HAYATI
Journal of Biosciences

Vol. 29 No. 4, July 2022 479-486
DOI:10.4308/hjb.29.4.479-486
ISSN: 1978-3019
EISSN: 2086-4094

Gynura procumbens Adventitious Root Extract Altered Expression of Antioxidant Genes and Exert Hepatoprotective Effects Against Cadmium-Induced Oxidative Stress in Mice

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ARTICLE INFO

Article history:
Received November 15, 2021
Received in revised form January 3, 2022
Accepted January 14, 2022

KEYWORDS:
Antioxidant,
Cadmium,
Gene expression,
Gynura procumbens,
Health care,
Hepatoprotective

ABSTRACT

Exposure to cadmium (Cd) could increase of reactive oxygen species (ROS) and changes in expression of antioxidant genes. *Gynura procumbens* is a medicinal plant that is rich in phenolic and flavonoid compounds. The aimed of study to evaluate the hepatoprotective effect of *G. procumbens* adventitious root (GAR) extract against Cd toxicity, especially expression rate of hepatic antioxidant genes. Twenty-five male mice were treated as follows: P1 (control), P2 (Cd100mg/L), P3 (GAR100mg/L + Cd100mg/L), P4 (GAR200mg/L + Cd100mg/L), and P5 (GAR300mg/L + Cd100mg/L). The samples (blood and liver) were collected for analysis of malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase (CAT) activities, and their relative gene expression were determined. The hematological assay showed Cd-treated administered with GAR extract increased the number of red blood cells (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH), but reduced the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, the GAR extract decreased the MDA production, but increased the activities of SOD and CAT. These enzymatic activities were positively correlated with their respective gene transcripts. Our study revealed that GAR extract administration showed marked hepatoprotective effects against Cd-induced oxidative stress.

1. Introduction

Gynura procumbens (Asteraceae) are mainly distributed in tropical countries such as Indonesia, Malaysia, Vietnam, Thailand, and China. *G. procumbens* (locally name is Sambung Nyawa), in Indonesia and Malaysia, which means longevity greens. Asian people, especially Indonesian, used *G. procumbens* as an appetizer. It is also used as a remedy for fever disease, kidney, migraines, hypertension, diabetes mellitus and cancer. In China, *Gynura* was approved by the government as a natural medicine for diabetes treatment since 2010 (Yam *et al.* 2008; Keng *et al.* 2009; Saeed *et al.* 2014; Tan *et al.* 2016).

The root of *G. procumbens* contains various beneficial secondary metabolites including ascorbic acid, phenolic, flavonoid (Krishnan *et al.* 2015), stigmasterol, β-sitosterol, daucosterol, (Hu *et al.* 2019), kaempferol and quercetin (Manuhara *et al.* 2019). According to Krishnan *et al.* (2015) roots of *G. procumbens* contain higher concentration of secondary metabolites with antioxidant activities compared to the other tissue, especially flavonoids. These secondary metabolites are essential for plant growth, development, and protection against pathogen. However, the concentration and composition of flavonoids isolated from the conventionally cultivated *G. procumbens* plants are unstable due to environmental effects. Therefore, it is desirable to enhance the production of flavonoids using *in vitro* culture technique. Several studies demonstrated that biomass and flavonoid contents could be increased by culturing adventitious roots of *G. procumbens* under *in vitro* conditions (Isah *et al.* 2018; Manuhara *et al.* 2017, 2019; Pramita *et al.* 2018).

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Flavonoids isolated from *G. procumbens* have been reported have the ability as antioxidant activity (Kaewseejan *et al.* 2015; Li *et al.* 2017; Ashraf *et al.* 2020). Our previous study showed that *G. procumbens* adventitious root methanol extract contains antioxidant properties. We found flavonoid compound such as myricetin, kaempferol, quercetin, and catechin are the major compounds in *G. procumbens* adventitious root extract, when analyzing by high-performance liquid chromatography (HPLC) (data was not shown).

