Cogongrass (Imperata cylindrical L.) Roots Ethanol Extract to Improve Hematological Profile in Carbon Tetrachloride-Injection Mice Model

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Abstract
Carbon tetrachloride (CCL₄) is widely used in industry, toxic to the environment and humans, and most often used as a model of acute liver damage and liver fibrosis in experimental animals. Liver damage can deteriorate the hematological profile. The roots of cogongrass (Imperata cylindrica L.) have been used as traditional medicine due to its antioxidant activity. This study was conducted at the Faculty of Medicine, Universitas Padjadjaran, from January to March 2019. The study aimed to investigate whether the cogongrass roots ethanol extract (CGRE) can ameliorate the disturbance in the hematological profile in acute CCl₄-injected mice. CGRE in dose 150 and 200 mg/kgBW was given orally to mice for four weeks before intraperitoneal injection of CCl₄, 1 mL/kgBW in olive oil (1:1 v/v). After 48 hours, mice were sacrificed, and the whole blood was drawn for hematological analysis. As a result, mean corpuscular volume (MCV) was reduced in CCl₄-induction mice treated with CGRE in dose 150 mg/kgBW (49.25±3.06 vs 43.38±2.13 fl, p<0.05). This condition was followed by the improved hematocrit (Hct) and mean corpuscular hemoglobin concentration (MCHC). Platelet and platelet crit (Pct) levels were tended to decrease in CCl₄-induction mice treated with CGRE in dose 150 mg/kgBW. In conclusion, CGRE dose 150 mg/kg BW can improve MCV, Hct, MCHC, platelet, and Pct in CCl₄-injection mice. The antioxidant level in CGRE might facilitate it.

Key words: Antioxidant, carbon tetrachloride, cogongrass roots, hematological profile, liver damage

Ekstrak Etanol Akar Alang-alang (Imperata cylindrical L.) Memperbaiki Profil Hematologi pada Mencit yang Diinjeksi Carbon Tetrachloride

Abstrak
Carbon tetrachloride (CCL₄) banyak digunakan pada industri, bersifat toksik bagi lingkungan dan manusia, serta sering digunakan pada hewan coba untuk kerusakan liver akut dan fibrosis. Kerusakan liver dapat menyebabkan gangguan profil hematologi. Akar alang-alang (Imperata cylindrica L.) telah digunakan sebagai obat tradisional karena memiliki aktivitas antioksidan. Penelitian ini dilakukan di Fakultas Kedokteran Universitas Padjadjaran pada bulan Januari hingga Maret 2019. Tujuan penelitian ini adalah meneliti apakah ekstrak etanol akar alang-alang dapat memperbaiki gangguan profil hematologi pada mencit yang diinduksi CCL₄, secara akut. Ekstrak etanol akar alang-alang (EAEA) dosis 150 dan 200 mm/kgBB diberikan per oral kepada mencit selama empat minggu sebelum injeksi intraperitoneal CCl₄ 1 mL/kgBB yang dilarutkan dalam minyak zaitun (1:1 v/v). Setelah 48 jam, mencit dikorbankan dan diambil darahnya untuk pemeriksaan hematologi. Sebagai hasil, mean corpuscular volume (MCV) menurun pada mencit yang diinduksi CCl₄ dengan perlakuan EAEA 150 mg/kgBB (49.25±3.06 vs 43.38±2.13 fl, p<0.05). Keduaa, ini diikuti dengan perbaikan hematokrit (Hct) dan mean corpuscular hemoglobin concentration (MCHC). Kadar platelet dan platelet crit (Pct) cenderung menurun pada mencit yang diinduksi CCl₄, dengan perlakuan EAEA 150 mg/kgBB. Sebagai simpulan, EAEA dosis 150 mg/kgBB dapat memperbaiki MCV, Hct, MCHC, platelet, dan Pct pada mencit yang diinduksi CCl₄. Kesimpulan: difasilitasi oleh antioksidan pada EAEA.

