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RESEARCH ARTICLE

The effect of antidiabetic combination of aqueous extracts of salam leaves (*Stevia rebaudiana* Bert.) and bitter leaves (*Andrographis folium*) in white male mice

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ABSTRACT

Background: Traditional usage and many studies show that leaves salam and leaves bitter on a single use have an antidiabetic effect**. Aims and Objectives:** The aim of this research is to know the combination of aqueous extract of salam leaves and bitter leaves in decreasing blood glucose level in white male mice-induced alloxan using pre-test and post-test group control design. **Materials and Methods:** White male mice are grouped into six treatment groups: Normal control, negative controls (CMC-Na 0,5%), positive control (metformin HCl dose of 20 mg/KgBW), SB 7,5:20, SB 15:40, and SB 30:80. All groups except normal control-induced alloxan dose of 200 mg/KgBW on day-0, treatment continued from day 1–day 24. The data obtained were blood glucose level of mice before and after treatment. Blood glucose level data were analyzed using Shapiro–Wilk test and continued by analysis of variance (ANOVA) with $\alpha = 0.01$. **Results:** The results showed that there was a decrease in blood glucose level a combination of aqueous extract salam leaves and bitter leaves at $P < 0.01$. **Conclusion:** Combination of aqueous extract salam leaves and bitter leaves is able to lower blood glucose level in male white mice.

KEY WORDS: Antidiabetic; Salam Leaves; Bitter Leaves; Aqueous Extract

INTRODUCTION

Diabetes mellitus is actually a collection of clinical symptoms (syndrome clinic) that occur due to pancreas not able to produce insulin. Decreased production of insulin will result in an increase in blood glucose levels.[1] Glucose in the blood continues to increase resulting in glucose in the blood becomes uncontrolled resulting in hyperglycemia in the blood. Hyperglycemia in the blood will further lead to the process of glucose autoxidation, glycation of non-enzymatic proteins, and accelerate the formation of reactive oxygen compounds.[2] Reactive oxygen compounds (free radicals) formed by hyperglycemia can be one factor causing the emergence of various diseases such as cancer coronary heart disease, atherosclerosis, and premature aging. Free radical compounds, if not stopped or reduced, will damage the body cells and give harmful effects to the health of the body.[3] High levels of glucose in the body can also trigger an increase in the amount of radical oxygen in the body. For that, the body needs antioxidant compounds that can help protect the body from free radical attack and reduce its negative impact.[4]

Diseases caused by excess glucose in the blood (diabetes mellitus) are one of the most dangerous diseases in Indonesia, and the number of sufferers continues to increase every year. Data from Perkeni in 2011 show that the number of diabetes mellitus patients in Indonesia from 2000 to 2030 continues to increase from 8.4 million to about 21.3 million.[5]

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Treatment of diabetes mellitus generally uses synthetic drugs with various side effects. To overcome various side effects of synthetic drugs, now, many researches have been done to find alternative medicine in overcoming diabetes mellitus, especially from natural material. Indonesia has many species of medicinal herbs such as salam leaves and bitter leaves. Conventionally, in Indonesia, salam leaves have been used in treating diabetes.[6] Phytochemical analysis showed that in the salam leaves contain essential oils, tannins, flavonoids, and terpenoids which is one group of compounds that can lower blood glucose levels by breaking free radicals in the body.[7,8]

Meanwhile, bitter (*Andrographis paniculata* Nees) is also one of the plants that have traditionally also been long used to treat diabetes in Indonesia. Bitter leaves have a chemical content including deoksiandrografolid, andrografolid, noeandrografolid, 12 didehidroandrografolid, and homoandrografolid.[9] The ethanol extract of bitter leaf dose of 20.5 mg/kgBW can give effect of decreasing blood glucose level in animal test.[10]

Based on preclinical data from both plants then conducted a study to determine the effects of antidiabetes from the combination of salam leaves and bitter leaves as an alternative drug diabetes mellitus. This combination is done to improve the effectiveness of herbal use. With this combination is expected at small doses will be able to lower blood glucose levels than its use in a single dose and has an effect equivalent to synthetic drugs. In addition, with the combination of herbs, the effect of therapy is greater and relatively safe because using natural ingredients without the addition of synthetic materials can be obtained.