Cadmium (Cd) is a highly toxic environmental pollutant for human and animals. High exposure to Cd could induce oxidative stress, causing an enhance production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This may cause a toxic effect on an organism (Genchi *et al.* 2020). Exposure to Cd can lead to fragility and damage of blood cells. It can induce anemia by reducing the number of red blood cells (RBC), haemoglobin concentration (HGB), hematocrit (HCT), liver weight and body weight (Sugiharto *et al.* 2020). In addition, a high concentration of Cd may inhibit the biosynthesis of DNA, induced DNA damage and chromosome aberrations in animal cells. To counteract the Cd toxicity, cells have a group of antioxidant enzyme systems function (Bertin and Averbeck 2006; Madejczyk *et al.* 2015; Zhu *et al.* 2020).

Cd-exposed cells have been found to produce a high concentration of malondialdehyde (MDA), but the low activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ayala *et al.* 2014; Sugiharto *et al.* 2019b). SOD is the first line defense antioxidant enzyme against excessive lipid peroxidation. According to metal co-factor of catalytic site, there are three different forms of SOD in mammalian genome, namely *SOD-1* (Cu/Zn SOD), *SOD-2* (Mn SOD), and *SOD-3* (extracellular SOD). It catalyzes the radical dismutation of superoxide (O_2−) into oxygen (O_2) or hydrogen peroxide (H_2O_2) molecules through a dismutation reaction with the Haber-Weiss reaction pathway. The resulted H_2O_2 is then converted into water (H_2O) by the CAT enzyme (Kim *et al.* 2018; Kumar *et al.* 2020). This implies that oxidative stress in tissues can be reduced by endogenous antioxidants. However, consumption of antioxidants supplements is also important as it may interact with and enhance endogenous antioxidant activities.

As *G. procumbens* adventitious root extracts contain flavonoids showing antioxidant properties, we hypothesized that the extracts could reduce the cadmium sulfate ($CdSO_4$) toxicity and enhance the expression of antioxidant genes. To test our hypothesis, we evaluated the hepatoprotective effect of *G. procumbens* adventitious root cultures against $CdSO_4$ toxicity. We examined the: (1) hematological parameters, (2) MDA level and antioxidant enzyme activities in blood serum and liver homogenates, and (3) relative gene expression of *SOD-1* and *CAT* in the liver.

2. Materials and Methods

2.1. Animals and Ethical Approval

A total of 25 mature and healthy male mice (*Mus musculus*, strains Balb/C), obtained from the Faculty of Pharmacy, Airlangga University, Indonesia, were maintained in plastic cages, being fed with standard commercial mice chow, and provided with drinking water ad libitum.

The use of animal subjects in this research does not violate of animal welfare and have been approved by Faculty of Veterinary Ethics Committee, Airlangga University (certificate no. 2.KE. 151.07.2019).

2.2. Chemicals and Location

SV total RNA isolation system (Promega), qRT-PCR (MyGo Pro), forward and reverse primers of β-actin, *SOD-1* and *CAT* (Macrogen), MDA TBARS kit (Bio Assay), mouse SOD Elisa kit (Bio Assay), mouse CAT Elisa kit (Bio Assay), $3CdSO_4{}^*8H_2O$ (Merck), Eppendorf micropipette, centrifuge Eppendorf 5424R, µDrop Thermo Scientific, microplate reader Multiskan Go–Thermo scientific, ABX Micros 60 and ABX Pentra 400 hematology analyzer. This research conducted in Molecular Genetic Laboratory and Plant Physiology Laboratory, Faculty of Sciences and Technology, Airlangga University, Surabaya.

2.3. Methanol Extraction of *G. procumbens* Adventitious Roots

Adventitious root of *G. procumbens* were cultured in Murashige and Skoog (MS) liquid medium supplemented with 5 mg/L indole butiric acid (IBA) in a balloon-type bubble laboratory-scale bioreactor. The cultures were incubated in the dark at room temperature. After four weeks of culture, *G. procumbens* adventitious roots (GAR) were harvested (Faizah *et al.* 2018; Manuhara *et al.* 2019) and homogenized with a mortar into fine powder. Extraction was carried out by the maceration method with methanol as a solvent. A total of 30 g of dry root powder were immersed in 300 ml of methanol. Maceration process was carried out for 24 h in a shaker. The resulted supernatant was then filtered through filter paperand the procedure was repeated for three times. The filtrates were then concentrated using an evaporator.