Kata kunci: Akar alang-alang, antioksidan, carbon tetrachloride, kerusakan liver, profil hematologi
Introduction

Tissue damage can be caused by an increase in oxidative stress, where the production of reactive oxygen species (ROS) is higher than antioxidants in the body. ROS originates from the body's biological process, such as by-products of the electron transport chain and oxidase and peroxidase enzymatic activity. ROS production can also come from external exposure, such as organic solvent chemicals and pollution. Carbon can also come from external exposure, such as peroxidase enzymatic activity. ROS production of the electron transport chain and oxidase and the body's biological process, such as by-products marker reduction and peroxidation activity. The production of ROS is higher than in oxidative stress, where the production of reactive oxygen species (ROS) is lower than in oxidative stress. Tissue damage can be caused by an increase in oxidative stress, where the production of reactive oxygen species (ROS) is higher than antioxidants in the body. ROS originates from the body's biological process, such as by-products of the electron transport chain and oxidase and peroxidase enzymatic activity. ROS production can also come from external exposure, such as organic solvent chemicals and pollution. Carbon can also come from external exposure, such as peroxidase enzymatic activity. ROS production of the electron transport chain and oxidase and the body's biological process, such as by-products marker reduction and peroxidation activity.

Studies showed that plant extracts benefit from protecting the liver from damage and further complication of liver damage. For example, basil extract (Ocimum basilicum) ameliorates liver damage in the CCl₄-injection rats model showed decreased fibrosis signs and liver enzyme marker reduction and peroxidation activity. Other extract plants, Cnidoscolus aconitifolius, leaves extract, Teucrium polium aqueous extract, and Mangifera indica leaves extract has been identified to protect rats from hematology deterioration caused by CCl₄-injection rat. These extract plants contain high flavonoids derivate that have the ability as an antioxidant to protect cells from free radical.

Cogongrass (Imperata cylindrica L.) or alang-alang (Indonesian language) is a weed species invasively cultivated in tropical areas. Cogongrass roots have been used as a traditional medicine to treat fever and as a tonic due to their antioxidant activity. The extract ethanol of cogongrass roots (CGRE) has bioactive compounds, including flavonoid, isoflavone, and flavonol that play an antioxidant role. We suggested CGRE can ameliorate disturbance in hematological profile due to liver damage. Thus, the study aimed to investigate whether CGRE can ameliorate disturbance in hematological profile in acute CCl₄-induced liver damage animal model.

Methods

Cogongrass roots were obtained from Garut, West Java, and were identified by the School of Life Sciences, Institut Teknologi Bandung. Cogongrass roots were made powder and extracted using 96% ethanol (Merck, Japan) through a 72-hour maceration process. Maceration results were filtered and concentrated with the freeze-drying technique. The yield of CGRE products was diluted using 0.5% carboxymethyl cellulose (CMC, Merck, Japan).

Eight-week-old male mice were obtained from the Animal Laboratory, PT Bio Farma, Bandung, Indonesia. The study was conducted at the Faculty of Medicine, Universitas Padjadjaran, from January to March 2019 after obtaining approval from the Research Ethics Committee Universitas Padjadjaran (Number 1353/UN6.KEP/EC/2018). Mice were placed in a temperature-controlled room, 12-hour light, and 12-hour dark cycle and had unlimited access to water and food. Mice were divided into four groups: group I (as normal control) and II (as a negative control) were given 200 μL 0.5% CMC orally. Group III was given CGRE at a dose of 150 mg/kg Body Weight (BW) in 200 μL CMC 0.5%, and group IV were given CGRE at a dose of 200 mg/kgBW in 200 μL CMC 0.5%. The treatment was carried out for 28 days. Considering the high damage caused by CCl₄, the dose was doubled from the previous study to gain more flavonoid compounds.
weeks of treatment, groups II, III, and IV were given an injection of CCL4 1 mL/kgBW in olive oil (1:1 v/v). After 48 hours of induction; mice were terminated for blood collection by injection of ketamine-xylazine. Blood was drawn out through the abdominal portal vein as much as 100 μL and transferred into the EDTA microtube (BD, U.S.A.). The examination of 14 parameters of the hematology profile was conducted using the hematology analyzer machine (Samsung Labgeo HC-10).

