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Gynura procumbens ameliorates cadmium-induced hematotoxicity in mice

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ABSTRACT

The objective of this research was to investigate the antioxidant activities methanolic extract of *G. procumbens* leaves ameliorates cadmium-induced hematotoxicity in mice. Research was conducted using twenty male mice, which were grouped into five treatments: P1 (control), P2 (Cd 100 mg/l), P3 (*G. procumbens* 100 mg/L + Cd 100 mg/l), P4 (*G. procumbens* 200 mg/l + Cd 100 mg/l), P5 (*G. procumbens* 300 mg/l + Cd 100 mg/l). The results showed Cd exposure significantly increasing concentration of MDA blood serum, ALT and AST levels, but reducing levels of RBC and HGB in mice. Methanolic extract of *G. procumbens* leaves administration can restore the damaged cell and mainly attributed to its antioxidant properties against Cd intoxication.

Key words: *Antioxidant, Cadmium, Gynura procumbens, Hematotoxicity*

Introduction

The use of heavy metal has increased from year to year, especially in big cities and industrial activities. Heavy metals such as cadmium (Cd) and lead (Pb) can be absorbed through food, drink and inhalation. In mantis shrimp (*Harpiosquillaharpax*) which in the eastern region of Java Sea, the concentration of Cd was 1.5-1.6 mg/kg BW. This value exceeds the threshold allowed for human consumption which is 1.0 mg/kg BW (Candra *et al.,* 2019). Increasing Cd and Pb levels in blood also found in children that are living in areas contaminated with heavy metals. It was responsible for the decrease in the enzyme 8-hydroxydeoxyguanosine (8-OHdG) and DNA damage (Xu *et al.,* 2018). The main mechanism of heavy metal toxicity is *via* oxidative stress induction. The metal is bound mostly to erythrocytes and blood plasma so that it can cause fragility and damage to blood cells. Accumulation Cd and Pb will trigger oxidative stress which causes the formation of reactive oxygen species (ROS) due to increasing malondialdehyde (MDA) levels and deactivation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPOD) which function as antioxidants. It leads damage some organs such as liver and kidneys (Kim *et al.,* 2018; Sugiharto *et al.,* 2019a; Sugiharto *et al.,* 2019b; Sugiharto *et al.,* 2020; Wani *et al.,* 2015).

Oxidative stress can be alleviated with exogenous antioxidants. The function of antioxidants is to eliminate ROS generated in the body. Antioxidant stops the oxidation process by neutralizing free radicals that formed during oxidation and convert free radicals into stable forms. Indonesia is a mega biodiversity country, has many plants that have possesing medicinal properties such as *Gynura procumbens. G. procumbens* is an annual shrub, a leaf

is an oval shape, and an aromatic odor. People cultivates *G. procumbens* in their yards, because they ussually consumed as a fresh vegetables and as an appetizer to increase their appetite. The leaves of *G. procumbens* are widely used of of health ailments such as fever, benign tumors, relieving itching due to caterpillars, diabetes mellitus, hypertension, and dysentery. These beneficial effects of *G. procumbens* are related to the presence of bioactive compound, such as phenolic, flavonoids, tannins, saponins, terpenes, coumarins, anthocyanins, and alkaloids (Hu *et al.*, 2019a; Puangpronpitag *et al.*, 2010; Tan *et al.*, 2016). Crude ethanolic extract of *G. procumbens* leaves shown to have antioxidant activity in the ferric reducing test and 2,2-Diphenyl-picrylhydrazyl (DPPH) assay (Kaewseejan *et al.*, 2015). Extract of various *G. procumbens* callus culture organs also indicated as antioxidant activity in DPPH assay (Krishnan *et al.*, 2015).

The aim of this research was to investigate the antioxidant activities methanolic extract of *G. procumbens* leaves ameliorates cadmium-induced hematotoxicity in mice, especially in concentration of MDA blood serum, red blood cell (RBC), hemoglobin concentration (HGB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in mice.

Materials and Methods

Plant material and extraction of *G. procumbens*

The fresh leaves of *G. procumbens* (1 kg) was obtained from Surabaya–Indonesia. The leaves were washed to remove surface pollutants and left at ambient temperature for 5-7 days. After that, leaves were dried in oven at 40 °C for 2 days and then sample was ground into a fine powder and stored at room temperature in desiccators.

Extraction was carried out by the maceration method with methanol as a solvent. A total of 25 grams of leaves powder were immersed in 250 mL of methanol, the maceration process was carried out for 24 hours in a shaker. The supernatant was filtered through filter paper and filtrates were concentrated by evaporator.

Animal preparation and cadmium exposure

The research used twenty male mice (*Mus musculus*, strains Balb/C), aged 8-10 weeks from Faculty of Pharmacy, Airlangga University. Mice randomly gathered into five treatment groups:

P1: 0.3 ml of distilled water (control)

P2: 0.3 ml of Cd solution 100 mg/l

P3: 0.3 ml of *G. procumbens* 100 mg/l and 0.3 ml of Cd solution 100 mg/l

P4: 0.3 ml of *G. procumbens* 200 mg/l and 0.3 ml of Cd solution 100 mg/l

P5: 0.3 ml of *G. procumbens* 300 mg/l and 0.3 ml of Cd solution 100 mg/l

G. procumbens treatment was given every morning (08:00 to 10:00 hours) and Cd treatment was given 2 hours after *G. procumbens* treatment. Treatment were administered orally for 30 days using injection syringe with a round tip. On the last day of treatment, mice were sacrificed, and blood samples were taken using intra-cardiac technique. The blood samples were centrifuged at 3,000 rpm (10 minutes, 10 °C) by centrifuge (Eppendorf 5424R) to harvest serum.

