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Detoxification of Cadmium on Albino Rats (*Rattus norvegicus*) with Natural Chelator of Fruiting Body Extract of *Ganoderma lucidum*

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ABTRACT. Cadmium is a heavy metal pollutant sourced from various industries and toxic to the kidneys. Cadmium exposure can be used natural chelator of ethanol extract of the fruiting body of *Ganoderma lucidum*. The aim of the study was to determine the effect and effective dose of the ethanolic extract of the fruiting body of *G. lucidum* on reducing the toxicity effect of cadmium in male albino rats (*Rattus norvegicus*) Wistar strain. The research design was experimental research, which consisted of 5 treatment levels. The research parameters were blood cadmium, β2-microglobulin, malondialdehyde, and superoxide dismutase levels. The ethanolic extract of fruiting body of *G. lucidum* was tested by gas chromatography-mass spectrometry (GCMS) to determine linalool as active compounds. The results of each parameter had a significant effect, which decreased blood cadmium, β2M and MDA levels, and increased SOD level that linear according to the addition of the dose of ethanol extract of the fruiting body of *G. lucidum*. Blood cadmium levels with β2M and MDA levels had a positive correlation, while blood cadmium levels with SOD levels had a negative correlation. The dominant compound detected was linalool which has potential as a radical scavenger. The dose of 750 mg.kgBW^{-1} is the effective dose of the ethanolic extract of fruiting body of *G. lucidum* based on a decrease in blood cadmium levels (54.10%), β2M (63.94%) and MDA (20.31%), as well as an increase in SOD levels (14.20%) compared to sick control.

Keywords: β2-microglobulin, cadmium, *G. lucidum*, malondialdehyde, superoxide dismutase.

INTRODUCTION

Environmental problems generally occur due to increased contaminants that can cause air, land, and water pollution. These problems occur when contaminants are not managed properly and wisely (Kelishadi, 2012). Cadmium is a type of heavy metal that is very stable (Sharma et al., 2015). Sources of cadmium exposure can come from the mining industry, metallurgical industry, burning fossil fuels, battery industry, and cigarette smoke (Genchi et al., 2020).

A case study of cadmium exposure occurred in welding workshop workers in Purwokerto which was interpreted from a blood cadmium level of 0.049 ppm (Ferdhiani et al., 2018). Another reported case of cadmium exposure occurred in active smokers at the Padang city bus terminal, which was indicated by a relatively high blood cadmium level of 0.016 ppm (Rosita & Andriyati, 2019). The two exposure cases described high levels of blood cadmium compared to the limit of blood cadmium levels of 0.0012 ppm (WHO, 2007).

Cadmium has a nephrotoxic effect or is toxic to the kidneys. Cadmium poisoning in the body will be detoxified by the liver and excreted by the kidneys (Baba et al., 2013). The effects of cadmium exposure on the kidneys occur due to damage to the glomerulus and proximal tubules of the kidney. This effect causes kidney function failure which is interpreted from a decrease in the value of the glomerular filtration rate (GFR) and tubular reabsorption capacity (Satarug et al., 2019). The decreased renal filtration rate causes the manifestation of several types of proteins in the proximal tubule such as β2-microglobulin (β2M) (Hwangbo, et al., 2011). β2M levels are an early indicator of dysfunctional detection in the glomerulus and proximal tubules of the kidney (Argyropoulos et al., 2017).

The accumulation of cadmium that bound to metallothonein (MT) in the kidneys triggers the production of reactive oxygen species (ROS) as free radicals, resulting in kidney membrane damage (Nedecky et al., 2013). Malondialdehyde (MDA) which increases in line with the level of kidney damage (Hardiany & Paramita, 2019). The effect of increasing ROS due to cadmium exposure triggers a decrease in the activity of an antioxidant enzyme, namely superoxide dismutase (SOD). SOD enzyme levels decrease when free radical levels due to cadmium are high (Hernayanti & Lestari, 2020).

