

## Subchronic Toxicity of Green Algae (*Spirogyra sp.*) Ethanolic Extract on Hematologic Parameters

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### ABSTRACT

Green Algae, an organism with active substance such as phytomelatonin, has potential to be developed as Indonesian traditional medicine. As the long term addition of Green Algae ethanol extract (*Ekstrak etanol ganggang hijau*, EEGH) influences the hematology system, in this paper, the safety test was done to ensure the safety of its use through subchronic toxicity test of EEGH on the hematology parameters of Wistar rats. The test group consisted of three groups treated with EEGH 100 mg/kg, 200 mg/kg, and 400 mg/kg, while the control group was given by 0.5% CMC-Na, with 8 rats each respectively. By using blood samples taken from orbital sinus on the 29<sup>th</sup> day, common hematologic parameters (erythrocytes, leukocytes, and hemoglobin level), the parameters of hemostasis (platelets, pT, aPTT, BT) and immune parameters (Differential Leukocytes Counts include neutrophils segment, lymphocytes, monocytes, and eosinophils) were finally observed and showed that the 28 days-addition of EEGH increase the hematological parameters of Wistar rats.

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## 1. INTRODUCTION

Green algae (*Spirogyra sp.*) are a thred like green algae. Its body is made up of cells that form elongated strands as a threadlike. Each of its cells has chloroplasts with a ribbon-shaped; Seeds spiral with a cell nucleus; Proliferation vegetatively through fragmentation and generative proliferation through conjugation. Green algae (*Spirogyra sp.*) live in sea water, fresh water, and humid place. Green algae could live free but there are also colonized. The algae have chlorophyll and yellow substance (carotene) that appear greenish-yellow. Green algae (*Spirogyra sp.*) have the active substance in the form of melatonin, which melatonin compounds present in plants is often referred to as phytomelatonin [1].

In a previous study reported that melatonin may help prevent breast cancer and prostate cancer [2]. Another benefit of melatonin is an antioxidant [3], to prevent kidney damage which is caused by smoking [4], to address the myocardial damage due to nicotine [5], preventing cerebral hemorrhage [6], inhibits neurotoxic of arsenic [7], as an anti-mouth cancer [8], and anti-hypertension [9]. Due to the importance of the function phytomelatonin compounds, therefore the study of compounds phytomelatonin needs to be conducted [10].

Based on the description above, researchers conduct research on the effects of long-term treatment (subchronic) ethanol extract of green algae (*Spirogyra sp.*) on the hematologic picture of Wistar male rats, and to identify the toxic effects after giving ethanol extract of green algae (*Spirogyra sp.*).

## 2. RESEARCH METHOD

This study used 32 male rats Wistar -2-3 months old with a weight range of 150-200 grams. The research design was, 32 rats divided into 4 groups and each group comprised 8 tails. Before treatment the rats have adapted for one week, then treated for 28 days with orally injected every day. The treatments of each group were as follows:

Group I : As a control treated with a solution of Na 0.5% CMC suspension

Group II : Given the suspension of ethanolic extract of green algae orally at a dose of 100 mg/kg bw (green algae ethanol extract 100 mg/kg).

Group III : Given the suspension of ethanolic extract of green algae orally at a dose of 200 mg/kg bw (green algae ethanol extract 200 mg/kg).

Group IV : Given the suspension of ethanolic extract of green algae orally at a dose of 400 mg/kg bw (green algae ethanol extract 400 mg/kg).

The last day (after 28 days treatment) rat have blood drawn through orbital sinus and analyzed of value of erythrocytes, leukocytes, leukocyte count, count the number of platelets, PT, aPTT, BT and hemoglobin levels. Then the results analyzed statistically and compared with normal hematologic values rat [11].

## 3. RESULTS AND ANALYSIS

Green algae that have been pollinated and macerated with 96% ethanol produced the extracts viscous solid green characteristic with odor of green algae.

Hematological examination is very important in the diagnosis of a disorder or functional damage to organs or tissues, this is due to hematologic examination may show an overview of the functions of organs and physiological status. Blood samples have taken via the orbital sinus white rat using capillary tube. A clean Ependorf that has been given Ethylene Diamine Tetra Acid (EDTA) has used to accommodate the blood. EDTA used as an anticoagulant. Blood sampling performed in all groups of rat on day 28 [12].

### 3.1. General Hematological Parameter

Test for common hematological parameters conducted to determine how the effect of subchronic EEGH can affect the erythrocytes, leukocytes, and hemoglobin concentration [13]. All results are summarized in Table 1. Values have shown in the reduction or increase in the number and levels that have adjusted to normal levels in the blood of Wistar rats.