The use of animal subjects for research have been approved by Ethics Committee of Faculty of Veterinary Medicine, Airlangga University (certificate no. 2. KE.151.07.2019).

Biochemical hematology parameters

Measurement of biochemical hematology parameters of the spectrophotometric method was carried

Table 1. *Gynura procumbens* ameliorates cadmium-induced hematotoxicity

Data	Control	Cd_{100}	$Cd_{100} + G_{100}$	$Cd_{100} + G_{200}$	$Cd_{100} + G_{300}$
MDA (ìM)	0.304 ± 0.06^{ab}	0.656 ± 0.17^{c}	0.392 ± 0.13^{b}	0.192 ± 0.04^{a}	0.224 ± 0.07^{a}
ALT (U/L)	46.5 ± 6.03^{a}	72.7 ± 9.29^{b}	63.8 ± 14.20^{b}	43.7 ± 4.35^{a}	39.0 ± 6.06^{a}
AST (U/L	104.7 ± 16.9^{bc}	119.5 ± 13.2^{c}	93.5 ± 12.1^{ab}	79.5 ± 5.00^{a}	107.0 ± 15.2^{bc}
HGB (g/dL)	13.0 ± 0.62^{a}	12.8 ± 0.48^{a}	14.1 ± 0.24^{b}	14.4 ± 0.37^{b}	14.5 ± 0.50^{b}
RBC (10^6/mm^3)	8.26 ± 0.59^{b}	7.61 ± 0.17^{a}	8.42 ± 0.31^{b}	8.44 ± 0.30^{b}	8.55 ± 0.27^{b}

MDA = malondialdehyde, ALT = alanine aminotransferase, AST = aspartate aminotransferase, RBC = red blood cell, HGB = hemoglobin concentration. Statistical analysis was performed by one-way ANOVA and Duncan's test. The different letters show significant differences in the Duncan's test ($p<0.05$).

out with an ABX Micros 60 Hematology analyzer. The ALT and AST was performed using ABX Pentra 400. Briefly, 10 µL serum sample mixed with 1,000 µL R1 (Tris, LDH, L-aspartate for AST and L-alanine for ALT) and incubated for five minutes. Then, the tube solution mixed with 250 mL R2 (2-oxoglutarat, NADH) and incubated for one minute. Absorbance measured at l 365 nm on ABX Pentra 400. Determination number of RBC and HGB using reagents ABX Minidil LMG (2.1 ml) and ABX Minilyse LMG (alphalize, 0.52 ml).

Analysis of MDA

The assay was performed using Bioassay TBARS Assay Kit (DTBA-100) according to the kit manufacturer instruction (BioAssay System). Briefly, 100 µl of serum sample and 200 µl of 10 % TCA were mixed in a microtube. The mixture was centrifuged at 14,000 rpm for 5 minutes. Then, 200 µl of the supernatant was transferred into a fresh tube. Standard MDA, serum sample and 200 µl TBA reagent were mixed through vortexing, then incubated at 100 °C for 60 min. The tubes were re-centrifuged after vortexing. Aliqouts of 100 µl mixture were loaded into 96 well plates, and absorbance measured at λ 535 nm on a microplate reader. The results were expressed as µM MDA levels.

Data analysis

All experiments were performed in triplicate. The statistical analyses were performed byANOVA and Duncan's test at 5% significance level using SPSS 16.0.

Results and Discussion

The main mechanism of heavy metals toxicity is *via* oxidative stress (Flora *et al.,* 2012). It leads to the increase in free radicals production and induces several biochemical responses in the bloods.*G. procumbens*, which has the potential as an antioxidant, is supposedly to be able to overcome this problem. The results methanolic extract of *G. procumbens* leaves ameliorates cadmium-induced hematotoxicity in mice are shown in Table 1.

Cadmium exposure increasing concentration of MDA blood serum, ALT and AST levels, but reducing levels of RBC and HGB in mice. The hematopoietic system is very susceptible to heavy metal poisoning, because the metal is bound mostly to erythrocytes and blood plasma. It lead to deficiency of glucose-6-phosphate dehydrogenase enzyme to protect hemoglobin and erythrocyte membrane from oxidative stress. It can cause fragility and damage to blood cells. Accumulation heavy metals especially Cd, will trigger oxidative stress which causes the formation of ROS due to increasing MDA levels and lead to damage some organ such as liver and kidneys. It can be shifted to hematological parameters (Andjelkovic *et al.,* 2019; ATSDR, 2012; Kim *et al.,* 2018; Sugiharto *et al.,* 2020; Xu *et al.,* 2018).

Antioxidant activity methanolic extract of *G. procumbens* leaves was strongly correlated with bioactive compound such as phenolic and flavonoids (Kaewseejan *et al.,* 2015; Krishnan *et al.,* 2015; Pramita *et al.,* 2018; Tan *et al.,* 2016). *G. procumbens* administration can restore the damaged cell and mainly attributed to its antioxidant properties against Cd in toxication. *G. procumbens* has antioxidant property by acting as ROS scavengers, hydrogen donors, decreasing lipid peroxidation and preventing glutathione depletion, also relate to high tendency of chelating heavy metals (Khan *et al.,* 2019; Kim *et al.,* 2018; Shah and Jain, 2016).

Conclusion

Cadmium exposure significantly increasing concentration of MDA blood serum, ALT and AST levels, but reducing levels of RBC and HGB in mice. Methanolic extract of *G. procumbens* leaves administration can restore the damaged cell and mainly attributed to its antioxidant properties against Cd intoxication.

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