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THE HEMATOLOGIC PROFILE IN THE ACUTE TOXICITY TEST OF COGON GRASS ROOTS ETHANOL EXTRACT IN WISTAR RATS

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ABSTRACT

Objective: This study aimed to investigate the hematologic profile of Wistar rats in the acute toxicity test of Cogon grass roots ethanol extract (CGEE).

Methods: Cogon grass roots were dissolved in 70% ethanol. An acute toxicity test was conducted based on The National Agency of Drug and Food Control of the Republic of Indonesia. Five female rats in the treatment group were administered a single high dose of 5000 mg/kg body weight (BW) of CGEE in 200 µl of 0.5% carboxymethyl cellulose (CMC), and the 5 female rats in the control group were administered 200 µl of 0.5% CMC. After 14 d, blood samples were collected, and 18 hematologic parameters were measured with a hematology analyzer. Statistical analyses were performed to compare the parameters between the two groups with the independent t-test for normally distributed data and the Mann Whitney test for non-normally distributed data.

Results: None of the hematologic parameters in the treatment group significantly differed from those in the control group after 14 d of observation ($P>0.05$).

Conclusion: A single high dose of 5000 mg/kg BW of CGEE did not change the hematologic profile of Wistar rats. These results indicate that CGEE does not have an acute hemotoxic effect, at least for hematologic parameters.

Keywords: Acute Toxicity, Cogon Grass root Ethanol Extract, Hematologic Profiles

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INTRODUCTION

Cogon grass (*Imperata cylindrica*), which is also known as Alang-alang, is widely distributed in tropical countries and empirically used as herbal medicine. Cogon grass roots have been used as a medicine for glomerulonephritis, fever, shortness of breath, and epistaxis and can reduce plasma lipids and glucose [1]. The phytochemicals results showed Cogon grass roots contain tannins, saponins, flavonoids, alkaloids, and terpenoids [2].

A study conducted by Ruslin *et al.* in 2012 revealed that Cogon grass roots have an anti-hypertensive effect [3]. Cogon grass roots can improve the production of nitric oxide in mice with streptozotocin-induced diabetes [4]. A study conducted by Robianto in 2019 demonstrated that Cogon grass root ethanol extract (CGEE) shortens the oestrous phase in female mice [5]. Anggraeni showed in 2017 that CGEE can reduce the serum cholesterol in mice and potentially be used as an anti-hypercholesterolemia agent [6].

CGEE is a potential standardized herbal medicine. As one of the requirements for standardized herbal medicines, a toxicity test was performed for CGEE. The toxicity test consisted of acute toxicity, chronic toxicity, and pharmacodynamic evaluations. The acute toxicity test was conducted based on The National Agency of Drug and Food Control of the Republic of Indonesia. An acute toxicity test is performed to determine the toxic effects that may arise from the administration of a single dose of a compound [7]. Hematologic parameters need to be investigated because some of the plant's medicine can influence hematologic parameters. For example, *Viscum album* (mistletoe) extracts from *Coffee arabica* (coffee) host plant reduced red blood cell (RBC) parameters and increased the number of white blood cells (WBCs) after 14 d of oral administration in Wistar rats [8]. Thus, this study aimed to determine the hematologic profiles of Wistar rats in an acute toxicity test of CGEE.

MATERIALS AND METHODS

Plant material and preparation of the extract

Cogon grass roots were dissolved in 70% ethanol for 72 h, and the end products were a supernatant and sediment. The supernatant was evaporated to obtain a concentrated extract. The CGEE was diluted with 0.5% carboxylmethyl cellulose (CMC) to obtain a concentration of 5000 mg/kg body weight (BW).

Experimental animals

Female Wistar rats with a body weight of around 200 g and age of 4-5 w were collected from xxxx in Indonesia. The Institutional Animal Care and Use Committee (Faculty of Medicine, xxxx) approved all study protocols. Ten female Wistar rats underwent acclimatization for 7 d. Before and during the experiment, the rats had unrestricted access to water and a chow diet. The rats were divided into two groups; the control group received 0.5% CMC, and the treatment group received 5000 mg/kg BW CGEE.

Acute toxicity test

The acute toxicity test was conducted based on The National Agency of Drug and Food Control of the Republic of Indonesia. After a 14-18 h fast, the treatment group received a single oral dose of 5000 mg/kg BW CGEE in 200 µl of 0.5% CMC, and the control group received 200 µl of 0.5% CMC. Blood samples were collected 14 d later.