2.4. Experimental Design, and *Gynura* Adventitious Roots Extract and $CdSO_4$ Treatment

Twenty-five male mice (*Mus musculus*) randomly divided into five treatment groups:

P1: 0.25 ml of distilled water (control)
P2: 0.25 ml of Cd 100 mg/L
P3: 0.25 ml of *Gynura* adventitious roots 100 mg/l and 0.25 ml of Cd 100 mg/L
P4: 0.25 ml of *Gynura* adventitious roots 200 mg/L and 0.25 ml of Cd 100 mg/L
P5: 0.25 ml of *Gynura* adventitious roots 300 mg/L and 0.25 ml of Cd 100 mg/L

Every morning between 09:00 and 10:00 a.m., *G. procumbens* adventitious roots (GAR) treatment was given, and $CdSO_4$ treatment was given 2 h following GAR treatment. An injectable syringe with a round tip was used to administer the treatment orally for 30 days. Mice were slaughtered under xylazine anaesthesia on the last day of treatment. To obtain serum, blood samples were taken intracardially and centrifuged at 3,000 rpm (10°C for 10 min).

Liver organs were collected for MDA, SOD, and CAT assays. About 70 mg of liver was rinsed with PBS to remove residual blood. The tissues was homogenized in PBS (1:10) with a glass homogenizer chilled with ice, followed by sonication for 6 × 15 s and centrifugation at 2,000 rpm (20 min, 4°C). The liver homogenates were collected and transferred into a 1.5 ml fresh tube.

2.5. Assessment of Hematological Parameters

An ABX Micros 60 hematology analyzer was used to measure the hematological parameters.

ABX minoxidil LMG (2.1 ml) and ABX minilyse LMG were used as reagents (ABX alphalize, 0.52 ml). Red blood cells (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular haemoglobin (MCH) are among the hematological characteristics studied. Meanwhile, ABX Pentra 400 was used to assess alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, the reagents utilized were tris, L-alanine, L-aspartate, LDH, NADH, and 2-oxoglutarate.

2.6. MDA Assay

The MDA assay was performed according to the manufacturer's instructions (Bio Assay), the measurement was carried out using MDA TBARS kit (DTBA-100). First, 200 µl of 10 % (w/v) trichloroacetic was mixed with 100 µl blood serum or liver homogenates, then the samples wereincubated for 5 min on ice. Next, the mixture was centrifuged at 14,000 rpm (5 min). Around 200 µl of the resulted supernatant was transferred into a new tube and mixed with the standard MDA and 200 µl thiobarbituric acid reagent, then reincubated for 60 min at 100°C. After cooled to room temperature, the mixtures were vortexed and briefly recentrifuged. As much as 100 µl of the final mixtures were then transferred to 96 well plates. Using microplate reader Multiskan Go–Thermo scientific, measured the absorbance at λ = 535 nm.

2.7. SOD Assay

The SOD assay was carried out using a mouse SOD Elisa kit (E0290Mo) according to the manufacturer's instruction (Bio Assay). First, 50 µl of a standard solution (without an antibody) was added to a standard well in a 96 well plate, while approximately 40 µl of serum and liver homogenates samples were added to a sample well along with 10 µl of anti-SOD antibody. After that, 50 µl streptavidin-HRP was added into each sample and control wells followed by incubation for 60 min at 37°C. Next, washed five times the plate for 1 min with 0.35 ml wash buffer. In each well, added 50 µl of substrate solution A and solution B. Incubated the mixture at 37°C (10 min) in the dark. Finally, around 50 µl of stop solution was added to each well, and the yellow color appear immediately. Using microplate reader Multiskan Go–Thermo scientific, measured the absorbance at λ = 450 nm.

