Effect of Stachytarpheta sp. Leaf Extract In Alloxan-Induced Mice

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Abstract. Diabetes is characterized by elevated blood sugar levels or hyperglycemia. Diabetes can lead to decreased erythrocyte and hemoglobin levels, as well as increased leukocyte counts. This study aimed to analyze the phytochemical content of *Stachytarpheta* sp. leaf extract and evaluate its protective effects on blood sugar levels and blood profiles of mice, including hemoglobin levels, total erythrocyte, and total leukocyte in alloxan-induced diabetic mice. The study included five treatment groups: K1 (normal), K2 (aquades 0.3 ml), and three treatment groups receiving varying doses of *Stachytarpheta* sp. leaf extract (P1: 200 mg/kg BW; P2: 300 mg/kg BW; P3: 400 mg/kg BW) administered for 15 days. Afterward, groups K2, P1, P2, and P3 were induced with alloxan 170 mg/kg BW and measured blood sugar levels 5 days after induction. Results indicated that *Stachytarpheta* sp. leaf extract contains phytochemical compounds such as flavonoids, alkaloids, tannins, saponins, and terpenoids. Blood sugar levels measured after alloxan induction showed hyperglycemia in K2, while P1, P2, and P3 showed normal blood sugar levels. The leaf extract demonstrated a protective effect against hyperglycemia and influenced erythrocyte and leukocyte counts, but had no significant effect on hemoglobin levels.

Keywords: Alloxan, Blood Sugar, Erythrocytes, Leukocytes, *Stachytarpheta* sp.

INTRODUCTION

Based on WHO (2023) diabetes was the direct cause of 2 million deaths in 2019. According to the International Diabetes Federation, people with diabetes generally reside in low and middle-income countries that are influenced by unhealthy diets such as frequent consumption of foods or drinks with high sugar content.

Diabetes mellitus is a condition of increased blood sugar levels above normal or hyperglycemia due todisruption ofthe body's metabolic system, such as the pancreas

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cause death directly, but can cause complications and if the handling is less precise can result in death [1]. Diabetes can affect blood components such as erythrocytes (red blood cells), leukocytes (white blood cells) and hemoglobin. Some individuals with diabetes can become anemic and susceptible to infections. The higher the level of sugar in the blood, the more sugar will be bound to hemoglobin which results in decreased hemoglobin levels [2]. Hyperglycemia causes erythrocytes in the blood to lysis easily so that there is a decrease in the number of erythrocytes which causes hemoglobin levels in the blood to also decrease [3]. An unhealthy diet triggers hyperglycemia, so hyperglycemia prevention therapy is needed, such as the consumption of herbal medicines made from plants.

On the other hand, diabetes treatment usually done by consuming chemical drugs, but it can have a bad impact, especially on the kidneys if consumed in the long term. Another alternative to reduce blood sugar levels can be using herbal plants. One of the herbal plants is the white snakeweed plant. The white snakeweed plant is a wild plant with the scientific name *Stachytarpheta* sp. Snakeweed leaves known contained flavonoids, alkaloids, steroids, glycosides, terpenoids, resins, tannins, and saponins [4]. Another research reported that white snakeweed leaf extract has antidiarrheal, antioxidant, and antibacterial properties [5]. Snakeweed leaves contain flavonoids and alkaloids that can reduce blood sugar levels by making insulin in the body more responsive in neutralizing blood sugar levels.

Research on the protective effect of white snakeweed leaf extract on blood sugar and blood profile in alloxan-induced mice is limited. This study analyzed the content of phytochemical compounds from white snakeweed leaf extract in the form of flavonoids, alkaloids, tannins, terpenoids, and saponins. Based on the above statement, research on the protective effect of white snakeweed leaf extract (*Stachytarpheta* sp.) on blood sugar levels and blood profiles of alloxan-induced mice is important to test the effect of white snakeweed leaf extract in preventing hyperglycemia and its effect on the blood profile of mice including hemoglobin levels, total erythrocyte, and total leukocyte in alloxan-induced diabetic mice. This study uses alloxan as a toxicant to cause hyperglycemia conditions in mice by damaging pancreatic beta cells.