Table 1. The Results of Calculation General Hematological Parameters of Rat after Ethanolic Extract of Green Algae were given for 28 Days

Group	Dosage (mg/kg bw)	X ± SD (g/dL)
Erythrocyte	Control	5.325 ± 0.54
	100	7.562 ± 0.25*
	200	6.466 ± 0.43*
	400	8.3 ± 0.37*
Leukocytes	Control	5.612 ± 2.47
	100	14.187 ± 3.87*
	200	9.037 ± 2.40*
	400	11.287 ± 4.08*
Hemoglobin	Control	9.78 ± 0.95
	100	13.68 ± 0.48*
	200	14.97 ± 0.47*
	400	14.67 ± 0.91*

Note :

\*= p < 0.05 (Has significantly differences to the control)

#### 3.1.1. Erythrocytes

Data on the number of erythrocytes that analyzed using parametric statistics, it showed differences between the treatment groups. However, if viewed from a normal mouse (5.00 to 10.00 million/mm<sup>3</sup>); the increasing in the value of the erythrocytes in the treatment group was still within the normal range, it concluded that the ethanol extract of green algae did not affect the number of erythrocytes.

### 3.1.2. Leukocyte

Normality test of leukocyte count has generated significant value ( $p > 0.05$ ), which means the data was homogeneous. Further analysis of parametric statistical one way ANOVA showed there was significant differences when compared with the control group ( $p < 0.05$ ). It means that the treatment of ethanol extract of green algae for 28 days did not affect the number of leukocytes. The situation which characterized by an increased number of leukocytes above normal number is called *leukositas*. If this increase is very high (up to 300,000 cells/mm<sup>3</sup>) is called leukemia while moderate decrease in leukocytes is called leukopenia [14].

### 3.1.3. Hemoglobin Level

The results of the analysis of the number of leukocytes after statistical tested by one way ANOVA showed that dose group of 100 mg/kg, a dose of 200 mg/kg and 400 mg/kg had a significant difference compared with the control group ( $p < 0.05$ ), which was an increased in value blood hemoglobin in rat. However, the increased in hemoglobin levels were still within the normal range (11.0 to 19.0 g/dL). Therefore it can be concluded that the ethanol extract of green algae did not give effect to changes in blood hemoglobin levels.

## 3.2. Blood Hemostasis Parameter

Hemostasis is the mechanism of the body to prevent and stop the bleeding. Overview of hemostasis were tested in this study include bleeding time/BT, prothrombin time/PT, activated partial thromboplastine time/aPTT and count the number of platelets. The hematological parameters of hemostasis were collected in Table 2. Bleeding Time / BT or bleeding time is an examination to determine the function of vasoconstriction of the blood vessels in the event of injury. Inspection plasma prothrombin time (Prothrombin Time/PT) is used to test coagulation factors through the extrinsic pathway and along paths that coagulation factors VII, X, V, prothrombin and fibrinogen. APTT examination serves to test the intrinsic pathway of coagulation factors and pathways along which the coagulation factors V, VIII, IX, XII, prekallikrein, kininogen, prothrombin, and fibrinogen. While the number of platelet count has a function to evaluate the function of platelets in order to clog of small holes in blood vessels.

Table 2. Results Calculated Hematological Parameters of Hemostasis Rats after Ethanol Extract of Green Algae were given for 28 Days

Group	Hemostasis Parameter			
	BT (second) (n=8)	PT (second) (n=8)	aPTT (second) (n=8)	Number of Thrombosis (10 <sup>9</sup> /L) (n=8)
Control	643.875 ± 131.280	30.125 ± 2.416	48.000 ± 4.071	223.625 ± 92.325
100 mg/kgBB	756.875 ± 145.840	23.125 ± 1.808*↓	39.875 ± 2.031*↓	795.750 ± 242.959*↑
200 mg/kgBB	587.875 ± 214.109	21.750 ± 8.137*↓	64.250 ± 5.445*↑	818.750 ± 206.259*↑
400 mg/kgBB	686.500 ± 232.408	13.250 ± 1.282*↓	40.625 ± 1.685*↓	821.125 ± 320.983*↑

Note: \* Significantly different from the control

↓ value is smaller than the control

↑ value is bigger than the control

The results of the ANOVA statistical test showed no significant difference for BT ( $p = 0.337 > 0.05$ ). This is due to differences in the vascular integrity of each rat were not the same and the human skills in conducting examinations even though the distance cuts already equated in 5 mm from the tip of the tail. The parameters of PT, aPTT and platelet count were significant differences between the treatment group and the control group ( $p < 0.05$ ). PT test results shorten on the treatment group compared to the control group. Shortening of PT was associated with melatonin dose given, the greater the dose given, the shortening of the PT is also getting bigger. It was because the increased of fibrinogen, von Willebrand factor increased, PAI-1 increased, and fibrinolysis decreased.