Handling of poisoning due to exposure to cadmium is generally done by administering an

antidote in the form of a chemical compound, namely dimercaprol as a chelator. The use of dimercaprol as a cadmium chelator has several side effects, namely hemolysis of red blood cells to an increase in blood pressure (Flora & Pachauri, 2010). Based on these side effects, it is necessary to have an alternative active compund from natural ingredients (Hernayanti et al., 2019).

Sources of active compounds can use the *Ganoderma lucidum* mushroom which is a medicinal mushroom (Ratnaningtyas, 2007). The use of a dose of 500 mg.kg BB^{-1} extract of *G. lucidum* was able to provide a protective effect for the kidneys (nephroprotective) against cadmium in terms of kidney function (Yang et al., 2016). The content of active compounds in the fruiting bodies of *G. lucidum* include flavonoids, gallic acid, and coumarin acid (Kim et al., 2008). The content of other active compounds that are dominant in the fruiting body of *G. lucidum* are terpenoids consisting of various derivatives (Cor et al., 2018). Flavonoid compounds have the potential as chelators to metals (Cherrak et al., 2016). The terpenoid content in *G. lucidum* has the ability to reduce free radicals such as superoxide radicals and hydroxyl radicals by triggering the production of the body's antioxidant enzymes including SOD, catalase (Cat) and glutathione peroxidase (Gpx) (Smina et al., 2011). Based on this description, a study was conducted to determine the effect and effective dose of the ethanolic extract of the fruiting body of *G. lucidum* on reducing the toxicity effect of cadmium in male albino rats (*Rattus norvegicus*) Wistar strain.

EXPERIMENTAL SECTION

Materials and Experimental Design

The research method was experimental which consisted of 6 treatment groups, namely P_0 (without cadmium induction and ethanol extract of *G. lucidum*), P_1 (cadmium induced), P_2 (induced cadmium and ethanol extract of *G. lucidum* 250 mg.kg^{-1}BW), P_3 (induced by cadmium and ethanol extract of *G. lucidum* 500 mg.kg^{-1}BW), and P_4 (induced by cadmium and ethanol extract of *G. lucidum* 750 mg.kg^{-1}BW). The test animals used were male albino rats (*Rattus norvegicus*) Wistar strain and simplisia strain fruiting bodies of *G. lucidum* which are a collection of CV. Asa Agro Corporation Cianjur number 21.

Extraction of *G. lucidum* Fruit Body

The fruit body sample of *G. lucidum* was in powder form which was extracted by maceration method. *G. lucidum* fruiting body powder as much as 100 g was soaked in 1,000 mL of 96% ethanol (1:10) (Lin et al., 2015). *G. lucidum* powder was soaked in a beaker glass which was stirred for 10 minutes, then covered with aluminum foil and left at room temperature for 24 hours. The extracted macerate was evaporated using a vacuum rotary evaporator at a temperature of 45°C to obtain a thick extract (Hernayanti et al., 2019). The thick extract obtained was then weighed to determine the weight of the extract obtained. The weight value of the extract was calculated using the equation for the percentage of extract yield (Susanto et al., 2018). the active compound in the ethanolic extract of the fruiting body of *G. lucidum* using the (GCMS) method. The results of the mass spectra were analyzed by comparing the results and the National Institute of Standards and Technology (NIST) databases 27 and 147, as well as the WILEY 7 database (Hikam et al., 2019).

Animal Treatment

Cadmium induction was carried out using $CdSO_4$ orally, with a dose of 25% of the LD_{50} $CdSO_4$ which was 14 mg.kg^{-1} BW (Lewis, 2004). Each dose was dissolved in 12.38 mL of distilled water. Cadmium induction was carried out for 10 days orally, using a 2 mL syringe for the treatment P_1, P_2, P_3, and P_4.

