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The Effect of Binahong Leaf (*Anredera cordifolia* [Ten] Steenis) Extract and Bay Leaf (*Eugenia polyantha* Wight) Extract Compound on Blood Glucose Level of Male Mice (*Rattus novergicus* L)

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Abstract: The aim of this study is to explain the effect of Binahong leaf (Anredera cordifolia [Ten] Streenis) extract and Bay Leaf (Eugenia polyantha Wight) extract compound on glucose level of male mice (Rattus novergicus L.) blood. The mice were divided into eight groups. The normal control group was not induced by alloxan and was not administered by Carboxy Methyl Cellulose (CMC) solution. The other groups were induced by alloxan 100 mg/kg BW in intraperitonial manners and administered by CMC (negative control group); glibenclamide (positive control group); 250 mg/kg BW Binahong leaf extract (Binahong treatment group); 750 mg/kg BW bay leaf extract (Bay Leaf treatment group); 1000 mg/ kg BW compound extract (treatment extract group 1); 500 mg/kg BW (treatment extract group 2); and 250 mg/kg BW compound extract (treatment extract group 3)**.** The administering of experimentation substances was carried out for 21 consecutive days. The result of Kruskal-Wallis and single factor ANAVA (P< 0.05) indicates that the three doses of compound significantly reduce the average glucose level on mice blood. The highest result was found on 1000 mg/kg BW dose with the normal glucose content 121.36 mg/dl on the 15th day and 85.37 mg/dl at the 22nd day.
Keywords: Alloxan, *Anredera cordifolia* [Ten] Streenis, *Eugenia polyantha* Wight, Blood glucose content, *Rattus novergicus* L.

INTRODUCTION

Diabetes Mellitus (DM) is a disease indicated by *hyperglycemia*, a condition when the glucose level in the bloodstream increases exceeding the normal level. DM is the disease with second highest mortality rate on 45-54 year-old age group living in Indonesian urban areas (Depkes, 2008). Indonesia is the country with the fourth highest diabetes cases after India, China, and USA. There are some ways to reduce metabolic disorders on diabetes patients, including by organizing eating pattern and administering synthetic oral hyperglycemic agents (OHA) [1]. The excessive use of synthetic hyperglycemic medication for a long period may bring adverse effects on the patient, such as acute hypoglycemia, damages on kidneys and liver and lactic acidosis [2]. Therefore, the use of natural substances as antidiabetic agents tends to become alternative solution for the society [1].

Natural antidiabetic medications are considered more benefitted for the society due to its relatively low adverse effects compared to the synthetic medications. The administration of two or more natural medications simultaneously has proven effective because of its holistic effect in maintaining health and curing illnesses [1]. Indonesians has practiced therapy utilizing natural substances (known as polyherbal therapy) based on their empirical experiences. Tiwari and Rao [3] explain that polyherbal therapy has synergistic effect due to the substance contained within each medicinal plant. Polyherbal therapies are very beneficial because of its ability to maximize the therapeutic effects of each medicinal plant with minimum adverse effect.

Several researches on the potentiality of medicinal plant extract compounds have been conducted. Agoreyo *et al*. [4] found that the administration of *Hibiscus sabdariffa* extract and *Zingiber officinale* extract compound potentially reduce glucose contents more optimally than the administration of single extracts. Ebong *et al*. [5] reported their findings indicated that the decreasing glucose contents was more optimal on the treatment group administered by *Azadirachta indica* extract and *Vernonia amygdalina*

extract compound compared to the treatment groups administered by single extracts.

Among the medicinal plants known for its function as antidiabetes agents are *Binahong (Madeira vine leaf*) and bay leaf. These plants are potentially used as materials for polyherbal experimentation. *Binahong* is an ornamental plant known empirically for its medicinal use to cure kidney damage, diabetes, cardiomegaly, hematemesis, typhus, stroke, mending wounds, stomach ulcer, intestinal inflammation, dyspnea, gout, and swelling liver [6]. Bay leaf is commonly used to cure diarrhea, influenza, pneumonia, pertussis, hypertension, and diabetes [7].

The research utilizing *Binahong* leaf extract and bay leaf extract as antidiabetes agents has never been conducted before. The combination of these two extracts is expected to provide more optimal effect in curing diabetes. Other medicinal functions of these plants (especially *Binahong*, which is commonly known for its function in mending wounds) are expected to benefit diabetes patients concerning the fact that diabetes patients take much longer time to cure his/her wounds compared to non-diabetes people.

