

THE EFFECT OF SAUCE OF *L. LEUCOCEPHALA* SEEDS (*LEUCAENA LEUCOCEPHALA* [LAM.] DE WIT) ON LOWERING BLOOD GLUCOSE IN HYPERGLYCEMIC MICE

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ABSTRACT

Objective: The research to investigate that the source of *Leucaena leucocephala* can lower blood glucose levels in hyperglycemic mice.

Methods: In this study, 42 mice were divided into 7 groups each consisted of 6 mice: Normal Group I, Group II (metformin positive control), Group III (negative control), Group IV (sauce of *L. leucocephala* at a dose of 0.1 ml), Group V (sauce of *L. leucocephala* at a dose of 0.2 ml), Group VI (sauce of *L. leucocephala* with doses of 0.4 ml), and Group VII (sauce of *L. leucocephala* at a dose of 0.8 ml). The dosage of Group II, IV, V, VI, and VII was orally administered. Blood glucose levels in mice were observed during normal conditions and after administration of 200 mg/kg bw alloxan intraperitoneally (alloxan-induced mice). On day 0, 3, 7, and 14, blood was taken from the tail and glucose levels were measured with a glucometer.

Results: Data were analyzed by one-way analysis of variance followed by LSD test. Based on the results, the sauce at a dose of 0.8 ml was able to lower the blood glucose levels up to normal blood glucose levels.

Conclusion: There was not significantly different between the groups given sauce of *L. leucocephala* at a dose of 0.8 ml with the group given metformin (positive control).

Keywords: Hyperglycemic, *Leucaena leucocephala* (Lam.) de Wit, Alloxan, Sauce.

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INTRODUCTION

Most Indonesian people's dietary habit does not pay attention to the nutrition balance which could bring negative consequences such as the rise of degenerative diseases, including diabetes mellitus; this disease is a disorder of carbohydrate metabolism which is characterized by high blood glucose levels. One of the characteristics of early disease is quickly feeling hungry then made them eat a lot [1]. Diabetes mellitus disease usually lasts for a lifetime. A recent study from the International Diabetes Federation in 2012 revealed that people with diabetes mellitus worldwide reached 371 million people. Whereas, Indonesia entered the order of seven countries with most diabetics. The first rank is China with 92.3 million people, India 63 million, 24.1 million, Brazil 13.4 million, Russia 12.7 million, Mexico 10.6 million, and Indonesia with 7.6 million people diabetic patients [2].

During this treatment, diabetes mellitus is usually performed by given an oral antidiabetes or with insulin injection. However, both treatments are less accessible to Indonesians as well as because of the economic factors are also due to the adverse side effects of treatment with insulin or orally, then many medicinal plants are developed to treat this disease as a cheaper alternatives for alternative healing [3]. Medicinal plants that have potential to be developed are associated with a decreasing blood's glucose level that is *Leucaena leucocephala*. Part of this plant that serves to lowering blood's glucose levels are the seeds [4,5].

Research on the effect of antidiabetic *L. leucocephala* has been carried out previously, in which extracts from *L. leucocephala* has active compounds such as flavonoids that can lower blood glucose [6]. Based on previous studies, methanol extract from *L. leucocephala* with doses of 0.25 g/kg BB given in oral mice, which were propagated with allot potential in lowering blood glucose levels [7]. In the study conducted by Tri Bowo, it was found that the fraction of ethyl acetate extract had a decrease in the blood glucose caused by alloxan in the dose of 100 mg/kg BW and 500 mg/kg BW [8].

In addition to having many benefits, *L. leucocephala* have also been known to have toxin mimosine compounds [9]. Mimosine can cause growth slowdown, hair loss, swollen thyroid gland, and reduce fertility [10]. However, based on the study conducted by Dita, mimosine levels can be minimized optimally through the fermentation process [11]. Sauce is one of the forms of preparation made through the fermentation process. Based on this description, in this study, a hypoglycemic impression test of sauce of *L. leucocephala* in mice that was first encouraged by alloxan where alloxan was a diabetogenic agent with a cheaper price compared with streptozotocin. The results of this study are expected to be used as information by the public about the effect of antidiabetic sauce of *L. leucocephala*.

MATERIALS AND METHODS

Materials

The material used in this research is sauce made from *Leucaena leucocephala* obtained from previous research [12]. The sauce composition can be seen in Table 1. The experimental animals used were male mice (*Mus musculus*) DDY strain 2-3 months old with average weight of 20-30 g of 42 heads. While the ingredients for hypoglycemic activity test are water, alloxan tetrahydrate, and metformin.

Tool

The tools used in the study were a syringe, oral sonde, cage of mice as well as food and beverage outlets, an analytical balance, pipette, cotton, scissors, glucometers and test strips glucose, flask, and glassware.