Giving the ethanol extract of the fruiting body of *G. lucidum*, the dosage of the extract was made first. The yield of the ethanol extract of the fruiting body of the fungus *G. lucidum* was made in doses of 250 mg.kg^{-1} BW (P_2), 500 mg.kg^{-1} BW (P_3), and 750 mg.kg^{-1} BW (P_4) (Yang et al., 2016). Each dose was dissolved in 10 mL of distilled water. The ethanol extract of the fruiting body of *G. lucidum* was administered orally for 14 days using a syringe, which was given as much as 2 mL per test animal every day.

Sample Preparation

Blood samples of test animals were taken after treatment (posttest) on the 32nd day. Test animals were anesthetized using ether before sampling was carried out. Blood samples were taken through the orbital plexus vein (Sharma et al., 2014). Blood samples were centrifuged to separate the natant and supernatant (serum) for 10 minutes at a speed of 6,000 rpm (Hernayanti et al., 2019).

Parameter Measurement

Examination of blood cadmium levels begins with the preparation of a standard solution. The concentration of the mother standard solution is 1.000 mg L^{-1}. A total of 0.5 mL of blood and 0.5 mL of each standard solution were added to a different Erlenmeyer flask, and 3 mL of HNO_3-H_2O_2 (2:1, v/v) was added, then closed. The solution was homogenized and incubated for 1-2 hours by heating at 60-70°C. A total of 2 mL of nitric acid and 3-4 drops of H_2O_2 were added, and again heated on a hot plate at 80°C until the solution turned clear. The solution was stored at 37°C and diluted with 1 mL 0.1 mol. dm^{-3} HNO_3. The solution was then filtered using Whatman paper no. 42 and diluted with distilled water to 10 mL. The standard solution and the sample were then read for their absorbance values at AAS λ 228,6 nm with a current of 3.5 mA (Hernayanti et al., 2019).

The examination of β2M levels was carried out using the Rat β2-*microglobulin* reagent kit, carried out by making a standard solution which was diluted using

a standard diluent. The mother standard solution had a concentration of 480 μg.mL^{-1}. A total of 50 μL of the standard solution was added to the standard well, and 40 μL of the sample and 10 μL of anti-β2M antibody were added to the sample well. Each well was added 50 μL of streptavidin-HRP and homogenized. All wells were closed using a sealer and incubated for 60 seconds at 37ºC. The sealer cover was opened and the well plate was washed using 0.35 mL of wash buffer solution for 30 to 60 seconds with 5 repetitions of washing, then the well plate was dried. A total of 50 μL of substrate solution A and 50 μL of substrate solution B were added to each well and closed with a new sealer and incubated for 10 minutes at 37ºC. in dark conditions. After incubation, the sealer was opened and added to each well as much as 50 μL of stop solution. A color change in the solution from blue to yellow was observed. The solution in the well was read for optical density (OD) using a microplate reader λ 450 nm (Bioassay Technology Laboratory, 2012).

MDA levels were checked using a commercial MDA reagent kit. The first stage of checking MDA levels is the preparation of a standard solution, where the standard solution used is a TEP solution that has a concentration of 1 mM. MDA levels were checked using the thiobarbituric acid reactive substance (TBARS) assay method (Kurniasari et al., 2017). A total of 400 μL of test animal serum samples and 400 μL of each concentration of standard solution were put into different measuring cups, then 400 μL of 20% TCA was added. The solution was homogenized and then centrifuged using a centrifuge for 10 minutes at 4,000 rpm. The supernatant was taken as much as 400 μL and added 1,000 μL of 0.67% TBA. The solution was heated in a water bath for 10 minutes at 95ºC. The solution was cooled at room temperature, then the absorbance value was read on spectrophotometry λ 532 nm.

Examination of SOD levels SOD was measured with the RanSod reagent kit. A total of 1,500 μL of buffer solution and 300 μL of xanthine oxidase were put into 3 different test tubes. The first tube is a blank, the second tube is added 60 μL of standard solution as a standard, and the third tube is added 60 μL of serum sample as a sample. Each solution in the tube was homogenized and the absorbance value was read using spectrophotometry 520 λ nm (Randox Laboratories, 2009).