2.8. CAT Assay

The assay was carried out using a mouse CAT Elisa kit (E0076Mo) according to the manufacturer's instruction (Bio Assay). In 96 well plate, 50 µl of a standard solution (without an antibody) was added to a standard well, while 40 µl of serum and liver homogenates samples were added to a sample well along with 10 µl of anti-CAT antibody. Then, it was added to the sample and control wells of 50 µl streptavidin-HRP, followed by incubation at 37°C (60 min). Washed five times the plate for 1 minute with 0.35 ml wash buffer. After that, 50 µl of substrate solution A and solution B were added into each well and the plate was reincubated at 37°C (10 min) in the dark. Finally, around 50 µl of stop solution was added to each well, and the yellow color appear immediately. Using a microplate reader Multiskan Go–Thermo scientific, measured the absorbance at λ = 450 nm.

2.9. RNA Isolation and cDNA Synthesis

According to the manufacturer's instruction (Promega), using SV total RNA isolation system (Z3100), total RNA was isolated. First, around 40 mg of liver tissue was mixed in a tube with 175 µl of RNA lysis buffer. Added the tubes of 350 µl RNA dilution buffer then inverted 3-4 times. The tubes were centrifuged at 13,000 × g (10 min), and transferred the cleared lysate

to a new tube. Next, added 200 µl of 95% ethanol into the lysate and transferred the mixture to a spin column and recentrifuged at 13,000 × g (1 min), and discarded the resulting eluate. Then added around 600 µl of RNA wash solution into the column and centrifuged again (1 min). DNase mix (50 µl) was added to the membrane and for the column was incubated for 15 min at room temperature. Next, as much as 200 µl of DNase stop solution was added and the mixture was centrifuged at 13,000 × g (1 min). The resulting supernatant was washed with RNA wash solution and centrifuged at 13,000 × g (1 min). This step was repeated once. The mixture in the spin column was then transferred to an elution tube. After mixing with 100 µl nuclease-free water (NFW), the mixture was centrifuged at 13,000 × g (1 min). The concentration of the eluted RNA was measured using µDrop Thermo Scientific and the quality of the extracted RNA was evaluated using agarose gel electrophoresis. Next, cDNA was synthesized from 1 µg total RNA using reverse transcriptase according to the manufacturer's instructions (Promega).

2.10. qRT-PCR Analysis (SYBR Green Method)

qRT-PCR for two antioxidant genes SOD and CAT were performed to evaluate their transcriptional level in the liver. The PCR mixtures contain 2.0 µl of cDNA, 5.0 µl of Go Taq Mix, 2.0 µl of nuclease-free water (NFW), 0.5 µl of forward and reverse primer primer, Three replicates were used for qRT-PCR (MyGo Pro) reaction using following thermal cycling conditions: preincubation (95°C, 10 min), then 45 cycles of 95°C for 10 s (denaturation), 60°C for 15 s (annealing), and 72°C for 20 s (extension). The expression levels of *SOD-1* and *CAT* were calculated by comparing it with β-actin expression level. The sequences of the forward and reverse primers (Macrogen) are: 5'-CAGAAGGCAAGCGGTGAAC-3' (*SOD-1* forward-19mer) 5'-CAGCCTTGTGTATTGTCCCCATA-3' (SOD-1 reverse-23mer); 5'-GGACGCTCAGCTTTTCATTC-3' (CAT forward-20mer) 5'-TTGTCCAGAAGAGCCTGGAT-3' (CAT reverse-20mer); and 5'-TTGCTGACAGGATGCAGAAG-3' (β-actin forward-20mer) 5'-GTACTTGCGCTCAGGAGGAG-3' (β-actin reverse-20mer). The percentage relative quantification of gene expression was determined using the comparative ΔΔCt method.

2.11. Statistical Analysis

All data are presented as mean ± SD from three replicates. Gene expression data were expressed in percentage relative gene expression using ΔΔCt values. The statistical analysis was performed using SPSS 25.0. one way ANOVA and Duncan's test at 5 % significance level, where p-values less than 0.05 is statistically significant.

