Potential dual effect anti-inflammatory and anti-platelet of cogon grass ethanol extract on diabetic mice a preliminary study

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Abstract. Cogon grass is traditional medicine empirically used in nephritis, fever, hypertension, dyspnea, epitaxy, as a hepatoprotector agent, and has function to lower cholesterol and blood glucose. The compound of cogon grass is potentially served as an herbs medicine. But, the effect on haematology profile is still well unknown. We demonstrated the effect of cogon grass ethanol extract in mice model diabetic induced with STZ. Eight weeks old of male balb/c mice were injected intraperitoneally with STZ dose 130 mg/Kg BW. Seven days later, after DM confirmed, mice were given ethanol extract of cogon grass with dose 90 mg/KG BW (treatment group 1) and dose 115 mg/Kg BW (treatment group 2). After 14 days of extract gavage, haematology profile were estimated using the direct current detection method. We found there is no anemia occur in diabetic mice. But, the platelet and WBCs, were tend to increase in diabetic control group and treatment group 1, in contrast in treatment group 2 was tend to decrease. In conclusion, we suggested that the extract ethanol of cogon grass suppress the leukocyte and platelet count. It has potentially effect as anti-inflammatory and antiplatelet on diabetic mice.

1. Introduction
Diabetes Mellitus (DM) is a metabolic syndrome characterized by hyperglycemic that occurs due to abnormalities of insulin secretion, defective insulin action or both, occurs when the pancreas does not produce enough insulin or when the body cannot use insulin effectively [1,2,3].

There are two main types of diabetes mellitus. Type 1 diabetes known as Insulin Dependent Diabetes Mellitus (IDDM) is characterized by a lack of insulin production due to the destruction of a
small or large portion of the beta cells of the Langerhans islets in the pancreas that produce insulin. Type 2 diabetes known as Non-Insulin Dependent Diabetes Mellitus (NIDDM) is characterized by the use of insulin that is less effective because of interference with the function of insulin in entering glucose into the cell. The disorder may occur because of resistance to insulin or other unknown causes [3,4].

Pathogenic processes involved in the development of diabetes include autoimmune destruction of pancreatic β cells with insulin deficiency as a disorder that causes resistance to insulin action. Abnormalities of carbohydrate, fat, and protein metabolism in diabetes are caused by a lack of insulin action in the target tissue. The act of insulin deficiency is caused by a lack of insulin secretion and/or reduced tissue response to insulin at one or more points in the complex pathway of hormonal action [5,6].

Chronic diabetes complications including retinopathy can lead to vision loss; nephropathy causes renal failure; peripheral neuropathy with a foot ulcer condition that ends in amputation, and autonomic neuropathy causes gastrointestinal, genitourinary, and cardiovascular symptoms and dysfunction. Diabetes may increase the risk of cardiovascular atherosclerotic events, peripheral arteries, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are common in diabetics [7-10]. To restrain late complications of diabetes, primary prevention and early treatment are needed. Due to its chronic symptoms, new treatments need to be developed, due to the limited effectiveness of current therapy. One of herbs commonly used as traditional medicine is cogon grass (Imperata cylindrica L.). It is empirically used in nephritis, fever, hypertension, dyspnea, epistaxis, as a hepatoprotector agent, and has function to lower cholesterol and blood glucose [11,12].

A study conducted by Cui Jue (2012) revealed that cogon grass reduce blood glucose in diabetic mice induced by streptozotosin. Research conducted by Thiantongin concluded that through in vitro experiments cogon grass extract has an α-glucosidase inhibitor activity [13,14]. Our previous study established that cogon grass ethanol extract had significant effect to reduce cholesterol level in serum, suggesting its potential effect as an antihypercholesterolemia therapy [15].

The compound of cogon grass is potentially serve as an effective antihyperglycemic and antihypercholesteremic agent. However, study about the effect of cogon grass on hematological profile is rare. As we known hematological profile is one of parameters in detecting abnormalities that occur in the immune system. Thus, the aim of this study was to investigate the effect of ethanol extract of cogon grass on haematological parameters in diabetic mice.

