Short Communications

The Protective Effect of *Gynura procumbens* Adventitious Root against Lead Acetate Toxicity in Mice

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**ABSTRACT**

Lead induced oxidative stress contributes to increase the productivity of reactive oxygen species (ROS) and to disrupt the antioxidant balance. *Gynura procumbens* adventitious root (GPAR) methanol extract contains abundant phenolic and flavonoids compounds as antioxidants and can be used as traditional medicinal plants. The objective of this study is to evaluate the protective effect of GPAR against lead acetate toxicity in mice to haematological parameter, histological of hepatic cells, and activities of antioxidant enzymes. The data obtained from five groups of treatment: P1 (control), P2 (Pb acetate-100 mg/L), P3 (GPAR-100 mg/L + Pb acetate-100 mg/L), P4 (GPAR-200 mg/L + Pb acetate-100 mg/L), P5 (GPAR-300 mg/L + Pb acetate-100 mg/L). The results indicated that administration of methanol extract of GPAR can prevent the decreasing of haematological parameter, maintain the percentage of normal hepatic cells, activities of superoxide dismutase (SOD), and catalase (CAT) due to lead acetate treatment. The effective dose of GPAR extract was 300 mg/L. This study provides that methanol extract of *G. procumbens* adventitious root exerts protective effects against lead acetate toxicity in mice.

Keywords: adventitious root of *Gynura procumbens*, antioxidant enzymes, hepatic cells, lead, hematological profile

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Lead (Pb) is non-biodegradable heavy metal. It can pollute the environment and endanger organisms, especially humans. Lead absorbed into the body through the respiratory tract, digestive tract, and skin; then be stored in tissues or organs, such as bone marrow, muscle, brain, kidney, heart, spleen, and liver. Most of leads accumulate in the liver, which are about 33% because they are related to the function of the liver, such as storage, biotransformation, and detoxification of toxic compounds (Mudipalli 2007; Haouas et al. 2014)

Exposure to high concentrations of lead is inducing oxidative stress because of the formation of reactive oxygen species (ROS) or as free radicals. If the ROS production is excessive and the endogenous antioxidants cannot overcome, it will trigger the occurrence of lipid peroxidation, which impacts changes in cell membrane integrity and eventually results in cell damage. Metwally (2015) reported swelling of the liver cells of mice lead-treated for 2-4
weeks. It is caused by swelling of intracellular organelles, particularly mitochondria and endoplasmic reticulum. Lead also inhibits the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). High production of ROS leads to the suppression of the antioxidant in the human body. SOD is an oxidoreductase that acts as a superoxide radical detoxifier by converting superoxide into a less reactive form. In humans, the lower the SOD activity, the more various trigger reactions to metabolic diseases. CAT includes hydroperoxidase enzymes that catalyzed the breaking down $\text{H}_2\text{O}_2$ into $\text{H}_2\text{O}$ (Ercal et al. 2001; Kumar et al. 2015; Patra et al. 2011).

Several previous studies reported that to compare the control group, treatment by lead was significantly decreasing red blood cell (RBC), hemoglobin concentration (HGB), hematocrit (HCT) (Sugiharto et al. 2019a) and reducing SOD and CAT activity (Carocho & Ferreira 2013). On the contrary, leads were increasing platelet (PLT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) (Sugiharto et al. 2020a), malondialdehyde (MDA) level, and lead concentrations in the liver (Sugiharto et al. 2019b), and were also significantly shifting Bowman's capsule diameters, and glomeruli–Bowman's ratio (Sugiharto et al. 2020b). The other studies showed that administration of 1000 ppm lead acetate for 30 days caused parenchymal degeneration, hydropic and liver cell necrosis in mice (Mauliku & Gustian 2019), while administration of 4% lead acetate for 17 days in mice could cause damage to several parts of the brain (Naqi 2015).