Statistical analysis was performed by using GraphPad Prism 8. Data normality analysis was performed using the Shapiro-Wilk test of the normality test method. Analysis of variance (ANOVA) was used to analyze data among groups, followed by the Bonferroni post hoc test. Values are expressed as mean±standard deviation with significant level p<0.05.

Results

The results of hematological examination related to the profile of red blood cells are shown in Table 1. The total red blood cell examination (RBC) and hemoglobin did not show significant differences between groups after CCL4 induction. There was a significant increase in hematocrit in CCl4 compared to group I (p<0.05), but no change in the group given CGRE (groups III and IV). There was a significant increase in MCV values and decreased MCHC in group II compared to group I. However, the administration of CGRE significantly improved MCV and MCHC to the normal value in group I. There were no significant differences between groups in the mean corpuscular hemoglobin (MCH) value.

The results of hematological parameters related to white blood cells showed no increase in white blood cells’ components 48 hours after CCL4 induction (Table 2). CGRE administration did not change the value of total white blood cells (WBC), lymphocytes, monocytes, and granulocytes. Consistent with these results, the percentage value of white blood cell groups, i.e., % lymphocytes, % monocytes, and % granulocytes, did not differ significantly after CGRE administration.

The profile of hematological examination related to platelet parameters is shown in Table 3. The induction of CCl4 significantly increased the platelet and platelet crit (Pct) values by almost three times (p<0.05). However, the mean platelet volume (MPV) and platelet distribution width

### Table 1 Red Blood Cells Parameters

| Groups  | RBC (10⁶/µL) | Hb (g/dL) | Hct (%) | MCV (fl) | MCH (pg) | MCHC (g/dL) |
|---------|--------------|-----------|---------|----------|----------|-------------|
| Group I | 8.60±1.85    | 13.69±2.03| 37.37±7.48 | 43.71±2.56 | 16.24±1.71 | 37.14±2.82  |
| Group II| 9.44±0.36    | 15.09±1.17| 46.61±4.24 | 49.25±3.06 | 15.96±0.93 | 32.17±1.11  |
| Group III| 9.72±1.00   | 14.86±1.38| 41.97±3.09 | 43.38±2.13 | 15.34±0.87 | 35.36±1.01  |
| Group IV| 9.24±1.06    | 14.10±1.99| 40.99±7.05 | 44.29±3.86 | 15.21±0.68 | 34.64±2.55  |

Note: the same letter in superscript at the same column indicates statistically significant (p<0.05). RBC=red blood cells; Hb=hemoglobin; Hct=hematocrit; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration

### Table 2 White Blood Cells Parameters

| Groups  | WBC (10³/µL) | Lymphocyte (%) | Monocyte (%) | Granulocyte (%) |
|---------|--------------|----------------|--------------|-----------------|
| Group I | 4.32±2.00    | 77.40±5.63     | 12.01±1.98   | 10.56±4.46      |
| Group II| 5.15±0.08    | 77.13±8.74     | 10.98±4.67   | 11.90±4.79      |
| Group III| 6.95±2.02   | 77.18±7.61     | 10.80±3.79   | 12.03±7.56      |
| Group IV| 5.80±2.26    | 79.63±6.94     | 9.78±4.12    | 10.60±3.76      |

Note: WBC=white blood cells
(PDW) values did not differ significantly. CGRE administration reduced the value of Pct, although the statistical calculation was not significant.

**Discussion**

CCl₄ is widely used in industry and has a toxic effect on humans.³,²⁰ The induction of CCl₄ in animal models is used to investigate the impact of organ damage in the brain and liver.¹,²⁰ The increase of reactive oxygen mostly facilitates liver damage by CCl₄ and further can cause hepatocyte apoptosis. A disruption in lipid metabolism also causes liver damage that leads to the increase of lipid peroxidation.²⁰ In the long term, liver necrosis signs appear, and the liver enters the cirrhosis state. Natural products can be used to improve liver conditions and prevent further liver damage. For example, oral soybean consumption reduced hepatocyte apoptosis signs in CCl₄-induction mice.²¹