2. Methods

2.1. Plant material and preparation of the extract

The powdered root of cogon grass was collected and authenticated by School of Ilmu Hayati, Institut Teknologi Bandung. The powder was successively extracted with ethanol 96% (Merck, Japan) by maceration process for 72 hours. The macerated pulp was filtered through a coarse sieve and the filtrate was concentrated in rotary vacuum evaporator. Ethanol extract of cogon grass was diluted whit CMC (Carboxyl methyl cellulose) 0.5% (Merck, Japan) to get concentrations of 90 mg/Kg BW and 115 mg/KgBW [12,15].

2.2. Animal

Eight weeks old of male balb/c mice (Mus musculus) were collected from Animal Laboratorium, Biofarma, Bandung, Indonesia. The Institutional Animal Care and Use Committee (Faculty of Medicine, Universitas Padjadjaran) approved all study protocols. The mice were place in a temperature-controlled room in a 12-hour light/12-hour dark cycle and had unrestricted access to water and chow. The mice were divided into four groups. First group was normal control, second group was diabetic control. Third group was diabetic mice given ethanol extract of cogon grass with dose 90 mg/KGBW (treatment group 1) and fourth group was diabetic mice given ethanol extract of cogon grass with dose 115 mg/KGBW (treatment group 2). The extract was orally administrated for 2 weeks.
2.3. Protocol of STZ induction
Mice were fasted for 7 - 8 hours before streptozotocin (STZ) induction. Immediately prior to injection STZ dissolved in sodium citrate buffer pH 4.5 (Merck, Japan) as previously described. The DM group were injected intraperitoneally with STZ dose 130 mg/KgBW. The control group were injected with sodium citrate buffer. After the induction, mice were given standard chow and 10% sucrose water for three days. Seven days later, the mice were fasted for 7-8 hours and measured blood glucose level to confirm the induction of DM.

2.4. Hematological parameters measurement
After 2 weeks ethanol extract of cogon grass administration, mice were fasted for 8 hours. All mice were anesthetized with ketamine-xylazine, the abdominal cavity was opened and blood was collected from aorta abdominal in EDTA micro-tube (BD, NJ-USA). Hematology profile were estimated using the direct current detection method (Sysmex Automated Hematology Analyzer; Sysmex corporation, Japan).

3. Results

3.1. Hematological analysis for 3 major categories of blood cells
We examined the hematology profile from all groups. First, we analyzed the amount of three major categories of immune cells, which are red blood cells (RBCs), White Blood cells (WBCs) and platelets (Fig. 1).

![Hematology analysis of (a) RBCs; (b) WBCs; (c) Platelets](image)

We found there is no anemia occur in diabetic mice. The amount of RBCs in treatment group was tend to higher than non-diabetic control group [9.21(3.24-10.41) vs 8.89(6.77-13.24), p>0.9999]. The platelet and WBCs, both were tend to increase in diabetic control group and treatment group 1 compared to control [WBC : 6287±2893 and 6600±2010 vs 2940±1955; platelet : 848.000(441.000-999.000) and 677.500(636.500-1.100.250) vs 765.000(301.500-813.500)], however in contrast in treatment group 2 was tend to decrease [3685±531.8 and 412.500(275.000-925.000)].

3.2. Hematology analysis of WBC’s categories
We further analyzed the WBCs categories, which are amount of lymphocyte, monocyte, granulocyte and lymphocyte, monocyte, granulocyte percentage (Figure 2).

There is no significant alteration in percentage of lymphocyte and monocyte (figure 3). However, the amount of lymphocyte was significantly differences between the treatment group 2 and the non-diabetic control group (2155±122.6 vs 4076±1801). Beside, in treatment group 2 the amount of monocyte was tend to decrease than the diabetic control group and the treatment group 1 (597.5±164.4 vs 1187± and 1105±712.2).
In contrast, the amount and percentage of granulocyte in treatment group 1 was tend to decrease among the group and significantly difference with treatment group 2 (absolute: 587.5±220.7 vs 935±442.8; percentage: 9.2±2.8 vs 24.5±8.8, p=0.0471).