To mitigate the impact caused by lead exposure, the exogenous antioxidants can be given because of their function to stop or to break the chain reaction of free radicals in the human body. The administration of endogenous antioxidants is expected to reduce cell or tissue damage caused by free radicals. *Gynura* is one of the plants that contain secondary metabolites which has potential antioxidants. Puangpronpitag et al. (2010) and Teoh et al. (2013) reported that rats treated with *Gynura* methanol extract did not show significant toxicity symptoms and were safe to use as natural medicinal ingredients. *G. procumbens* is rich in phenolic and flavonoid compounds, which are also known to contribute to antioxidant properties. The results of adventitious roots culture of *G. procumbens* influenced by phenylalanine and tyrosine could increase in flavonoid compounds (Manuhara et al. 2019). Based on the High-Performance Liquid Chromatography (HPLC) assay, we found that methanol extract of the adventitious root of *G. procumbens* contains abundant of flavonoids compounds, such as myricetin, kaempferol, quercetin, and catechins (Sugiharto et al. 2021). Krishnan et al. (2015) reported that the root extract of *G. procumbens* had higher antioxidant potential than leaves and stems extract.

The present study was aimed to investigate the protective effect of *G. procumbens* adventitious root extract against lead acetate toxicity in mice. RBC, HGB, AST levels, ALT levels, histological analysis of hepatic cells, and activities of antioxidant enzymes (SOD and CAT) were also determined. There
are limited reports regarding the application of *G. procumbens* adventitious root extract produced from tissue culture for treatment in animal as well as to be used for traditional medicinal plants.

*G. procumbens* adventitious root in a laboratory-scale bioreactor was harvested after 28 days of culture (Faizah et al. 2018; Manuhara et al. 2019). Briefly, 25 g of dry root powder of *G. procumbens* were immersed in 250 mL of methanol (1:10) and macerated in a shaker for 24 h and supernatant filtered through filter paper. This procedure was treated three times using methanol as solvent and the filtrates were concentrated using an evaporator.

The use of animal subjects has been approved by Faculty of Veterinary Ethics Committee, Airlangga University (certificate no. 2.KE. 151.07.2019). Total of 25 (8-10 weeks old) male mice (*Mus musculus*, strains Balb/C) obtained from Faculty of Pharmacy, Airlangga University. Mice were randomly gathered into five treatment groups:

- P1: 0.25 mL of distilled water (control)
- P2: 0.25 mL of Pb acetate 100 mg/L
- P3: 0.25 mL of GPAR 100 mg/L and 0.25 mL of Pb acetate 100 mg/L
- P4: 0.25 mL of GPAR 200 mg/L and 0.25 mL of Pb acetate 100 mg/L
- P5: 0.25 mL of GPAR 300 mg/L and 0.25 mL of Pb acetate 100 mg/L

*G. procumbens* treatment was given every morning around 09:00 to 10:00 a.m., and lead treatment was given 2 h after that. Preparation of 100 mg/L Pb stock solution was carried out by dissolving 10 mg of lead acetate into 100 mL of distilled water. Meanwhile, the preparation of a 300 mg/L stock solution of methanolic extract GPAR was carried out by dissolving of 3 mg the GPAR extract into 10 mL of distilled water. The same method was also used to make 100 and 200 mg/L GPAR solutions. Treatment by oral administration for 30 days using *cannula*. Blood samples were taken using the cardiac puncture technique. The blood samples were mixed and incubated (3000 rpm) for 10 min at 10 °C to harvest serum for measurement of hematological parameters and activities of antioxidant enzyme (SOD and CAT) analysis. In addition, the preserved liver analyzed using hematoxylin eosin (HE) staining that embedded into paraffin for histological analysis. The histological hepatic cell observed by light microscopic (Olympus CWHK) with graticulae (magnification 400x). Histological observations of the liver were carried out in four (4) fields of view, namely superior, median right side, median left side, and inferior. The observed cells were normal cells (cells with oval shape, solid round nucleus in the middle); swollen cells (cells have clear and wide cavities, irregular cytoplasm); and necrotic cells (cells are small, nucleus not very clear and eosinophilic). The total number of cells in one field of view is assumed to be 100% (Sugiharto et al. 2019b).

\[
\% \text{ normal cell} = \frac{\text{normal cell}}{\text{total number cell}} \times 100\% \quad \ldots (1)
\]

All data were expressed as means ± standard deviations. The statistical analyses (p < 0.05) for the RBC, HGB, AST level, SOD activity, percentage of swollen and hepatic necrosis cells were subjected by SPSS 25.0. ANOVA
and Duncan’s test were applied. The Kruskal-Wallis and Mann-Whitney tests are used to determine ALT level and percentage of normal hepatic cells because the data are not normally distributed based on the Kolmogorov-Smirnov test (p < 0.05).