SUBJECT AND METHOD

Extract Preparation and Qualitative Phytochemical Compound Test

The white snakeweed was collected from Beteng Sari Village, Jabung District, East Lampung Regency, Lampung Province, Indonesia. The leaves extract was extracted using the ethanol maceration method, where the white snakeweed simplicia was soaked for 3x24 hours with once stirring every day. The extract was evaporated using a rotary evaporator with a temperature of 50˚C to obtained crude extract.

In this study, a qualitative test of phytochemical compounds was carried out. There were flavonoids, alkaloids, tannins, saponins, and terpenoids. The flanovoid test was carried out with 1 g of extract added to 10 ml of distilled water and then heated, 1-2 spatulas of Mg powder and 1 ml of HCl were added, a positive test if an orange, purple or red color solution was formed. Alkaloid test was done with 1 g of extract added 3-5

drops of Dragendroff reagent, a positive test is indicated by the formation of a brown or orange precipitate. Tannin test was done with 1 g of extract with 10 ml of distilled water added 3-4 drops of $FeCl_3$, positive test characterized by a blackish green color. The terpenoid test is carried out with 0.5 g of extract added 10 drops of anhydrous acetic acid and 2 drops of concentrated sulfuric acid, a positive test is characterized by purple or red color [6]. The saponin test is carried out with 1 g of extract added to 10 ml of hot distilled water, then shaken 10 seconds, a positive test is characterized by the formation of stable bubbles for 10 minutes [7].

Animal Experimental

This study has obtained ethical approval from Faculty of Medicine University of Lampung (726/UN26.18/PP.05.02.00/2024). This study used 15 male mice (*Mus musculus*). Acclimatization of mice was carried out for 7 days followed by treatment. Mice were given extracts at doses of 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW orally for 15 days. On the 15th day, mice were induced with alloxan at a dose of 170 mg/kgBB subcutaneously. During the study measurement of blood sugar levels were carried out 5 times (1st measurement on day 1 before being given the extract; 2nd, 3rd, and 4th measurement on day 5, day 10, and day 15 during the administration of the extract). The last blood sugar level (5th measurement) was carried out about 5th day after alloxan induction (day 20). Measurement of hemoglobin levels using hemoglobin just like the examination of blood sugar levels described in the previous section.

Calculation of the number of erythrocytes and leukocytes of mice was done at the end of the study. Blood samples were taken through the tail vein of the mice. Erythrocyte and leukocyte counts were performed using a hemocytometer counting chamber. The method of counting the number of erythrocytes is done by taking 0.5 µl of blood, then homogenized at 100.5 µl of Hayem solution (200 as the dilution is made). The leukocyte count was calculated by taking 0.5 µl of blood, then dissolved in 10.5 µl of Turk's solution (20 as the dilution is made). Furthermore, the blood suspension was dripped as much as ± 10 µl on the hemocytometer and then covered with a cover glass. The solution in the pipette was dripped into the hemocytometer and observed on a microscope with a magnification of 10 x 40. The following is the formula to calculate the number of erythrocytes and leukocytes:

$$number\ of\ cells\ (per\ mm^3) = (N \times Dilution) : (volume\ of\ hemocytometer)$$

RESULTS AND DISCUSSION

Phytochemical tests in this study were carried out to test the presence or absence of secondary metabolite compounds including flavonoids, alkaloids, tannins, saponins, and terpenoids. The results of phytochemical tests that have been carried out show that the ethanol extract sample of white snakeweed leaves contains flavonoids, alkaloids, tannins, saponins, and terpenoids.

Table 1. Phytochemical test results of white snakeweed leaf extract

Phytochemical compound	Result	Description
Flavonoids	+	Orange color
Alkaloids	+	Brownish orange precipitate
Tannins	+	Blackish green color
Saponins	+	Foam stable 10 min
Terpenoids	+	Purple color

This study in line with Rante et al. that extracts from snakeweed leaves cultivated in Manado, North Sulawesi contains flavonoids, alkaloids, saponins, tannins, and terpenoids [8]. In addition, research conducted by Estella et al. also stated that snakeweed leaf extract obtained from Orba, Udenu LGA, Enugu State, Nigeria contains flavonoids, alkaloids, saponins, tannins, and terpenoids [4].