Data Analysis

The absorbance values of the standard solutions of cadmium, β2M and MDA from each dilution series were analyzed to produce a regression curve. Measurement of blood cadmium, β2M, and MDA levels using the regression equation formula generated from the absorbance readings for each series of dilutions of standard solutions of cadmium, β2M, and MDA (Ardhya, Wardah, & Rachmawati, 2017). The formula for the regression equation is as follows: y = a + bx. Description: y= sample absorbance value, a= intercept (intercept of the curve line on the Y axis), b= slope (slope), x= blood cadmium level, β2M, or sample MDA.

Calculation of SOD levels was obtained from the standard absorbance results and each sample obtained, then entered into the equation for measuring SOD levels as follows (Randox Laboratories, 2009): SOD levels = (Sample absorbance/standard absorbance) × 30,65 U.mL^{-1}.

Statistical analysis was obtained from the results of calculating blood levels of cadmium, β2M, MDA, and SOD from each sample, then statistically processed using SPSS software. The resulting data were analyzed using the analysis of variance (ANOVA) method at an error rate of 1%. If the results of the ANOVA analysis are significant, then proceed with DMRT (Duncan multiple range test) analysis to determine the most effective dose of the ethanolic extract of the fruiting body of *G. lucidum* in reducing cadmium toxicity.

RESULTS AND DISCUSSION

The mean blood cadmium levels, β2M, MDA and SOD interpreted that each treatment had different values (**Figure 1.**). Treatment P_0 which is a healthy control, with the lowest mean blood cadmium, β2M, and MDA and the highest SOD compared to the other four treatments. The mean blood cadmium, β2M, and MDA levels were the highest in the P_1 treatment, while the lowest mean SOD levels in the P_1 treatment indicated that cadmium had been induced in the body.

Measurement of blood cadmium levels at the beginning of cadmium induction had relatively high levels. Cadmium is still quite high in the blood and then decreases after several months post-induction. The decrease in levels was because cadmium had been distributed to several tissues and accumulated in the kidneys as an excretory organ (Nasiadek et al., 2019). Cadmium levels in the body are distributed in the blood and some tissues to accumulate in the kidneys (Satarug et al., 2000).

Cadmium induction in this study which lasted for 10 days and examination at 15 days post-induction (day 32) could still measure blood cadmium levels from test animals. This is in accordance with the study of blood cadmium levels were still measurable after cadmium induction in Sprague-Dawley rats (Cho et al., 2010). The study showed a decrease in blood cadmium levels followed by an increase in kidney cadmium levels in line with the length of the induction time for 30 days. The decrease in blood cadmium levels for a long time occurs because the Cd-MT binding has been reduced in the kidneys.

The effect of cadmium exposure on the kidneys in this study was seen from the levels of β2M. Blood cadmium levels in line with an increase in serum β2M levels (Chen et al., 2018). β2M is a biomarker with high sensitivity to kidney damage due to cadmium exposure (Hong & Lim, 2012). The use of serum β2M