Hematological analysis

The rats were fasted overnight before blood sample collection. After proper anesthetization, blood samples were collected from the abdominal vein and transferred to an EDTA microtube. The following hematological parameters were measured with an automated hematology analyzer (Sysmex Automated Hematology

Analyzer; Sysmex Corporation, Japan): WBCs, a leukocyte differential (lymphocytes, monocyte, granulocyte), RBCs, hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), Platelets, mean platelet volume (MPV), platelet crit (PCT) and platelet distribution width concentration (PDWC).

RESULTS

After 14 d of treatment, the hematologic profiles were measured in both groups. The results showed that the RBCs and WBCs of the treatment group did not significantly differ from those of the control group (fig. 1). The platelets did not significantly differ between the treatment and control groups either (P>0.05). The analysis of the WBC components, including lymphocytes, monocytes, granulocytes, and the percentage of lymphocytes, monocytes, and granulocytes, did not significantly differ between the treatment and control groups (P>0.05) (fig. 2). The analysis of the RBC components, including Hb, Hct, MCV, MCH, MCHC, and RDW, did not differ between the treatment and control groups (fig. 3). In addition, the platelet components, including MPV, PDWC, and PCT, did not differ between the treatment and control groups (fig. 4).

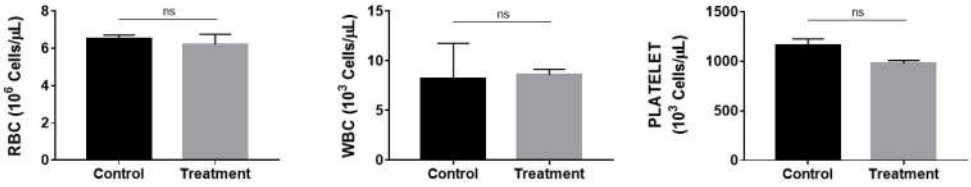

Fig. 1: Hematologic analysis of red blood cells (RBCs), white blood cells (WBCs), and platelets. ns= not significant different

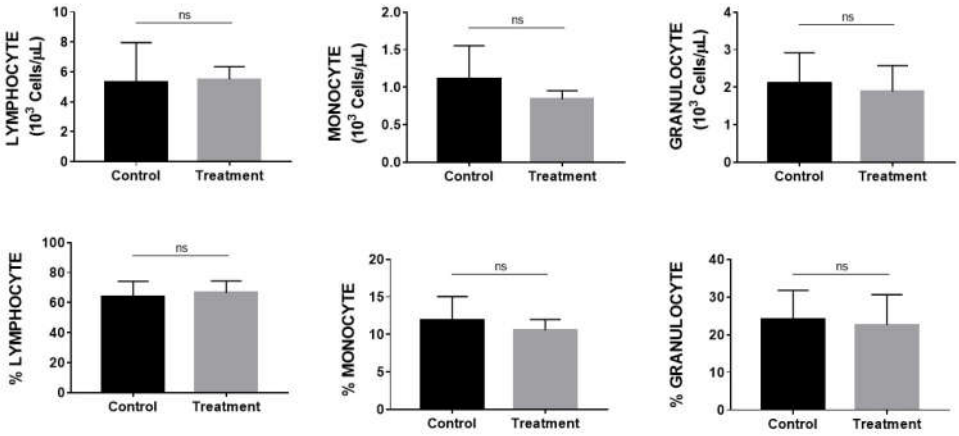

Fig. 2: Hematologic analysis percentage of white blood cell (WBC) components. ns= not significantly different