Based on **Fig.** 1**.** the average initial blood sugar levels before treatment in all groups were in the range of 70-104 mg/dL which is classified as normal blood sugar levels. After that, mice in groups P1, P2, P3 were given white snakeweed leaf extract at doses of 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW respectively for 15 days while mice in the normal control group (K1) and alloxan-induced group (K2) were only given distilled water. Within 15 days of giving the extract, blood sugar levels were measured three times, day 5, day 10 and day 15. Of the three measurements of blood sugar levels, all mice blood sugar levels are still in the range of normal blood sugar levels of 74-140 mg/dL (see **Fig.** 1). On day 15, groups K2, P1, P2, and P3 were induced by alloxan while K1 was not induced by alloxan because it was a normal control. After alloxan induction, the last measurement of blood sugar levels was carried out, where the average blood sugar levels in group K2 increased blood sugar to 402 mg/dL which indicates hyperglycemia because blood sugar levels are above 200 mg/dL [9]. While the average blood sugar levels in groups K1, P1, P2, P3 are 71-124 mg/dL (see **Fig.** 1). The results of *One Way ANOVA* and *LSD* statistical analysis, there is a significant difference in blood sugar levels of mice after alloxan induction. K2 group has a significant difference of sugar levels with a *p value* = 0.01 ($p<0.05$). In this study the white snakeweed leaf extract can provide a protective effect against hyperglycemia conditions in mice after alloxan induction.

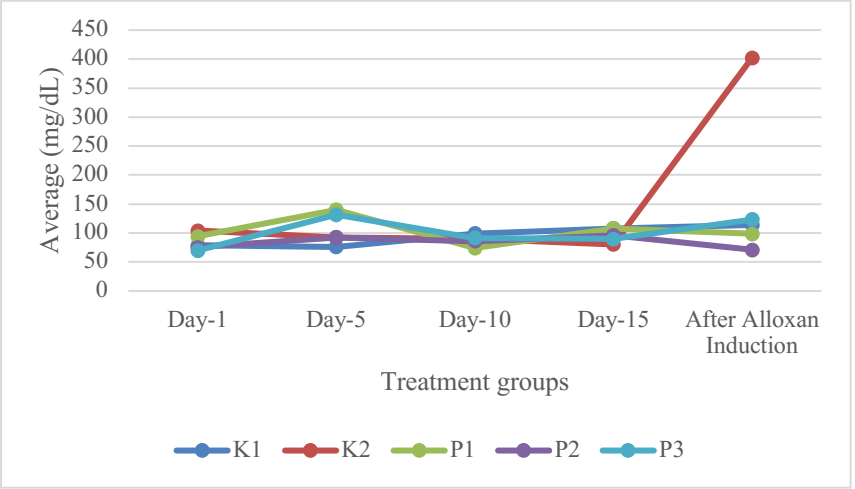

Fig. 1. Average blood sugar levels of mice (K1: control, K2: Induced alloxan 170 mg/kgBW, P1: white snakeweed leaf extract dose of 200mg/kgBW + induced alloxan, P2: white snakeweed leaf extract dose of 300mg/kgBW + induced alloxan, P3: white snakeweed leaf extract at a dose of 400mg / kgBW + induced alloxan)

Measurement of hemoglobin levels was carried out 5 times (see **Table 2**). Based on the results of the measurement all of the hemoglobin levels, all mice are still in the normal range (11.77-16.40 g/dL). The normal hemoglobin levels in male mice range from 10.9-16.3 g/dL [10]. This shows that the administration of white snakeweed leaf extract and alloxan induction did not affect the hemoglobin levels of mice as shown in **Table 2**.