The findings of preliminary research carried out by The Agency for Assessment and Application of Technology (*Indonesian: Badan Pengkajian dan Penerapan Teknologi/BPPT*) state that the ethanol content of *Binahong* leaf extract administered at the dose of 250 mg/kg BW may potentially reduce the glucose content of mice blood. Kemila [8] explains that flavonoid contents of *Binahong* leaf infusion may probably function as antioxidant absorbing free radicals contained within alloxan as DM inducer substance. This antioxidant activity is probably the mechanism that inhibits the function of alloxan and reduces the blood sugar contents of the alloxan-induced mice. Dewi LL *et al*. [10] stated that the ethanol content of bay leaf extract at the dose of 0.3-1.2 gram/kg BW might reduce the glucose level on mice blood.

The dose of extract administration refers to the findings of previous research conducted by Subramanian *et al*. [9] and BPPT's preliminary research. The dose of compound extract administered in this study is obtained by adding the single dose of each extract, namely 250 mg/kg BW of *Binahong* leaf extract and 750 mg/kg BW of bay leaf extract. The dose of administered compound extract is gradually decreased according to the pattern of $^{1}/_{2}^{n}$, thus the doses varies from 1000 mg/kg BW; 500 mg/kg BW; and 250 mg/kg BW.

Based on these backgrounds, the research question proposed in this study is how the effect of *Binahong* leaf extract and bay leaf extract compound administration on the glucose level of mice induced by alloxan. This study aims to identify the effect of *Binahong* leaf extract and bay leaf extract compound administration on the blood glucose level of the mice induced by alloxan. The writers hope that the data findings of this study can serve as preliminary information for further researches on formulation of natural extract compounds used as natural diabetes medication.

MATERIALS AND METHODS

This experimental research applied Completely Randomized Design (CRD). The treatments were administered randomly on the 8 (eight) treatment groups with 4 (four repetitions. The number of repetitions was determined based on Frederer formula: (t-1)(n-1) ≥15, with t was the number of treatments and n was the number of repetitions [14]. The treatment groups of this research were:

Normal Control Group (KC-1). the mice not induced by alloxan were administered by CMC 0.5% (the dose followed the body weight of each mouse) orally for 21 consecutive days.

Negative Control Group (KC-2). The mice were induced by alloxan (intraperitoneal) and administered by CMC 0.5% orally for 21 consecutive days.

Positive Control Group (KC-3). The mice were induced by alloxan (intraperitoneal) and administered by *glibenclamide*® orally (the dose 0.45 mg/kg BW orally for 21 consecutive days.

Binahong Leaf Treatment Group (KPB).The mice induced by alloxan (intraperitoneal) and administered by *Binahong* leaf extract 250 mg/kg BW orally for 21 consecutive days

Bay Leaf Treatment Group (KPS).The mice induced by alloxan (intraperitoneal) and administered by bay leaf extract 750 mg/kg BW for 21 consecutive days

Extract Compound Treatment Group Dose 1 (KPD-1).The mice induced by alloxan (intraperitoneal) and administered by combined bay leaf and Binahong leaf extract compound 1000 mg/kg BW orally for 21 consecutive days.

Extract Compound Treatment Group Dose 2 (KPD-2). The mice induced by alloxan (intraperitoneal) and administered by combined bay leaf and Binahong leaf extract compound 500 mg/kg BW orally for 21 consecutive days.

Extract Compound Treatment Group Dose 3 (KPD-3).The mice induced by alloxan (intraperitoneal)

and administered by combined bay leaf and Binahong leaf extract compound 250 mg/kg BW orally for 21 consecutive days.

Diabetic Animal Model

Thirty-two male mice were kept within a cage and divided into eight treatment groups (KC-1, KC-2, KC-3, KPB, KPS, KPD-1, KPD-2, and KPD-3). Each treatment group consisted of 4 (four) mice chosen randomly. Each mouse was marked using marker. The mark might be on its tails, backs, or feet [11]. The mark was used to differentiate a mouse from other mice. The mice were fed using pellets 20 gram/mice/day. Mineral water was provided as drinking water for the mice, provided in container with pipette *ad libitum* (unlimited). The cage was cleansed using disinfectant twice a week, rinsed, and dried up

Extract Preparation

The extract preparation was started by washing and crushing fresh *Binahong* leaf and bay leaf into powder. The powdered leaves were weighed. The powdered leaves were extracted separately using ethanol solvent to produce thick extract. The thick extract was stored in refrigerator before use.