Figure 2. Absolute count cells of WBC parameters

Figure 3. Percentage cells of WBC parameters

3.3. RBC’s-related parameters evaluation
We also analyzed RBCs-related parameters like hemoglobin concentration (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDWC) (figure 4).

There is no significantly alteration of RBCs-related parameters among the group. This is similarly with RBCs count, anemia was not occur in diabetic group.

4. Discussions
Hematological parameters examination could be used to determine the deleterious effect of drug, including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, normal functioning and histomorphology of the organs [17].

The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia [18]. Oxidation of these proteins and hyperglycemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to hemolysis of RBC [19]. In this study, the RBC membrane lipid peroxide levels were not measured. However, RBCs-related parameters like HB, HCT, MCV, MCH, MCHC, and RDWC were analyzed to investigate effect of cogon grass extract on the diabetic mice. The result of RBCs-related parameter were not significantly different among the group. The non-significant effect of the extract on the RBC may be an indication that the balance between the rate
of production (erythropoiesis) and destruction of the blood corpuscles was not altered. MCHC and MCH relate to individual red blood cells while HB, RBC and PCV are associated with the total population of red blood cells. Therefore, the absence of significant effect of the extract on RBC, HB, PCV, MCH and MCHC could mean that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered [20]. In previous study there was significantly alterations of these parameters in diabetic rats [21]. There were many study revealed that anemic occurred in diabetic rats induce with STZ [18,19,21]. Interestingly in our study, the result of RBC and HB that indicate the anemic on diabetic control group were not decrease compare with non-diabetic group. Our suggested, there is physiological difference between mice and rat. In previous study about altered red cell in diabetic mice showed that no obvious signs of anemia were found although red cell, haemoglobin content and cell volume were abnormal [22].

![Figure 4. RBC parameters analysis](image)

Peripheral WBC count has been shown to be associated with insulin resistance, type 2 diabetes, coronary artery disease, stroke, and diabetes micro- and macro-vascular complications [23]. We observed the WBCs were tend to increase in diabetic control group and treatment group 1, but in contrast in treatment group 2 was tend to decrease. WBC was found to be increased in diabetic group due to pathophysiological conditions including autolysis caused by some hydrolytic enzymes released by plasma under stress. WBCs were tended to decrease in treatment group 2 may because the active compound of cogon grass ethanol extract.

Cogon grass contains carbohydrates, glycosides, triterpenoids and flavonoids [15]. In previous study, flavonoids well known as anti-inflammatory caused decrease WBSC in treatment group 2. Flavonoids inhibit the release of chemical mediators; further suppress interleukin (IL)-4 and IL-13 synthesis (Th2 type cytokines) by allergen- or anti-IgE antibody-stimulated receptor-expressing cells (e.g., peripheral blood basophils or mast cells). They can also affect the differentiation of naive glycoprotein CD4 (cluster of differentiation 4) T cells (white blood cells) due to the inhibitory effect on the activation of the aryl hydrocarbon receptor [24]. The inhibitory activity of flavonoids on IL-4
and CD40 ligand expression is probably related through their inhibitory action on activation of nuclear factors of activated T cells and AP-1 (activator protein-1) [25].

The platelet count as same as WBCs was tend to increase in diabetic control group and treatment group 1, but in contrast in treatment group 2 was tend to decrease compare with non-diabetic control group. Flavonoid has been demonstrated to act at the blood platelet level by preventing platelet activity-related thrombosis [26]. Some studies suggested various mechanisms by which flavonoid exert its antiplatelet property that is, by lowering intracellular Ca2+ levels; alteration in the metabolism of cAMP, and thromboxane A2 [27].

5. Conclusions
In conclusion, we suggested that ethanol extract of cogon grass have a potentially effect as anti-inflammatory and antiplatelet in diabetic mice. However, further research is needed with a larger number of subjects to investigate the mechanism.

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