Methods

Hypoglycemic activity test

- All mice maintained for 1 week (7 days), given the same food and drinks.
- On the 7th day, each mouse that will be used for research is fasted for ±16 h, then taken early blood samples by cutting the tail of the mouse. Levels obtained are normal blood glucose levels.

Table 1: Composition of sauce *L. leucocephala*

Material	Formula (g)
<i>L. leucocephala</i>	750
Inokulum <i>A. oryzae</i> (%)	5
Salam leaf	0.3
Lime leaves	0.3
Galangal	1.4
Fennel	1
Palm sugar	300
Water (liter)	2
Salt	500

L. leucocephala: *Leucaena leucocephala*, *A. oryzae*: *Aspergillus oryzae*

- c. All mice were divided into seven groups, each group (consisting of six mice) was treated as follows:
 - 1. Group I normal control (not injected alloxan).
 - 2. Group II positive controls were given metformin.
 - 3. Group III negative control.
 - 4. Group IV was given sauce of *L. leucocephala* with dose 0.1 ml.
 - 5. Group V was sauce of *L. leucocephala* at a dose of 0.2 ml.
 - 6. Group VI was given sauce of *L. leucocephala* at a dose of 0.4 ml.
 - 7. Group VII was given a sauce of *L. leucocephala* at a dose of 0.8 ml.
- d. Mice made diabetes by injection of alloxan tetrahydrate (dose 200 mg/kg bw) intraperitoneally, except Group I (normal group), then maintained for 3 days for hyperglycemia.
- e. On the 3rd day, blood sampling is taken from the tail of each mouse before it is given test preparation and measuring the blood glucose level of each mouse. After the measurement of blood glucose levels, each of the mice received one kind of dose with the appropriate dosage administered orally. All are given daily for 14 days.
- f. Blood samples of mice from each group were taken and measured blood glucose levels with glucometer. Measurements were performed on days 0, 3, 7, and 14.
- g. Every time blood sampling of mice, before the mice should be fasted for approximately 16 h.

Data analysis

Data were analyzed by normality test and homogeneity test. Data were analyzed by one-way analysis of variance (ANOVA) test if the data were normal and homogeneous. If the data are not normal and homogeneous, then the data are analyzed by Kruskal-Wallis test. If there is a significant difference in the one-way ANOVA test, then it is continued with the smallest significant differential (BNT) test. Moreover, if there is significant difference in Kruskal-Wallis test, then it is continued with Mann-Whitney test.

RESULTS

Before use in the experiment, all mice from each group were weighed, then maintained for 1 week. Then, all the mice were given treatment according to the division of the group for 14 days. On day 0 (hyperglycemia), 3, 7, and 14, weigh the mice to obtain data of weight.

DISCUSSION

From Tables 2 and 3, it can be seen that before treatment hyperglycemic mice of Group II, IV, V, VI, and VII decreased body weight on day 0 and after treatment Group III hyperglycemic mice lost weight on day 7. This is due to the depletion of fat cells and proteins to meet energy needs because they cannot be met from glucose metabolism. In Groups II, III, and IV increased weight until day 14 after treatment.

In the initial condition of the experiment, intraperitoneal alloxan induction was performed for the whole group at a dose of 200 mg/kg bw, but Group I (normal) was not induced with alloxan because it was used as a comparison. After 3 days from the time of alloxan administration in Groups II, III, IV, V, VI, and VII, there was an increase in blood glucose levels, which averaged 130–158 mg/dL. This shows that alloxan can show

hyperglycemic effect on day 3. After 3 days of alloxan administration, treatment began to be given to Group II, IV, V, VI, and VII.

On the 3rd day, blood glucose levels of the mice given sauce of *L. leucocephala* with a dose of 0.1 ml did not decrease blood glucose levels. After the administration sauce of *L. leucocephala* with doses of 0.2 mL, 0.4 mL, and 0.8 mL, the average blood glucose level decreased by 133.8–137.2 mg/dL, but the blood sugar level still categorized as hyperglycemic.

On the 7th day after treatment, blood glucose levels in mice Group V, VI, and VII and metformin group decreased. On the 7th day after treatment, Group mice II, IV, V, and VI still had hyperglycemia. The group of mice who had decreased blood glucose level until they reached normal blood glucose level only Group VII, where the average blood glucose level on the 7th day was 99.8 mg/dL. However, for Group IV, mice given sauce of 0.1 ml until day 7 continued to increase blood glucose levels. Increased blood glucose levels were also experienced by Group III (negative control), in which the group was not treated.