3. Results

3.1. Assessment of Hematological Parameters

Generally, Cd treatment caused significant decrease in hematological parameters such as RBC, HGB, HCT, MCV and MCH, but significantly increased hepatic serum AST and ALT levels (Table 1). RBC, HGB, MCV, and MCH in Cd-treated mice was slightly increased after adding *G. procumbens* adventitious root methanol extract. The level of AST and ALT in mice administered with *G. procumbens* extract was slightly decreased.

3.2. MDA Levels and Antioxidant Activities of SOD and CAT

In this study, the MDA level of Cd-treated blood serum and liver homogenates was significantly higher than control (Figures 1A and B). After adding 100 mg/L *G. procumbens* adventitious root methanol extract, the MDA levels was significantly reduced in blood serum (Figure 1A). However, a high concentration of *G. procumbens* adventitious root methanol extract (200 mg/L) caused an increased level of MDA (Figure 1A). The MDA level of the liver

Table 1. Effect of *G. procumbens* adventitious root extract on hematological parameters

Data	P1	P2	P3	P4	P5
RBC (10^6/mm^3)	9.79±0.69^b	7.33±1.56^a	9.79±0.69^b	7.33±1.56^a	7.33±1.56^a
HGB (g/dl)	15.70±0.74^b	10.43±1.56^a	15.70±0.74^b	10.43±1.56^a	10.43±1.56^a
HCT (%)	39.15±2.82^b	31.45±2.25^a	39.15±2.82^b	31.45±2.25^a	31.45±2.25^a
MCV (fL)	48.50±2.89^b	41.25±2.75^a	48.50±2.89^b	41.25±2.75^a	41.25±2.75^a
MCHC (g/dl)	33.15±0.74^a	33.45±0.26^a	33.15±0.74^a	33.45±0.26^a	33.45±0.26^a
MCH (pg)	16.00±1.04^b	13.68±1.01^a	16.00±1.04^b	13.68±1.01^a	13.68±1.01^a
AST (U/L)	121.75±21.3^a	212.25±50.9^b	121.75±21.3^a	212.25±50.9^b	212.25±50.9^b
ALT (U/L)	52.62±9.21^a	82.00±13.64^b	52.62±9.21^a	82.00±13.64^b	82.00±13.64^b

RBC = red blood cell, HGB = hemoglobin concentration, HCT = hematocrit, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, AST = aspartate aminotransferase, ALT = alanine aminotransferase. P1 = Control, P2 = Cd-100, P3 = GAR-100 + Cd-100, P4 = GAR-200 + Cd-100, P5 = GAR-300 + Cd-100. Statistical analysis using one-way ANOVA and Duncan's test. The different letters show significant differences in Duncan's test ($p<0.05$)