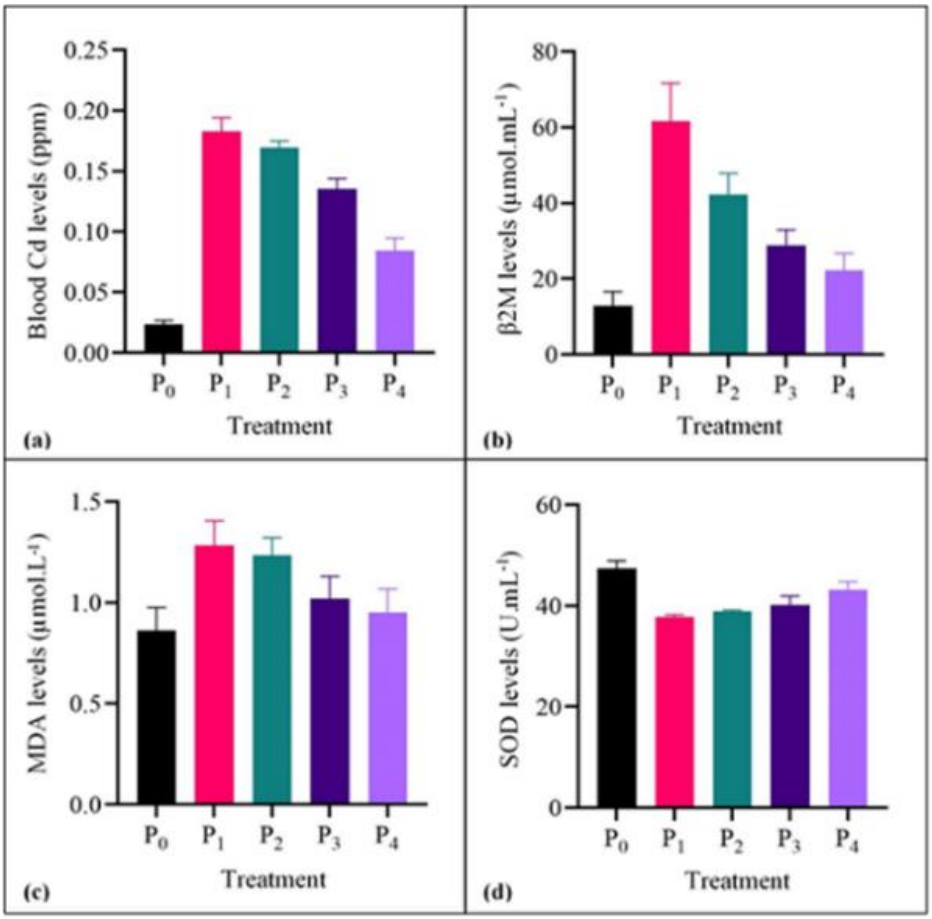

Figure 1. Histogram of mean levels of research parameters in male albino rats; **a)** average blood Cd levels; **b)** mean grade β2M; **c)** the average level of MDA; **d)** the average level of SOD; P_0: Healthy control (without induced cadmium and ethanol extract of *G. lucidum* fruiting body); P_1: negative control (cadmium induced); P_2: induced cadmium and 250 mg.kgBW^{-1} ethanol extract of the fruiting body of *G. lucidum*; P_3: induced cadmium and 500 mg.kgBW^{-1} ethanol extract of the fruiting body of *G. lucidum*; P_4: induced cadmium and 750 mg.kgBW^{-1} ethanol extract of *G. lucidum* fruiting body

Table 1. Analysis of variance test for blood cadmium, β2M, MDA and SOD levels

Parameter	*p-value*	Remarks
Blood Cd	0.000	Significant
β2M	0.000	Significant
MDA	0.000	Significant
SOD	0.000	Significant

Table 2. DMRT test 99% confidence level of blood cadmium, β2M, MDA and SOD

Treatment	Blood Cd (ppm)	β2M (µg.mL^{-1})	MDA (µmol.L^{-1})	SOD (U.mL^{-1})
P_0	0.023 ± 0.003^{a}	12.87 ± 3.71^{a}	0.86 ± 0.11^{a}	47.38 ± 1.54^{a}
P_1	0.183 ± 0.011^{d}	61.65 ± 9.97^{d}	1.28 ± 0.12^{c}	37.83 ± 0.38^{c}
P_2	0.169 ± 0.006^{d}	42.29 ± 5.53^{c}	1.23 ± 0.09^{c}	38.87 ± 0.28^{c}
P_3	0.136 ± 0.008^{c}	28.71 ± 4.16^{b}	1.17 ± 0,05^{bc}	40.15 ± 1.76^{c}
P_4	0.084 ± 0.011^{b}	22.23 ± 4.47^{ab}	1.02 ± 0.11^{ab}	43.20 ± 1.59^{b}

Note: the numbers accompanied by the same letter do not differ based on the DMRT test at a 99% confidence level

biomarkers for short-term exposure to cadmium was considered appropriate because serum β2M levels decreased with increasing dose and exposure time (Kawasaki et al., 2004).