Alloxan (Diabetogen) Induction on Mice

The mice were weighed to determine the volume of alloxan administered. Alloxan was injected at the dose of 100 mg/kg BW (intraperitoneal). The volume of the injection was 0.5 ml/100 grams BW. The results of preliminary research indicated that at that dose, the injection had been enough to trigger hyperglycemic effect.

Alloxan was diluted on cold *aquabides* at the temperature near 0 °C because alloxan was stable at that temperature. Alloxan was administered at fasting period (8-12 hours) [12]. Blood glucose level of the mice at the 0^{th} day will be measured four days after alloxan induction. The mice were considered having diabetes if its blood glucose content exceeded 200 mg/dl. The mice with diabetes will be used as experimentation animal on this anti-diabetes examination.

CMC 0.5% Solution Preparation

0.5 grams CMC powder was diluted on 50 ml aquadest inside 100 ml Erlenmeyer flask. The solution was homogenized on magnetic stirrer and added by aquadest until 100 ml volume.

Extract Suspension Preparation

250 mg/kg BW *Binahong* leaf extract suspension was prepared by mixing 250 mg *Binahong* leaf with 10 ml CMC 0.5% solution in 50 ml Erlenmeyer flask. The mixture was homogenized until suspension formed. Similar procedure applied on the preparations of other extract suspension with different doses (i.e. 750 mg/kg BW bay leaf extract suspension, 1000 mg/kg BW, 500 mg/kg BW, and 250 mg/kg BW compound extract suspensions).

Treatment

The mice were induced by alloxan (except for the normal control group) so that the mice experiencing hyperglycemia. The hyperglycemia mice were administered by experimentation plant extract for 21 consecutive days (once/day). The administration of experimentation plant extract was carried out at the same time daily (between 11 am-12 pm). The extract was administered orally using feeding tube. The normal control group (KC-1) and negative control group (KC-2) were only administered by CMC 0.5% solution. The volume of CMC 0.5% administered depending on the body weight of each mouse. The positive control group (KC-3) was administered by *glibenclamide* ® solution at the dose of 0.45 mg/kg BW [8]. The treatment groups (KPB, KPS, KPD-1, KPD-2, KPD-3) were administered by extract suspensions at the determined doses [9]. The volume of administered extract suspensions was 1 ml for every 100 mg body weight, for example, the bodyweight of a mouse is 200 mg, the volume of suspension administered will be 2 ml.

Blood Sample Collection

Mice Blood samples were collected at 0^{th}, 8^{th}, 15^{th}, and 22^{nd} days of examinations. The blood sample collection was carried out after the mice were kept in fasting condition for 12 hours.

The first blood sample collection was carried out at the fourth day after alloxan induction before treatment (considered as the 0^{th} day).

The second blood sample collection was carried out at the eighth day (after seven days of treatment through experimentation material administration).

The third blood sample collection was carried out at the 15^{th} day (after 14 days of treatment through experimentation material administration).

The second blood sample collection was carried out at the 22^{nd} day (after 21 days of treatment through experimentation material administration).

Blood Sample Analysis

The analysis of blood sample was carried out based on enzymatic method.

Data Processing and Analysis

Analysis on the research data was carried out based on statistical approach considering the nature of this research as experimental research. The blood

glucose level of the samples taken in 0th, 8th, 15th, and 22nd days were measured using computer software.