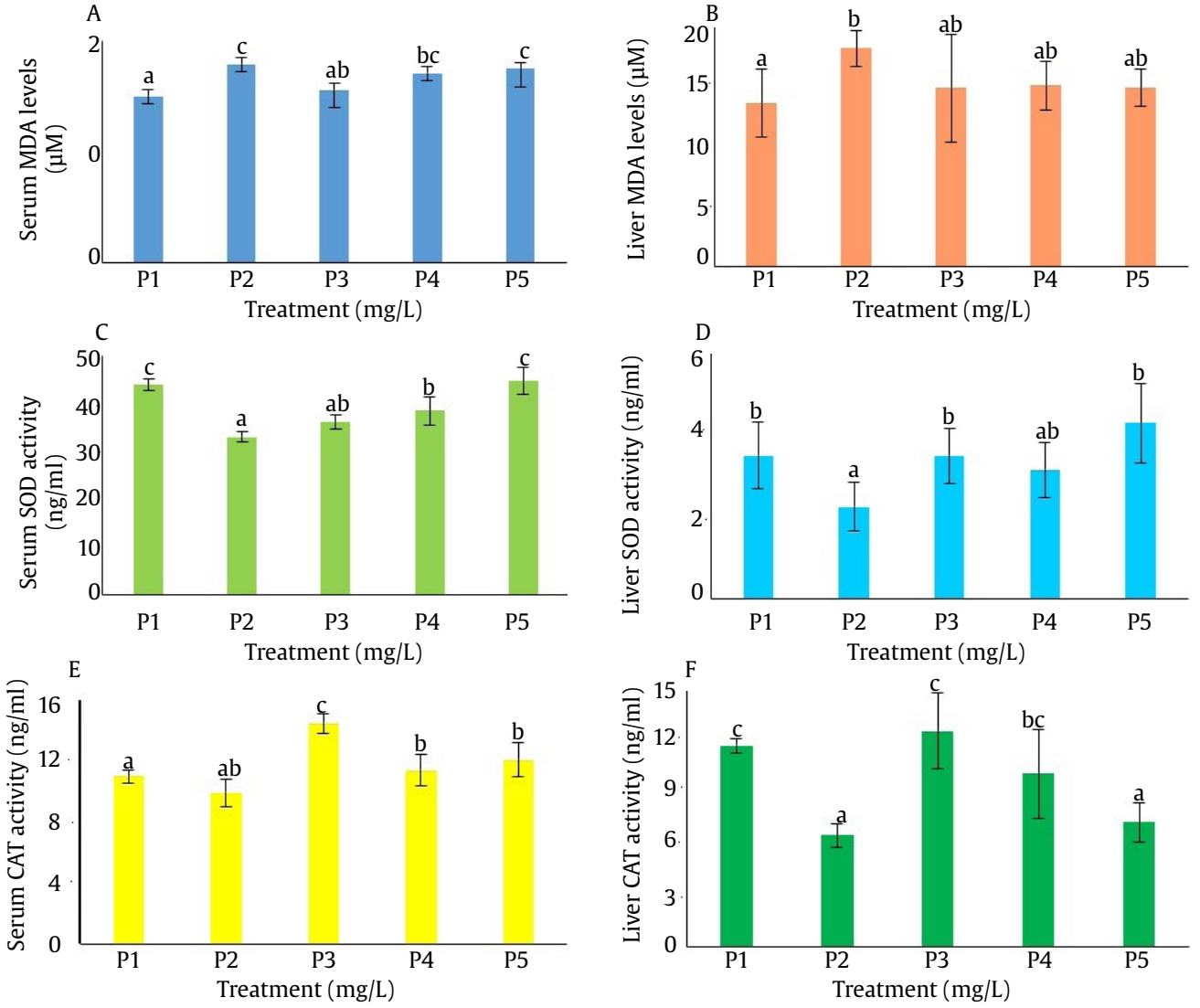

Figure 1. The malondialdehyde (MDA) level and antioxidant enzyme activities of mice administered with or without cadmium and *G. procumbens* adventitious root extract. (A) the MDA levels in blood serum, (B) the MDA level in liver homogenates, (C) the superoxide dismutase (SOD) activity in blood serum, (D) the SOD activity in the liver homogenates, (E) the catalase (CAT) activity in blood serum, and (F) the CAT activity in liver homogenates. P1 = Control, P2 = Cd-100, P3 = GAR-100 + Cd-100, P4 = GAR-200 + Cd-100, P5 = GAR-300 + Cd-100. Statistical analysis using one-way ANOVA and Duncan's test. The different letters show significant differences in Duncan's test ($p<0.05$).

homogenates from the mice supplemented with Cd and *G. procumbens* adventitious root methanol extract was slightly reduced compared to Cd-treated (Figure 1B).

Antioxidant enzyme activities, namely SOD and CAT, was determined. The SOD activity in the blood serum of mice administered *G. procumbens* adventitious root methanol extract at 200 and 300 mg/L was significantly increased compared to Cd-treated mice (Figure 1C). Similarly, the application of 100 and 300 mg/L *G. procumbens* adventitious root methanol extract significantly increased the SOD activity in liver homogenates of the Cd-treated mice compared to mice treated with Cd alone (Figure 1D). For CAT activity, the blood serum of mice administered with 100 mg/L *G. procumbens* extract recorded significantly higher CAT activity than Cd-treated mice, while 100 and 200 mg/L *G. procumbens* extracts enhanced the CAT activity in liver homogenates (Figures 1E and F).