The mechanism of cadmium exposure in the kidney, due to the release of the Cd-MT complex in the proximal renal tubule. The Cd-MT complex is released, with MT being degraded by endosomes and lysosomes, while cadmium accumulates in the cytosol of the proximal tubule (Fels et al., 2019). Studies exposure to cadmium in the kidneys on pregnant female mice, that increases the production of free radical compounds that trigger cell damage through the mechanism of lipid peroxidation (Chater et al., 2008). The event of lipid peroxidation in the kidneys due to exposure to cadmium increased MDA levels in serum, liver, and kidney of rats (Kara et al., 2005).

Exposure to cadmium results in a decrease in the body's antioxidant enzymes such as SOD. Studies of cadmium exposure in rats showed that SOD levels decreased in line with a decrease in the body's antioxidant activity. A decrease in SOD levels interprets the effects of cadmium exposure and an increase in ROS in the body (El-Boshy et al., 2015). The role of SOD as an antioxidant enzyme in the body decreases, causing oxidative stress that triggers an increase in ROS. The body's inability to neutralize ROS, triggers a continuous effect of lipid peroxidation and decreases SOD levels (Hormozi et al., 2018).

Table 1. shows that each parameter has a significant effect on changes in blood cadmium levels, β2M, MDA and SOD. The results of the DMRT test at the 99% confidence level (**Table 2.**), interpreted that the P_4 treatment or a dose of 750 mg.kgBW^{-1} ethanol extract of the fruiting body of *G. lucidum* was an effective dose in reducing blood cadmium levels (54.10%), β2M (63.94%) and MDA (20.31%) and increased SOD levels (14.20%) compared to sick control (P_1), because they tended to have levels towards healthy control values (P_0). Based on **Figure 2.**, blood cadmium levels have a positive correlation with β2M and MDA levels, and a negative correlation with SOD levels. The extract weight obtained in this study was 52.34 g, so the percentage yield obtained was 5.23%. Induction of the ethanolic extract of the fruiting body of *G. lucidum*, showed a decrease in blood cadmium levels accompanied by a decrease in β2M and MDA levels and an increase in SOD levels. This is because the ethanol extract of the fruiting body of *G. lucidum* contains various compounds. The results of the identification of compounds in the ethanol extract of the fruiting body of *G. lucidum* using the GC-MS method, identified the dominant compounds is linalool (56.88 percent).