RESULT

Table-1: The average blood glucose levels of the blood samples were taken at day 0, 7,15 and 22 of the normal control group, positive control group, negative control group,

Glucose levels in Blood (mg/dL)				
	Day 0	Day 7	Day 15	Day 22
KC-1	114.18±2.94	113.04±1.91	116.63±3.05	117.09±1.54
KC-2	233.98±21.56	245.89±16.48	226.32±24.93	276.19±2.43
KC-3	266.15±20.81	113.99±2.50	113.61±24.09	85.56±10.87
KPB	277.45±19.63	177.64±18.89	138.01±7.34	104.20±4.71
KPS	262.25±30.17	172.48±10.00	129.78±2.86	96.38±9.87
KPD-1	246.97±16.94	164.39±2.91	121.36±11.76	85.37±7.44
KPD-2	262.22±31.39	168.51±25.18	128.03±9.40	110.69±5.19
KPD-3	245.94±18.33	170.23±5.23	138.68±*28.47*	119.27±8.94

Note

KC-1: Normal Control Group
KC-2: Negative Control Group
KC-3: Positive Control Group *(Glibenchlamide®)*
KPB: Binahong Leaf Extract 250 mg/kg BW
KPS: Bay leaf Extract Treatment Group 750 mg/kg BW
KPD-1: Compound Extract Treatment Group Dose-1 (1000 mg/kg BW)
KPD-2: Compound Extract Treatment Group Dose-2 (500 mg/kg BW)
KPD-3: Compound Extract Treatment Group Dose-3 (250 mg/kg BW)

The results of Shapiro-Wilk test indicated that the glucose level of the male mice blood samples taken at the 0th day were not distributed normally with $\alpha = 0.05$ ($p < 0.05$). The results of Levene homogeneity examination indicated that the data were homogenous variance with $\alpha = 0.05$ ($p > 0.06$). The results of Kruskal-Wallis examination indicated significant differences among the treatment groups.

The results of Shapiro-Wilk test indicated that the glucose level of the male mice blood samples taken at the 7th day were distributed normally with $\alpha = 0.05$ ($p > 0.05$). The results of Levene homogeneity examination indicated that the data were not homogenous variance with $\alpha = 0.05$ ($p < 0.06$). The results of Kruskal-Wallis examination indicated significant differences among the treatment groups as the effect of treatment administered.

The results of Shapiro-Wilk test indicated that the glucose level of the male mice blood samples taken at the 7th day were distributed normally with $\alpha = 0.05$ ($p > 0.05$). The results of Levene homogeneity examination indicated that the data were not homogenous variance with $\alpha = 0.05$ ($p < 0.06$). The results of Kruskal-Wallis examination indicated significant differences among the treatment groups with $\alpha = 0.05$ ($p < 0.05$).

The results of Shapiro-Wilk normality test indicated that the blood glucose level of mice blood taken at the 22nd day was normally distributed with $\alpha = 0.05$ ($p > 0.05$). The results of Levene homogeneity examination indicated the data was homogeneous variance with $\alpha = 0.05$ ($p < 0.05$). the results of single factor ANAVA examination indicated that the treatment administered had affected the blood glucose level at the 22nd day as indicated by significant differences among the treatment group $\alpha = 0.05$ ($p > 0.05$). There are significant differences among the treatment groups: KC-1, KC-3, KPB, KPS, KPD-1, KPD-2, and KPD-3 compared to KC-2, KPD-3 compared to KC-1, KPD-3 compared to KPS, KPD-2 and KPD-3 compared to KPD-1; and KPD-2 and KPD-3 compared to KPD-1.

DISCUSSION

Data on blood glucose level at the 0th day of this research was needed to determine the uniformity of blood glucose level of the experimentation animals used in this research. Hyperglycemia condition found on the experimentation animal is the result of alloxan induction at the dose of 100 mg/kg BW. The determination of the alloxan dose used in this research was based on initial orientation. Alloxan is one of diabetogen (substance that causes diabetes) that is selective in causing damages on Langerhans β-cells of pancreas. Szkudelski [15] state that alloxan induction may trigger permanent hyperglycemia condition in quick time (for about 2-3 days). The examination of the blood glucose contents of the male mice blood samples confirmed this statement. The blood samples of the 32 male mice indicated that the mice were at hyperglycemic condition 4 days after alloxan induction.

The normal control group (KC-1) was not administered by experimentation extract. However, the

group was administered by Carboxy Methyl Cellulose (CMC) 0.5% during the treatment period, similar to the other treatment groups in order to prevent research bias. The administration of CMC 0.5% on KC-1 also aims to prove that CMC 0.5% is not the factor that affects blood glucose level of *Rattus novergicus L.* Murray *et al.* [2] stated that CMC could not be digested by mammals because mammals did not have certain enzyme to carry out hydrolysis on the cellulose. Therefore, the blood glucose content is not affected by CMC.