This study showed that lead could induce toxicity due to an increase in ROS and lipid peroxidation. Lead toxicity can cause changes in several parameters such as haematological parameter, enzyme activities of SOD and CAT in blood serum, as well as histological hepatic cells (Table 1, Figure 1, Figure 2, and Figure 3). Most of the lead accumulate in the liver, generating changes in cell membrane integrity and inhibition of endogenous antioxidant enzymes activity, cell or organ damage, and metabolic disorders (Assi et al. 2016; Mudipalli 2007).

![Figure 1. Effect of G. procumbens adventitious root against lead acetate on histological hepatic cells. P1=control, P2=Pb acetate-100 mg/L, P3=GPAR-100 mg/L + Pb acetate-100 mg/L, P4=GPAR-200 mg/L + Pb acetate-100 mg/L, P5=GPAR-300 mg/L + Pb acetate-100 mg/L. One-way ANOVA and Duncan's test performed statistical analysis for the percentage of swollen and hepatic necrosis cells. The Kruskal-Wallis and Mann-Whitney tests were conducted for the normal cells (p < 0.05).](image)

Table 1. Effect of G. procumbens adventitious root against lead acetate on hematological parameters.

<table>
<thead>
<tr>
<th>Data</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10⁶/mm³)</td>
<td>9.93 ± 0.72c</td>
<td>5.96 ± 0.93a</td>
<td>8.08 ± 0.36b</td>
<td>8.25 ± 1.36b</td>
<td>8.12 ± 0.58b</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>15.57 ± 0.85c</td>
<td>8.63 ± 1.67a</td>
<td>12.25 ± 0.66b</td>
<td>12.73 ± 2.53b</td>
<td>12.33 ± 2.03b</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>119.75 ± 23.96c</td>
<td>230.00 ± 32.32c</td>
<td>199.25 ± 3.30bc</td>
<td>202.25 ± 49.20bc</td>
<td>167.75 ± 34.73ab</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>50.75 ± 10.11a</td>
<td>149.00 ± 47.95b</td>
<td>71.50 ± 8.89bc</td>
<td>68.75 ± 18.54bc</td>
<td>78.25 ± 8.77bc</td>
</tr>
</tbody>
</table>

RBC=red blood cells, HGB=hemoglobin concentrations, AST=aspartate aminotransferase, ALT=alanine aminotransferase. P1=control, P2=Pb acetate-100 mg/L, P3=GPAR-100 mg/L + Pb acetate-100 mg/L, P4=GPAR-200 mg/L + Pb acetate-100 mg/L, P5=GPAR-300 mg/L + Pb acetate-100 mg/L. Statistical analysis one-way ANOVA and Duncan's test performed to RBC, HGB and AST levels. The Kruskal-Wallis and Mann-Whitney tests were conducted for the ALT level. Both analysis were significant at p < 0.05.
Figure 2. Effect of *G. procumbens* adventitious root against lead acetate on SOD activity. P1=control, P2=Pb acetate-100 mg/L, P3=GPAR-100 mg/L + Pb acetate-100 mg/L, P4=GPAR-200 mg/L + Pb acetate-100 mg/L, P5=GPAR-300 mg/L + Pb acetate-100 mg/L. One-way ANOVA and Duncan’s test performed statistical analyses (p < 0.05).

Figure 3. Effect of *G. procumbens* adventitious root against lead acetate on CAT activity. P1=control, P2=Pb acetate-100 mg/L, P3=GPAR-100 mg/L + Pb acetate-100 mg/L, P4=GPAR-200 mg/L + Pb acetate-100 mg/L, P5=GPAR-300 mg/L + Pb acetate-100 mg/L. One-way ANOVA and Duncan’s test performed statistical analyses (p < 0.05).

Compared to the control group, the administration of lead acetate decreased RBC, HGB, percentage of normal hepatic cells, and activity of SOD and CAT. Whereas, lead treatment increased AST levels, ALT levels, the percentage of swollen and necrotic of hepatic cells significantly. Lead toxicity could induce oxidative stress and caused lipid peroxidation in cell membrane. It can be increased cells damage, especially in liver and kidney. Similar to present study, Haouas et al. (2014) reported administration of lead acetate 2 g/L for 35 days increased hypertrophy of hepatic cells and accompanied by an increase in AST and ALT levels. Administration of lead acetate 20 mg/kg BW for 3 weeks increased necrotic of hepatic cells, MDA levels, AST, and ALT levels as indicators of damage in liver cells (Yuniarti et al. 2021). Met-
wally (2015) reported swollen hepatic cells and intracellular organelles for 2-4 weeks due to lead administration. Lead exposures were significant to reduce SOD and CAT activity (Carocho & Ferreira 2013; Sugiharto et al. 2019a). Exposure of 150 mg/kg BW lead acetate for mice showed decreasing of SOD activity and significantly increased H₂O₂ formation (Andjelkovic et al. 2019).