3.3. Antioxidant Gene Expression in Liver

Total RNA extracted from each treatment was assessed for its quality to check whether the RNA is intact. The ribosomal RNA bands (28S and 18S) were clearly observed in agarose gels after electrophoresis, indicating a good integrity of total RNA. To determine the antioxidant gene expression in the liver, we selected *SOD-1* and *CAT* because these genes are highly expressed in the liver and kidney. Our results showed that the expression of *SOD-1* and *CAT* in the Cd-treated sample was slightly lower than control. However, the mice administered *G. procumbens* adventitious root at 100 mg/L increased the expression of *SOD-1* and *CAT* (Figure 2). This result is consistent with SOD and CAT activity assay, where *G. procumbens* treatments were able to increase the activity of both enzymes (Figure 1D and F). Together our result shows that in Cd exposed mice, *G. procumbens* adventitious root could enhance the expression of antioxidant genes and the activity of antioxidant enzymes in liver, again indicating the hepatoprotective effect of *G. procumbens* against Cd.

4. Discussion

Cd is a well-recognized top 10 environmental pollutant with numerous adverse effects on human health even at low concentrations. It is a toxic metal group that could be absorbed and accumulated in the cytosol by forming metal-ligand complexes. Cd binds to erythrocytes and blood plasma, leading to fragility and damage to the blood cells (El-Boshy *et al.* 2015; Zou *et al.* 2020). Here, we show that RBC, HGB, HCT, MCV, and MCH in the Cd- treated group were decreased. The decrease of hematological parameters is an indicator of anemia symptoms (Andjelkovic *et al.* 2019; Sugiharto *et al.* 2020).

Exposure to heavy metals, such as cadmium (Cd) and lead (Pb) has been shown to reduce RBC, HGB, body and liver weights in mice (Sugiharto *et al.* 2019a). For instance, water polluted by Cd increased the levels of Cd in shrimp (Candra *et al.* 2019). In humans, exposure to Cd can lead to hemolysis of peripheral red blood cells, iron deficiency due to competition with iron absorption in the duodenum, and reduction of erythropoietin (EPO) production. Severe health problems might occur due to inhibition of enzyme activities, kidney and liver failure (Sim *et al.* 2017). Our previous study found that the number of swollen and necrotic cells in Cd-treated hepatic cells was significantly higher than in control (data was not shown).

The damage of hepatic cells can be detected by determining the level of AST and ALT. This is because AST and ALT are released into the circulatory system when hepatic cells are injured (Laamech *et al.* 2017; Yuniarti *et al.* 2021). We found that the level of AST and ALT in Cd-treated mice serum was increased but slightly reduced after administering *G. procumbens* adventitious root extract. Other studies showed that the hepatic cells in the mice treated with heavy metals produced a higher level of ALT and AST than control. Cd exposure led to the formation of ROS and MDA (Sugiharto *et al.* 2019b). MDA is a biomarker that provides an indication of lipid peroxidation level. Several studies reported that Cd treatment increased the MDA level in mice (Andjelkovic *et al.* 2019). Similarly, our