The average blood glucose contents of *Rattus novergicus L.* induced by alloxan at the day 0 was ranged from 233.98 ± 21.56 mg/dl to 277.65 ± 19.63 mg/dl while the average blood level content of the normal control group was 114.18 ± 2.94 mg/dl. This condition confirms the theory stating that the normal glucose blood level of Rattus novergicus L. was 50-135 mg/100 ml [13]. Based on the blood glucose levels of the samples collected at day 0, it can be concluded that the blood glucose level of *Rattus novergicus L.* after alloxan induction tends to be uniform. This finding is supported by the results of Kruskal-Wallis non-parametric test with $\alpha = 0.05$ ($p > 0.05$) indicating there is no significant difference among the treatment group.

The data on *Rattus novergicus L.* blood glucose content of the normal control group (KC-1) indicates that there is no blood glucose level exceeds 200 mg/dl. The administration of compound extract for 14 consecutive days shows significant effect on the decreasing of blood glucose content of *Rattus novergicus.* This condition can be found on Table 3 and the results of Kruskal Wallis test ($p < 0.05$, $\alpha = 0.05$). the administration of compound extract in various doses (1000, 500, and 250 mg/dl) for 14 consecutive days has proven decreasing the blood glucose level of *Rattus novergicus L.* induced by alloxan.

Data on blood glucose content of the samples taken at 15th day indicated that average levels of blood glucose on the KPD-1 and KPD-2 was lower than KPB and KPS. This finding proves that the combined (compound) extract seems to be more effective in reducing blood glucose level compared to the single extracts. The decreasing dose of compound extract (as seen on KPD-2) turns out to provide greater results compared to KPB and KPS. Mutual interactions between bioactive substances contained within each medicinal plant might be the factor explaining why the compound extract is more effective in reducing blood glucose level.

Based on the data of blood glucose level of the experimentation animals taken at the 22nd day of examination (as presented on Table 3.4 above) and the results of ANAVA test with $\alpha = 0.05$ ($p < 0.05$) (see Appendix), it is found that there was significant differences of the average blood glucose content on KPD-3 compared to KC-1; KPD-3 compared to KPS; KPD-2 and KPD-3 compared to KC-3; KPD-2 and KPD-3 to KC-1; KPD-1 compared to KPD-2 and KC to KPD-3. The decreasing of compound extract dose still potentially reduced the blood glucose content although the average blood glucose level of KPD-2 and KPD-3 at the 22nd day seemed to be higher than single extracts (KPB and KPS). The dose of each extract combined as KPD-2 and KPD-3 may contain a number of active compounds that reduce blood glucose content lower than KPD-3.

Although the average blood glucose levels in KPD-3 = 119.27 ± 8.94 mg / dl is still higher than KC3 = 85.56 ± 10,87mg / dl and KC1 = 117.09 ± 1.54 mg / dl, KPD- 3 is capable of approaching the average value of blood glucose levels in a single extract group (i.e. the single extract KPB = 104.20 ± 4,71mg / dl and KPS = 96.38 ± 9.87 mg / dl). It shows that the smallest dose on the compound extract (KPD-3) is effectively able to lower blood glucose level better. This is consistent with the theory that states the benefits of polyherbal therapy because it can increase the ability of therapeutic doses and side effects as small as possible [3].

CONCLUSION AND RECOMMENDATION

Based on the findings of this study examining the effect of combined (compound of) Binahong leaf and bay leaf extract in reducing blood glucose level of male mice (*Rattus novergicus L.*) induced by alloxan, the conclusion of this study are: The administration of Binahong (*Anredera cordifolia [Ten] Steenis)* leaf extract and bay leaf (*Eugenia polyantha Wight)* compound extract at the doses of 1000 mg/kg BW, 500 mg/kg BW, and 250 mg/kg BW is able to lower the blood glucose levels of male mice *(Rattus novergicus L.)* induced by alloxan.

Further researchers should focus their studies on toxicity effect of Binahong *(Anredera cordifolia [Ten] Steenis)* and bay leaf (Eugenia polyantha Wight) of the mice with diabetes and the impacts of the extract administration on internal organs such as kidneys, liver, and pancreas. These studies need to be conducted in order to gain more comprehensive information on the benefits and safety of Binahong leaf extract and bay leaf extract as natural treatment for diabetes mellitus.

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