Lead is a lipophilic compound and turns into Pb²⁺ in the body. Lead is extremely poisonous and devastating liver cells, induced lipid peroxidation and associated with increase of ROS. When the production of ROS is excessive, it is directly suppressed the body’s antioxidative system. Lipid peroxidation due to lead administration could disrupt membrane integrity in the cell, increase cell membrane permeability and the distribution of the ions, it causing extracellular fluid easily through to the cell. As a result, the cells become swollen and then undergo necrosis in hepatic cells (Sipos et al. 2003). Meanwhile, lead bound to the SH group inducing deficiency of the enzyme glucose 6-phosphate dehydrogenase (G6PD) in erythrocytes, and inhibition of the enzymes coproporphyrinogen, δ-aminolevulinic acid dehydratase (δ-ALAD), and ferrochelatase in the bone marrow. It leads to the decreasing of the life span of erythrocytes, increasing the fragility of the erythrocyte membrane, decreasing the number of erythrocytes, and impairing hemoglobin synthesis. In addition, high levels of ROS are an inhibitor of antioxidative enzyme activities such as SOD and CAT. The function of the SOD is to convert two superoxide radical anions (O₂⁻) into hydrogen peroxide molecules (H₂O₂) and oxygen molecules (O₂). Moreover, CAT as an antioxidative enzymes produced by peroxisomes, decomposed H₂O₂ molecules into H₂O (Sharma & Singh 2014; Sugiharto et al. 2019a; Sugiharto et al. 2020a; Wani et al. 2015).

*G. procumbens* adventitious root extract can be an antioxidant because it contains flavonoid and phenolic compounds (Krishnan et al. 2015; Pramita et al. 2018). Flavonoid and phenolic compounds showed inhibitory effects on ROS and radical scavenging activity by hydrogen atom transfer (HAT) or single electron transfer (SET) that producing more stable free radical. Chelating properties of OH group is probably responsible for the inhibitory effect of flavonoids and protection from oxidative stress; it has been implicated in several of degenerative diseases and exhibited anticancer activities. Wang et al. (2013) and Chaichana et al. (2019) reported that in cancer cell line assay, *Gynura* ethanolic extract (40 – 400 µg/mL) was able to inhibit human cells such as osteosarcoma cell line (U2-OS), gastric carcinoma cell line (KATO-III, HTB-103), human liver hepatocarcinoma cell line (HepG2, HB-8065), human colon adenocarcinoma cell line (Caco-2, CCL-227), and human breast cancer cell line (MCF-7). Furthermore, the myricetin and quercetin compounds in the methanol extract of the adventitious root of *G. procumbens* have exerted potent inhibition to lipid peroxidation and free radical scavenging activity, and it is thought to increase the of the antioxidative enzyme activity such as SOD and CAT by inactivating superoxide (Akan & Garip 2013; Tan
et al. 2016; Terao 2009; Yuniarti et al. 2021). The results of our study indicated that the decreasing of RBC, HGB, percentage of normal hepatic cells, and activity of SOD and CAT could prevent by administration of *G. procumbens* adventitious root extract. *G. procumbens* also significantly prevent increasing of AST and ALT levels, and the percentage of swollen and necrotic hepatic cells in mice exposed to lead. The effective dose of *G. procumbens* extract in this study was 300 mg/L, because there was an increasing in the activity of SOD and CAT enzymes as well as a decreasing in the number of necrotic hepatic cells and AST levels compare to that of *G. procumbens* at the doses of 100 and 200 mg/L. This study provided the scientific potential of *G. procumbens* to be used for traditional medicinal plants.

**AUTHORS CONTRIBUTION**

S.S designed the research, wrote the manuscript, and supervised all the process, D.W. collected and analysed data especially for histological analysis, U.I designed the research and supervised the data, A.H.M and A.B.M. analysed the data, Y.S.W.M. prepared *G. procumbens* adventitious root extracts and supervised all the process.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**