The test employed aPTT test results has Shortened at a dose of 100 mg/kg and 400 mg/kg, it is due to increased fibrinogen, von Willebrand factor increased, fibrinolysis decreased. However, on examination of aPTT at dose of 200 mg/kg, the aPTT become lengthwise, it caused by endothelial dysfunction which would degrade the coagulation factors V, VIII, IX, X, XI, XII, prothrombin, fibrinogen, prekallikrein, kininogen, which increase fibrinolysis activity and decreased the levels of fibrinogen which are usually caused by the presence of inhibitors and deficiency coagulation factor VIII, IX, XI, XII, prothrombin, fibrinogen, prekallikrein, and kininogen. The examination results of count the number of platelets in the hematology

analyzer tool showed the significant differences, the number of platelets have significantly increased due to the effect of melatonin which is also consistent with studies that have been conducted [15].

### 3.3. Parameters Blood Immunity

Immunity refers to the body's ability to resist or eliminates the abnormal cells or foreign objects that have potential to damage. Leukocytes and its derivatives (lymphocytes, monocytes, eosinophil, and neutrophil segments) can resist an invasion by pathogens (disease-causing microorganisms, such as bacteria and viruses) through the process of phagocytosis, identify and destroys cancer cells that appears in the body and cleaning the body with debris phagocytosis which comes from the dead or injured cells [16]. The parameters of immunity were calculated in Table 3.

Table 3. The result of the Parameters Calculation on Immunity of Rat after Ethanol Extract of Green Algae was given for 28 Days

Group	Dosage (mg/kg bw)	X ± SD (g/dL)
Neutrophil segment	Control	82.875 ± 3.441
	100	78.125 ± 5.866
	200	80.500 ± 7.309
	400	76.125 ± 9.234
Monocytes	Control	2.000 ± 0.756
	100	1.000 ± 1.069
	200	0.750 ± 1.035
	400	1.250 ± 1.581
Eosinophils	Control	1.750 ± 2.187
	100	1.750 ± 1.069
	200	1.250 ± 1.035
	400	1.500 ± 1.581
Lymphocytes	Control	1.750 ± 2.187
	100	1.750 ± 1.069
	200	1.250 ± 1.035
	400	1.500 ± 1.581

Note :

\*=p<0.05 (has significantly different to the control)

#### 3.3.1. Number of Neutrophils

The number of average neutrophils segment in all dose groups of ethanol extract of green algae was higher than the control and there are no significant differences that mean the ethanol extract of green algae did not affect on the number of neutrophil segments. The normal value of neutrophils segment in rat is 18.0 to 35% [16].

#### 3.3.2. Number of Lymphocytes

The average number of lymphocytes in the group dose of 200 mg/kg of ethanol extract of green algae was higher compare the control, it causes the ethanol extract of green algae at a dose of 200 mg / kg has effected on lymphocyte count. But overall there are no significant differences, it means the ethanol extract of green algae does not affect to the number of lymphocytes. According Schalm's Veterinary Hematology [17] the normal value of lymphocytes in rat is 84.8±8.3%, while the number of lymphocytes of normal rat is 62-75% [16].

#### 3.3.3. Number of Monocytes

The average number of monocytes in a dose group of 400 mg/kg of ethanol extract of green algae was higher compare the control; it caused the ethanol extract control of green algae at a dose of 400 mg / kg affected on lymphocyte number. In overall there were no significant differences, the ethanol extract of green algae did not affect the number of monocytes. The normal value of monocytes is 0-6%.

#### 3.3.4. Number of Eosinophils

The average number of eosinophils in all dose groups of ethanol extract of green algae was lower compares the control and there were no significant differences in the ethanol extract of green algae did not affect the number of eosinophils. The normal value of eosinophils is 1-4% [18].

#### 4. CONCLUSION

Based on hematological examination and statistical analysis of ethanol extract of green algae for 28 days has increased the general hematological parameters (erythrocytes, leukocytes, and hemoglobin concentration), and may affect the function of hemostasis by activating the coagulation of extrinsic and the intrinsic pathway, based on PT parameters and aPTT. Giving EEGH has increased the number of platelets but has not affected to BT, and has a role to stimulate immune activity by increasing the number of leukocytes, especially neutrophils segments in all treatment groups, the lymphocytes in the group of 200 mg/kg, monocytes in a dose group of 400 mg/kg. The increase in the number of leukocytes is acting as an effort to maintain the body's immune system when a foreign substance possessed. Therefore it can be concluded that the ethanol extract of green algae has no harmful effect.

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