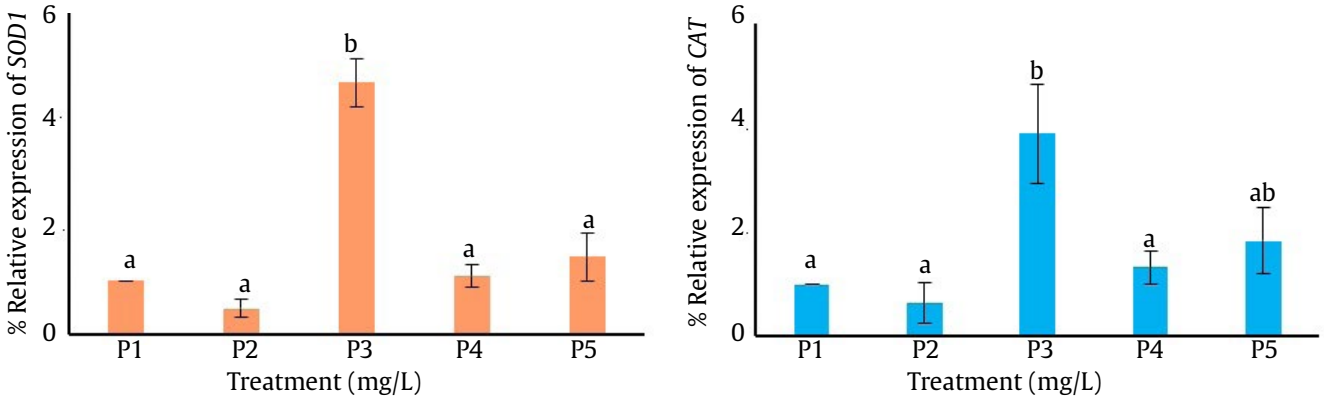

Figure 2. Percentage relative gene expression of *SOD-1* and *CAT* in the liver were determined by comparing to β-actin as the endogenous control performed by ΔΔCt values. P1 = Control, P2 = Cd-100, P3 = GAR-100 + Cd-100, P4 = GAR-200 + Cd-100, P5 = GAR-300 + Cd-100. Statistical analysis using one-way ANOVA and Duncan's test. The different letters show significant differences in Duncan's test ($p<0.05$)

results showed that the MDA level of blood serum and liver homogenates was increased after being treated with Cd but reduced by *G. procumbens* adventitious root methanol extract. This reduction might be due to the activation of antioxidant enzyme activities. To confirm this, we analyzed the activities of SOD and CAT in the blood serum and liver homogenates of the mice treated with or without Cd and *G. procumbens* adventitious root methanol extract. We found that *G. procumbens* adventitious root methanol extract could increase the SOD and CAT activities in blood serum and liver homogenates. The enhanced antioxidant activities might be due to the polyphenols and flavonoids present in *G. procumbens* adventitious root methanol extracts. Our previous study found that *G. procumbens* adventitious root methanol extracts rich in phenols and flavonoids, namely myricetin, kaempferol, quercetin, and catechin. These compounds have been shown to increase antioxidant activity and could function as chelating agents to Cd ion (Lamidi and Akefe 2017). The results of this study strengthen our previous research which reported that *G. procumbens* leaves methanolic extract possesses hematoprotective effect against Cd toxicity by reducing MDA, ALT, and AST levels, as well as increasing HGB and RBC levels in mice (Sugiharto *et al.* 2021).

We validated the antioxidant activities in hepatic cells. Our results showed that *G. procumbens* adventitious root extracts increased the expression of *SOD-1* and *CAT* in Cd-treated mice. This observation suggests that the *G. procumbens* adventitious root extracts might have a hepatoprotective effect against Cd. The study conducted by Xu *et al.* (2017) found the gene expression of SOD was increased continuously when *Gynura* exposed to oxidative stress. Thus, *Gynura* extract could alleviate the oxidative stress injury in the liver and promote the activities of CAT and GSH-Px as an antioxidant enzymes (Liu *et al.* 2019), we speculate that it could be useful in the preventing diseases due to oxidative damage, such as cancer and diabetes. The similar study showed that *Gynura* extract reported upregulation of antioxidant enzymes such as *SOD-1* and *GST-1* in culture human gingival cells (Kantawong *et al.* 2021).

In conclusion, this study showed that *G. procumbens* adventitious root methanol extract possesses hepatoprotective effects against Cd toxicity. The Cd-treated mice administered with *G. procumbens* extract produce a higher number of RBC, HGB, HCT, MCV, and MCH than Cd-treated mice. In addition, the supplementation of *G. procumbens* root extract reduced the MDA production in blood serum and liver, and increased the activities of CAT and SOD. These enzymatic activities were positively correlated with their respective gene transcripts. Taken together, these results demonstrate that *G. procumbens* adventitious root methanol extract could be potentially used in treating and preventing hepatic genotoxicity and oxidative stress.

Acknowledgements

We gratefully acknowledge to Universitas Airlangga for supporting via a Mandatory Research grant (No.1408/UN3/2019) and Ministry of Education and Culture (PDD grant No. 853/UN3.14/PT/2020).

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