On day 14 after treatment, blood glucose levels in Groups II, V, VI, and VII decreased. In the positive group of mice given metformin decreased blood glucose levels to reach normal blood glucose levels. Where the mean blood glucose levels of the positive group on day 14 were 102.2 mg/dL. In Group IV, mice given sauce of *L. leucocephala* with a small dose of 0.1 ml, blood glucose levels continued to increase until day 14, where the average blood glucose level from Group IV was 151.7 mg/dL. Meanwhile, Groups V and VI continued to decrease blood glucose levels but were still categorized in hyperglycemic states because the mean blood glucose levels of Groups V and VI were 129.7 mg/dL and 120.6 mg/dL. For Group III (negative control), blood glucose levels decreased when compared with day 7, where the average blood glucose level from Group III was 130.7 mg/dL and still categorized hyperglycemic. In Group VII mice, there was a decrease in glucose levels, where the average blood glucose level was 97.8 mg/dL and was said to have achieved normal blood glucose levels.

Based on these data, it can be seen that sauce of *L. leucocephala* with a dose of 0.8 ml can lower blood glucose levels of mice to normal. This can be seen from the statistical analysis of the difference of blood glucose level of mice before and after treatment, where blood glucose level from Group VII there is no significant difference with Group II which is a positive control.

On the results of weight measurements from day 0 (after alloxan feeding) until day 3, all groups on average gain weight unless Group III is a negative control. On the 14th day, weight gain was experienced by Groups I, II, IV, and V, but weight gain was not experienced by Groups VI and VII. In the Group VII, initially gained weight from day 0 (hyperglycemic state, after alloxan induction) up to day 7, but on the 14th day, all mice the group is on average experiencing weight loss. This may be due to the side effects of mimosine that still exist in *L. leucocephala*, which are used as the base ingredients in sauce, because according to research conducted by Syamsudin et al., the extract of *L. leucocephala* given to pregnant mice can reduce fetal weight along with increasing dosing [9]. *L. leucocephala* is also known to cause young cows that eat *L. leucocephala* in the long term will decrease body weight [10].

In previous studies, it has been proved that *L. leucocephala* can lower blood glucose levels in hyperglycemic mice [5-8]. *L. leucocephala* are known to have active compounds that can stimulate insulin secretion in the pancreas, the active compound is flavonoids [6]. Cahyono et al. in his research said that *L. leucocephala* has an active compound that is flavonoids that can lower blood glucose levels. The study also used alloxan as an inducer. Alloxan has a destructive effect on pancreatic β cells, in which alloxan is a powerful oxidizer that produces high amounts of free radicals that give rise to oxidative stress. Oxidative stress is a condition where there is an imbalance between free radicals and antioxidants. The situation damages pancreatic β cells,

Table 2: Mean weight data of mice (g)

Time (day)	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Initial	27.8±2.2	27.4±1.6	26.4±2.1	26.4±1.7	27.9±1.9	27.9±1.3	27.1±1.9
0	29.3±2.6	24.9±2.1	29.8±2.1	25.5±1.4	26.4±1.9	27.3±1.2	26.9±1.5
3	29.3±3.0	26.5±1.6	30.1±1.3	26.9±1.9	27.5±2.8	27.2±1.7	26.6±1.4
7	31.8±1.4	27.2±1.2	26.8±1.5	29.9±2.7	30.2±4.1	28.3±2.8	24.8±2.4
14	34.03±2.2	28.6±1.3	25.2±1.4	32±2.7	32.2±5.8	27.7±3.9	23.2±1.6

Table 3: Mean data on blood glucose levels of mice (mg/dL)

Time	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Initial	81.3±10.2	90.5±8.7	79±12.9	80±12.5	85.2±15.2	75.8±10.1	85.8±12.3
0	91.7±7.2	158±23.2	130±9.1	136.5±19.1	156.8±15.3	155.2±19.4	144±15.2
3	82.7±10.9	130.5±17.5	137.5±10.6	140±9.7	137.2±11.9	133.8±12.6	137.3±12.7
7	77.2±5.1	123±13.3	142.2±7.7	140±8.8	134.8±11.3	132.2±28.5	99.8±9.4
14	87.5±12.7	102.2±11.9	130.7±14.8	151.7±9.3	129.7±12.9	120.7±26.5	97.8±8.1

causing increased blood glucose levels; whereas, flavonoids found in *L. leucocephala* have antioxidant activity that can inhibit free radicals so that *L. leucocephala* has hypoglycemic effect [8].

CONCLUSION

Sauce of *L. leucocephala* with dose 0.2 ml, 0.4 ml, and 0.8 ml can decrease blood glucose level of hyperglycemic mice. However, only sauce with a dose of 0.8 ml alone can reduce blood glucose to normal levels.

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