Table 2. Average and standard deviation of hemoglobin levels

Treatment groups	Average hemoglobin level (g/dL) ± St.Dev				
	Day-1	Day-5	Day-10	Day-15	After Alloxan Induction
K1 (control)	15,73 ± 0,51	15,97 ± 0,99	12,87 ± 1,88	15,97 ± 0,91	12,33 ± 0,51
K2 (alloxan)	13,23 ± 1,79	11,97 ± 0,50	13,80 ± 0,35	12,43 ± 1,46	11,80 ± 0,95
P1 (extract 200mg/kgBW)	16,40 ± 0,17	13,33 ± 1,71	14,23 ± 1,53	13,57 ± 1,58	13,57 ± 2,91
P2 (extract 300mg/kgBW)	13,43 ± 1,88	11,77 ± 1,91	15,17 ± 2,49	13,67 ± 2,49	11,50 ± 1,00
P3 (extract 400mg/kgBW)	15,50 ± 3,83	13,67 ± 1,96	13,43 ± 3,49	13,67 ± 2,06	13,23 ± 0,91

The number of erythrocytes in all mice is still in the normal category, but group K2 which is only induced by alloxan has the least number of erythrocytes among the other

four groups, namely 5.16 million cells/mm^3 (**Table 3**). The number of normal erythrocytes in male mice ranges from 5.0-9.5 million cells/mm^3 [10]. The results of *One Way ANOVA* statistical analysis of the number of mice erythrocytes showed no significant difference between all treatment groups with *p value* = 0.352 (p>0.05). In other side, the number of leukocytes in all mice is still in the normal category, but group K2, which is only induced by alloxan, has the highest number of leukocytes among the other four groups, namely 10.47 thousand cells/mm^3 (**Table 3**). Normal leukocyte counts in male mice range from 3.0-14.2 thousand cells/mm^3. The results of *One Way ANOVA* statistical analysis of the number of mice leukocytes showed no significant difference with *p value* = 0.273 (p>0.05). In this study, the administration of *Stachytarpheta* sp. leaf extract can provide a protective effect on the number of erythrocytes and the number of leukocytes of mice after being induced by alloxan.

Table 3. Average the number of erythrocytes and leukocytes

Treatment groups	Erythrocytes (million cells/mm^3) ± St.Dev	Leukocytes (thousand cells/mm^3) ± St.Dev
K1 (control)	7,34 ± 2,07	8,63 ± 4,39
K2 (alloxan)	5,16 ± 1,36	10,47 ± 0,68
P1 (extract 200mg/kgBW)	7,87 ± 0,34	7,02 ± 1,58
P2 (extract 300mg/kgBW)	8,19 ± 2,88	5,45 ± 1,76
P3 (extract 400mg/kgBW)	7,94 ± 0,54	6,15 ± 3,91

The results stated that after alloxan induction, the K2 group experienced an increase in blood sugar levels above 200 mg/dL as a condition of hyperglycemia. Alloxan is a chemical compound used in research as a toxic agent to induce diabetes in experimental animals that specifically targets and damages pancreatic beta cells responsible for insulin production. The mechanism of action of alloxan is by producing excessive reactive oxygen species (ROS) in pancreatic beta cells, when alloxan is processed in the body it produces free radicals, mainly superoxide and hydrogen peroxide. These radicals attack cell membranes and cause oxidative damage to pancreatic beta cells. In addition, alloxan induces oxidative stress, resulting in DNA damage, activation of apoptotic pathways, and a decrease in the number of pancreatic beta cells. As a result, insulin production decreases significantly, leading to the condition of hyperglycemia which is an early hallmark of diabetes [11].

