 CdSO4 and 400 mg / kgBW mistletoe extract).

Based on Figure 4.2 the mean creatinine levels differ in each treatment. The lowest and normal creatinine levels were found in the negative control treatment of 0.67 ± 0.01 mg.dL⁻¹. The magnitude of these levels is in accordance with the statement of Derelanko (2000), that the normal creatinine level of Wistar rats is 0.3-0.8 mg.dL⁻¹[18]. Creatinine level of positive control treatment was 2.20 ± 0.02 mg.dL⁻¹ which was the highest level compared to other treatments. Creatinine levels of cadmium induction treatment, cadmium induction and 100; 200 and 300 mg.dL⁻¹ tend to decrease according to the increase in the dosage of induced mistletoe extract. The value of negative control creatinine treatment, the dose of 100 mistletoe extract; 200; 300 and 400mg.dL⁻¹ sequentially ie 2.15 ± 0.01 ; 2.10 ± 0.04 ; 1.93 ± 0.06 ; and 1.74 ± 0.07 mg.dL⁻¹. These four levels indicate that mistletoe extract can reduce creatinine levels due to induction of cadmium.

Based on variance analysis (ANOVA) it was found that the mean levels of urea and creatinine were significantly different or very significant. This shows that the treatment tried affects the levels of urea and creatinine in Wistar rats. The Duncan test results at an error rate of 1% showed that the average urea level of negative and positive control treatment was significantly different than the ureum level of the mistletoe extract extract 100; 200; 300 and 400 mg.dL⁻¹. The negative control treatment had normal urea levels compared to other treatments. Urea levels treated with

mistletoe extract 100; 200; 300 and 400 mg.dL⁻¹ are not too different. It was interpreted that the administration of mistletoe extract at doses of 100, 200, 300 and 400 mg.kg⁻¹ BB was able to reduce cadmium-induced Wistar urea levels. Administration of mistletoe extract with all four doses slightly reduced Wistar rat urea levels.

Based on the Duncan test results at an error rate of 1%, average creatinine levels showed that negative control creatinine levels, as well as treatment of mistletoe 300 and 400mg.dL⁻¹ doses of extract were significantly different than other treatments. Positive control creatinine levels, as well as the treatment of mistletoe 100 and 200 mg.dL⁻¹ extracts had a not too significant value. This shows the presence of 300 and 400 mg dL⁻¹ KgBW of mistletoe extract of cadmium-induced rat significantly affects the decrease in creatinine levels. The mean creatinine level of the treatment of the dose of mistletoe 300 and 400 mg.dL⁻¹ extract had the most effect on the decrease in the dose of creatinine in Wistar rats compared to other treatments. The difference in the Duncan test results at a 1% error rate of urea and creatinine levels, because the increase in creatinine is considered to be more stable and better to give a figure of kidney damage[19].

Urea and creatinine can an indicator of kidney damage due to the induction of cadmium. Urea and creatinine levels will increase in line with the level of acute kidney damage [20]. Martono and Satino (2014) state that urea and creatinine are excreted from the kidneys through filtration at the glomerulus [21]. Blood with urea and creatinine will be filtered diffusion through the glomerulus, where creatinine and urea will be excreted and both levels in the blood will decrease. Cadmium induction is associated with disruption of kidney function as an excretory organ with a process of filtration of materials such as urea and creatinine.

Cadmium in the body causes a decrease in glomerular filtration rate (LFG) [22]. Decreasing LFG is an indication of kidney damage. The lower the LFG, the higher the level of damage to the kidneys. This according to Hernayanti et al. (2017) because toxic substances such as cadmium will accumulate in kidney cell tubules [9]. Decline in function and biochemical changes in the kidneys as the level of nephron damage by cadmium. The mechanism of renal glomerular destruction is caused by free radical formation or ROS [23].

The existence of ROS as a trigger for oxidative stress due to reduced complex CdMT bonds in the kidneys. Kidney oxidative stress results in lipid peroxidation of the renal cell membrane. This is what triggers a decrease in LFG and an increase in blood urea and creatinine levels. The results of the levels of urea and creatinine treated with mistletoe extract at doses of 100, 200, 300, and 400 mg. Kg⁻¹ BB showed a decrease in both levels in the normal direction. This indicates that mistletoe extract can reduce blood urea and creatinine levels of cadmium-induced rats. The role of mistletoe extract is not direct, but to prevent oxidative stress in the kidneys.

Mistletoe acts as a natural kelator and restores the role of GSH as a cofactor of the body's detoxification enzyme, GST. Mistletoe has a content of quercetin of 9.6 mg / g. Bu et al. (2011) added, quercetin compounds act as chelator of cadmium[24,25]. Quercetin protection activity against cadmium induction through the mechanism of free radical reduction by binding of free cadmium ions. The administration of quercetin increases endogenous antioxidants, including the body's GPx and SOD, and prevents lipid peroxidation that can cause damage to cadmium's target organs such as kidneys and liver. The quercetin mechanism as a natural chelator according to Symonowicz & Kolanek (2012) is by chelating metal ions in its 3' and 4' phenolic groups [26]. Quercetin compounds are said to be able to chelate metal ions, such as cobalt (II) and aluminum (III). The dose of mistletoe extract 400 mg. Kg⁻¹ BW rat is an effective dose, where the levels of urea and creatinine decrease tend to be normal. Quercetin has high potential antioxidant activity to prevent free radicals [27]. According to other research, the content of quercetin in mistletoe can be used to prevent degenerative diseases due to exposure to free radicals such as cadmium[28].

CONCLUSION

Based on the results of the study it can be concluded that mistletoe extract can reduce urea and blood creatinine levels on rat induced Cd, and the effective dose of mistletoe extract as a natural chelator which is 400 mg.dL⁻¹, with mean urea and creatinine levels of 38.46 ± 1.06 mg / dL and 1.74 ± 0.07 mg / dL.

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