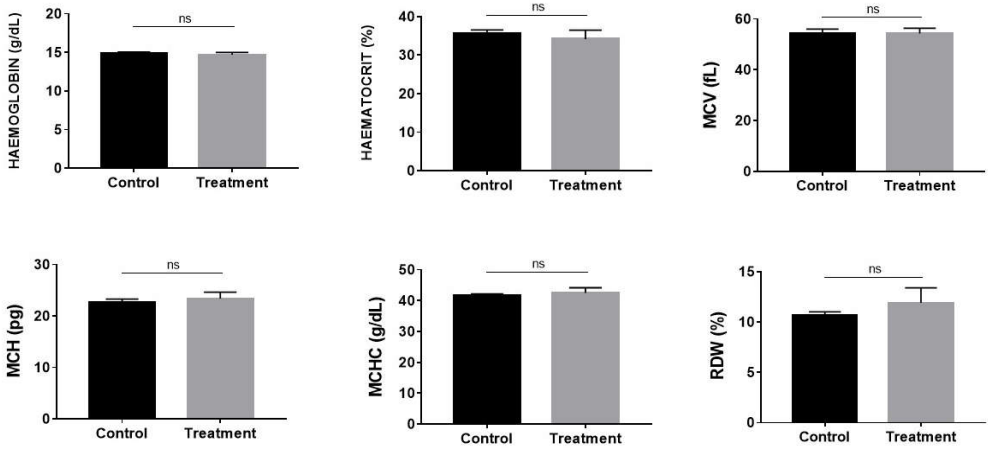

Fig. 3: Hematologic analysis of red blood cell (RBC) components. Hemoglobin (Hb); Hematocrit (Hct); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC) and red distribution width (RDW). ns= not significantly different

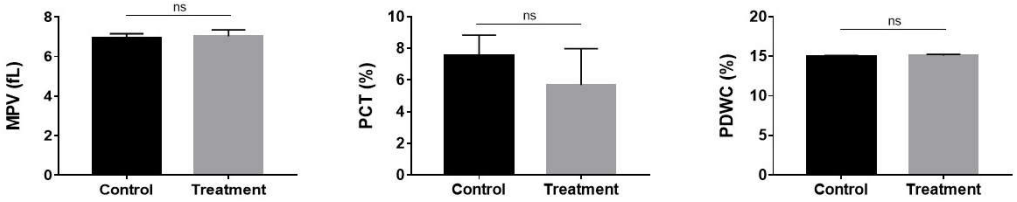

Fig. 4: Hematologic analysis of the platelet components. Mean platelet volume (MPV); plateletcrit (PCT); and platelet distribution width concentration (PDWC). ns= not significantly different

DISCUSSION

One of the issues of herbal medicines is the safety and toxicity of herbs. Some traditional herbal medicines have shown toxic effects and could be harmful in the short or long term [9]. To confirm the safety of herbal medicine and/or its constituents, toxicity tests, including acute toxicity, sub-chronic toxicity, and chronic toxicity tests, must be conducted. This study aimed to investigate the hematologic profiles of female Wistar rats after an acute toxicity test. The purpose of a hematologic test during an acute toxicity test is to determine the toxicity of a component of a drug, including plant extracts [10, 11]. Our results indicated that a high dose of CGEE had a consistent effect on the hematologic parameters.

The results of our study are consistent with those of a previous study conducted by Chunlaratthanaphorn in 2007 [13]. They showed no significant difference in RBCs, platelets, and WBCs in rats that received 1200 mg/kg BW of an *Imperata cylindrica L* water extract. The results of the RBC tests showed no difference in the number of RBCs between the treatment and control groups, which means the CGEE did not affect erythropoiesis or the osmotic pressure on erythrocyte cells [14]. Some bioactive substances in a plant can induce anemia, which is characterized by low RBC and Hb levels [15, 16]. The administration of 5000 mg/kg BW CGEE was not able to induce anemia during treatment. WBCs are the first body defense that will respond when there is an infection, inflammation, or tissue injury [10, 17]. The results showed no significant differences in the neutrophils, lymphocytes, or monocytes between the treatment and control groups. Lymphocytes are cells that mediate the immune response to foreign substances [12]. The results of this study did not show a change in lymphocytes, although CGEE contains flavonoid compounds that can modulate WBC components [18]. The results of the platelet evaluations showed no difference in the number of platelets between the treatment and control groups, although flavonoids in CGEE to inhibit platelet aggregation and prevent thrombosis [19, 20].

Our study demonstrated that CGEE did not change the hematologic profiles in the acute toxicity test. However, further investigation into the effects of orally administered CGEE in the long term are necessary to confirm the safety of this extract. These types of studies may vary; for example, a 40-day study of oral administration of *Corrigiola telephiifolia* extract showed no effects on hematologic profiles [21]. A study on the ethanolic extract of *Marsdenia tenacissima* leaves with an acute toxicity test and sub-acute toxicity test (after 28 d of oral administration) did not show significant changes in the hematologic profile [22].

CONCLUSION

A single high dose of 5000 mg/kg of CGEE did not change the hematologic profile of Wistar rats after 14 d of observation, which suggests that CGEE does not have an acute hemotoxic effect, at least for hematologic parameters.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

All authors declare there are no competing interests in this study.

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