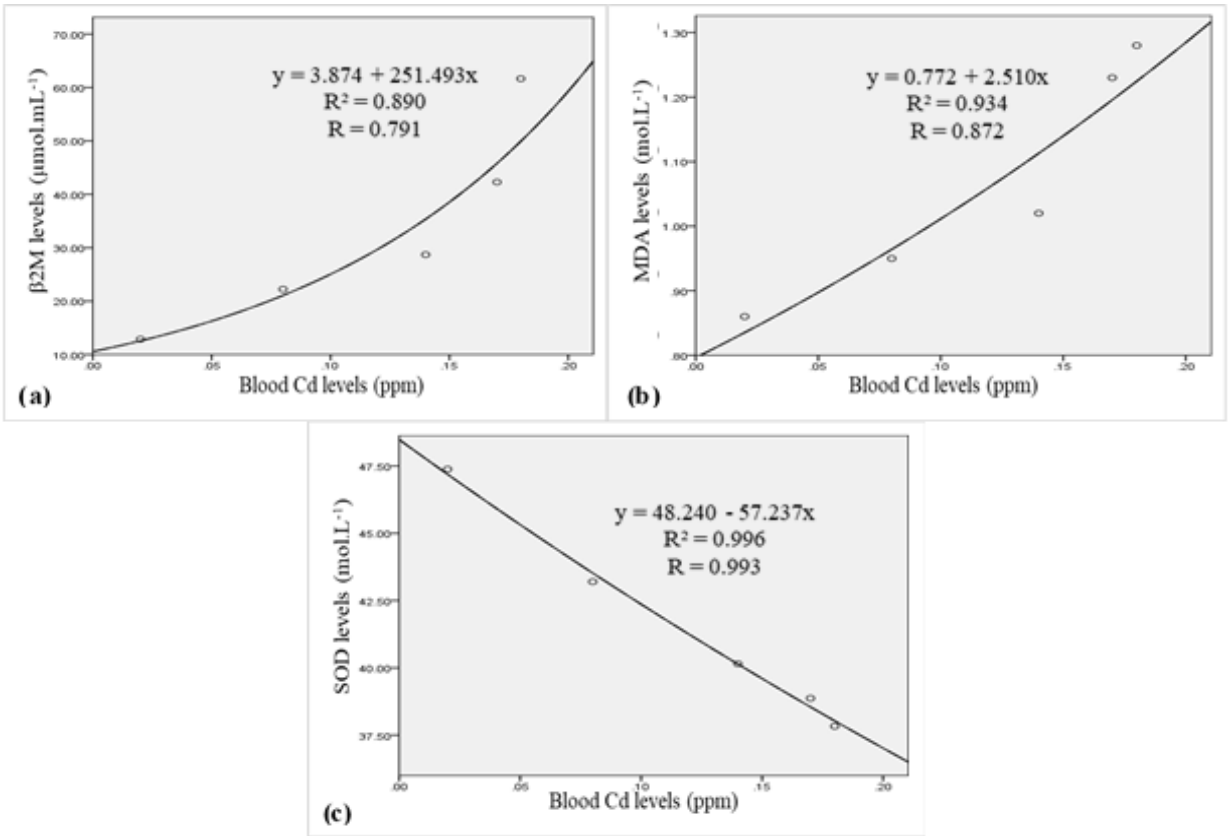

Figure 2. Regression curve of blood Cd levels with parameters β2M, MDA and SOD; **a)** positive correlation of blood cadmium - β2M; **b)** positive correlation of blood cadmium – MDA; **c)** negative correlation of blood cadmium – SOD

The decrease in MDA levels occurs because linalool is able to suppress oxidative stress in the body. Linalool has an antioxidant effect by reducing levels of free radicals in the body, so that the rate of lipid peroxidation decreases (Altinoz et al., 2021). Oxidative stress conditions were returned to neutral by linalool, triggering an increase in SOD. This results in the inhibition of superoxide anion free radicals from being oxidized to hydroxy peroxide (Xu et al., 2017).

Identification of the compound content of the ethanol extract of the fruiting body of *G. lucidum* using GC-MS in this study did not detect any flavonoid content. On the other study, the flavonoid compound was identified. The ethanolic extract of *G. lucidum* has a high terpenoid content compared to phenolic compounds such as flavonoids (Lin et al., 2015). Qualitative screening of flavonoid content of the mycelium of *G. lucidum* was detected as weak (Ratnaningtyas et al., 2018b). The levels of flavonoids in *G. lucidum* tend to be lower and only one type of flavonoid derivative was detected in this study, namely catechins (Yahia et al., 2017).

The undetected flavonoid content could be caused by the instrument used was not proper for flavonoid analysis. The GC-MS method can be used for nonpolar and volatile compounds analysis, such as fatty acids, essential oil and terpene (Jiang et al., 2016). The phenolic compounds like flavonoid only detected by LC-MS method (Pukalski & Latowski, 2022).

CONCLUSIONS

Ethanol extract of the fruiting body of *G. lucidum* has 56,88 percent linalool as active compound, was able to reduce cadmium-induced toxicity in albino rats by decreasing blood cadmium, β2M and MDA, and increasing SOD levels. The effective dose of the ethanolic extract of the fruiting body of *G. lucidum*, namely 750 mg.kgBW^{-1}, was able to reduce blood cadmium (54.10%), β2M (63.94%) and MDA (20.31%), as well as increase SOD levels (14.20%) compared to sick control.

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