Mice given white snakeweed leaf extract at doses of 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW after alloxan induction did not show hyperglycemia conditions with blood sugar levels of 70-140 mg/dL which are normal blood sugar levels. This shows that white snakeweed leaf extract can provide a protective effect against hyperglycemia conditions after alloxan induction. The protective effect of white snakeweed leaves against hyperglycemia suspected that the presence of secondary metabolite compounds contained in white snakeweed leaf extract. Flavonoids is a compound that could protect against pancreatic beta cell damage, increase insulin response and release, and reduce blood sugar levels with the role of antioxidants [12]. This process is accompanied by

the prevention of apoptosis of pancreatic beta cells by antioxidants that bind free radicals. Alkaloids become protective agents of blood sugar levels by regenerating damaged pancreatic beta cells, providing stimulation to sympathetic nerves that stimulate increased insulin production. Alkaloids can also increase sugar transport by inhibiting the absorption of sugar in the intestine so that it is not flowed into the blood, glycogen synthesis is stimulated and the synthesis of new sugars from non-carbohydrate substrates is inhibited [13]. In addition, tannins have properties that can inhibit the activity of the enzyme glucosidase which plays a role in breaking down carbohydrates into simple sugars, so as to keep blood sugar levels stable.

The administration of white snakeweed leaf extract and alloxan induction in this study showed no effect on hemoglobin levels in mice, where all mice had hemoglobin levels in the normal category. The results of this study differ from the research of Wijayanti (2022) which showed that hyperglycemia mice after alloxan induction experienced a decrease in hemoglobin levels with a waiting period or length of research for 15 days [14]. In this study, after the mice experienced hyperglycemia (K2 group), the mice were immediately dissected, so the effect of decreasing hemoglobin levels due to alloxan induction has not been seen. On the other hand, Candrarisna & Kurnianto (2018) mentioned that the administration of extract which contained flavonoid has a role in increasing hemoglobin levels that previously decreased due to alloxan induction. The flavonoid content in white snakeweed leaves affects iron release and absorption during hemoglobin production, so it can increase hemoglobin formation [15].

The lowest average number of mice erythrocytes is owned by the K2 group induced by alloxan, which is 5.16 g/dL. This is because the condition of hyperglycemia in mice causes erythrocytes in the blood to be easily lysed resulting in a decrease in the number of erythrocytes, the ability of erythrocytes to stick together increases (aggregation), the ability of erythrocytes to change shape decreases (deformability), the erythrocyte membrane can be damaged and the regulation of water content in erythrocytes is disturbed [16]. The number of erythrocytes in groups P1, P2, and P3 given white snakeweed leaf extract is almost similar to the number of erythrocytes in the normal group (K1) with the number of erythrocytes 7.34 - 8.19 million cells/mm^3.. The flavonoid content in white snakeweed leaf extract is able to increase the number of erythrocytes. Flavonoids in extract can inhibit erythrocyte damage by increasing erythropoesis (formation of erythrocytes in the bone marrow) and inhibiting hydroxyl and superoxide radicals in blood components [17].

The highest average number of leukocytes was found in group K2 which was induced by alloxan. Tiana et al. (2021) stated that hyperglycemia conditions cause an increase in the number of leukocytes because this condition causes an inflammatory response in the body [18]. Excess blood sugar can cause oxidative stress and damage to blood vessel endothelial cells, thus triggering the vascular endothelial system and immune system to respond. The immune system will send more leukocytes to the affected area to fight potential infection and repair the damage. Groups P1, P2, and P3 given white snakeweed leaf extract have leukocyte counts that are still within normal limits. The terpenoid content in white snakeweed leaf extract is thought to have anti-inflammatory activity that can inhibit the activity of cytokines (signal carrier proteins for increased leukocytes) which can reduce the number of leukocytes that increase in

tissue and blood circulation and suppress leukocyte production to stay within normal limits [19].

CONCLUSION

Based on the results of the research that has been done, it can be concluded that white snakeweed leaf extract is positive for phytochemical compounds such as flavonoids, alkaloids, tannins, saponins, and terpenoids. White snakeweed leaf extract doses of 200 mg/kgBW, 300 mg/kgBW, and 400 mg/kgBW have a protective effect on hyperglycemia conditions after alloxan induction, so that the blood sugar levels of all mice remain in normal conditions. In addition, the extract can provide a protective effect on the number of erythrocytes and the number of leukocytes but had no significant effect on hemoglobin levels of mice after being induced by alloxan.

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