Liver damage can influence the hematological profile seen by anemia, leucocytosis, and thrombocytopenia. Our study focused on the role of CGRE to improve hematological profile in CCl₄-induced liver damage mice model in an acute state. According to the previous study, the assessment of hematological profile was performed after 48 hours of the induction after 4 weeks of oral CGREE treatment.⁷ After two days of CCl₄ induction, the circulation liver enzyme, aspartate transaminase (AST), and alanine aminotransferase (ALT) were dramatically increased, and necrosis signs appeared in the liver histopathologically.⁷

The number of RBC, WBC, and thrombocyte did not change after two days of CCl₄ injection. These results indicated that hematological profile deterioration, such as anemia, leucocytosis, and thrombocytopenia, resulted from chronic liver damage. This suggestion is supported by previous studies that showed chronic CCl₄ injection disrupts the number of RBC, WBC and thrombocyte and the giving of natural products such as *Cnidoscolus aconitifolius* leaf extract, *Teucrium polium* aqueous extract, and *Mangifera indica* leaf extract can ameliorate the hematological profile.¹²,¹³,¹⁵ Adebayo et al.’s²² study conducted acute liver damage by administering a solution of CCl₄: olive oil (1:1) with a dose of 2 mL/kgBW for four times in consecutive days before treatment. They showed that hemoglobin, RBC and platelet were reduced. However, no studies describe the hematological profile in a single dose of CCl₄ injection for an animal model of acute liver damage. Despite our findings, several red blood cell and platelet cell parameters had been disrupted in CCl₄-injection mice.

Hct and MCV were increased in CCl₄-induction mice and decreased after CGRE treatment with a dose of 150 mg/kgBW. The increase of Hct reflecting the increase of the ratio of red blood cells to the blood total volume and correlates with the increase of MCV, that calculated by multiplying Hct by ten and divided by RBC count.²³ The increase of MCV, suggesting macrocytic anemia. The condition is found in vitamin B12 deficiency or liver disease.²¹ Injection of CCl₄ causes disruption in lipid metabolism and further compromise in a lipid bilayer in RBC. CGRE treated mice showed the reduction of MCV and Hct, suggesting antioxidant compounds in CGRE rescued lipid metabolism. MCHC level reflecting the level of hemoglobin in RBC. Hemoglobin is a protein carrier for nutrients and oxygen derived from iron (Fe) metabolism. Considering the normal hemoglobin level and increase the MCV level, the low level of MCHC is secondary to the increase of RBC volume and less

| Groups  | Thrombocyte (10⁹/µL) | Pct (%) | MPV (fl) | PDWc (%) |
|---------|----------------------|---------|----------|----------|
| Group I | 494.29±178.58⁺       | 0.15±0.05⁺ | 3.10±0.39 | 35.17±1.86 |
| Group II| 709.25±96.09⁺        | 0.28±0.12⁺ | 3.93±1.29 | 35.13±1.89 |
| Group III| 583.75±91.55        | 0.18±0.03  | 3.16±0.33 | 34.91±1.81 |
| Group IV| 604.43±164.66        | 0.18±0.10  | 2.87±0.89 | 34.06±1.58 |

Note: the same letter in superscript at the same column indicates statistically significant (p<0.05). PCT=platelet crit; MPV=mean platelet volume; PDW=platelet distribution width
likely due to iron deficiency. Improvement of Hct and MCV facilitated by CGRE dose of 150 mg/kgBW might increase the level of MCHC. Further analysis of the iron level and iron-related protein could confirm this hypothesis.

Thrombocytopenia is commonly found in chronic liver damage conditions both in human and animal models. However, the results of the platelet count is not stable. The platelet count was increased in CCl₄-induction mice and agreed with the study conducted by Gazwi and Mahmoud. However, the increase of platelet count and Pct might cause thrombocytosis leads to organ disruption. A recent study showed that Pct could be used as a predictor of liver fibrosis. Administration of CGRE before CCl₄-induction can protect the liver from further liver damage due to its antioxidant effect indicating Pct's reduction. Liver histopathology and inflammation marker examination are necessary to confirm this hypothesis.

Conclusions

A CGRE dose of 150 mg/kgBW can improve MCV, Hct, MCHC platelet, and Pct in CCl₄-injection mice. A high antioxidant level in CGRE might facilitate it.

Conflict of Interest

All authors state there is no conflict of interest in this article.

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