MATERIALS AND METHODS

Materials and Devices Research

Plant materials used in this study are salam leaves and bitter leaves obtained in Koto Marapak, IV Angkek, West Sumatra. Chemicals used include metformin HCl (Phapros), aquabidest (Ikapharmindo Putramas), and CMC-Na 0.5% (Phapros).

Equipment used include analytical scales, filter paper, glass tools (Pyrex), injection syringe, oral syringe, scalpel, magnetic stirrer, mouse weight scales (Daema), blood glucose meter *Easy Touch*® GCU and blood glucose strips (*Easy Touch*®), food standard BR2, and water *ad libitum*.

Animals

Male white mice (20–40 g) were obtained from the central animal house of Andalas University, Padang, West Sumatra. They were kept in the animal house pharmacological laboratory at ambient temperature of 26°C ± 2°C and relative humidity of 40–60%, with light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (BR2) and the food was withdrawn 18–24 h before the experiment though water was allowed *ad libitum*. "Principles of laboratory animal care" (NIH Publication No. 82–23, revised 1985) guidelines were followed. Approval from the institutional animal ethical committee was taken before the experimental work (Notification No.: 013/KEP/FK/2015).

Experimental Procedure

Salam fresh leaves 34 g and fresh bitter leaves 60 g each are mashed and extracted using a water solvent. The aqueous extract is then concentrated to obtain a thickened extract with constant weight calculated the rendemen of each extract obtained.

Aqueous Extract Salam Leaves and Bitter Leaves

Fresh Salam leaves 34 g and fresh bitter leaves 60 g each are mashed and extracted with aqueaous. the solvent is evaporated until the extract is obtained with a constant weight.

Antidiabetic Activity Test

The method used to examine the antidiabetic effect of alloxan-induced mice is 30 mice that were divided into six groups. Group I (normal control) is given aquadest, Group II (negative control) was given CMC-Na 0.5%, Group III (positive control) was given metformin HCl dose of 20 mg/kgBW, Group IV (SB 7,5: 20) was given a combination dose of leaves and bitter extract with a ratio of 3%:8%, Group V (SB 15: 40) was given a combination dose of leaves and bitter extract with a ratio of 6%:16%, and Group VI (SB 30:80) was given a combination dose of leaves and bitter extract with a ratio of 12%:32%.

All groups of animals induced with alloxan 200 mg/kgBW except Group 1 (normal control) with intraperitoneal on day 0, followed by animal testing from day 1–day 24.[11] Test preparations were administered to each treatment group mice for 24 days orally. After the end of the test preparation, The blood glucose levels were measured to see the presence of antidiabetic effects. The effects of antidiabetes are seen by comparing blood sugar levels before and after administration of the test preparation.

Measurement of Blood Glucose Level

Mice to be measured glucose levels are not given food for ± 18 h. The rat tail was then rubbed with 70% of alcohol cotton then scratched transversely with a scalpel to form a small wound. Blood used is blood on the 4th drip.[12] Blood is dripped on the end of the black strip. After ± 10 s, the results of blood glucose readings will be seen and displayed on the glucometer screen. Data of difference of blood sugar level of mice were

analyzed using Shapiro–Wilk test followed by ANOVA (analysis of variance) test with α = 0,01. The significant difference is indicated by the significance value <0.01.

RESULTS

The findings of the results are recorded in Figure 1 and Table 1.

DISCUSSION

The preparation of aqueous extracts of salam leaves and bitter leaves refers to the traditional use and single dose use of each plant. Extraction is intended to attract secondary metabolite compounds from the sample and also to remove unwanted impurities in the sample.[13] The results of the extraction process obtained rendemen aqueous extract salam leaves as much as 5.7% and bitter leaves 10.33%.

This research uses test method of type II antidiabetic effect. The test animals were white male mice of 20–40 g Wistar strain induced using alloxan at a dose of 200 mg/KgBW until blood glucose levels reached blood glucose levels of diabetes.

Treatment in test animals was done for 24 days for all groups. The results of testing blood glucose levels from day 0 to day 24 are shown in Figure 1.

On day 0–day 7, all groups experienced elevated blood glucose levels, especially negative controls that experienced the highest increase. While on day 7 until day 14, all groups have decreased blood glucose level except for negative control still increase. Negative controls were not given the drug treatment but were given only CMC-Na 0.5% which had no treatment effect. It aims to determine the effect of alloxan as an inducer in increasing blood sugar levels in animals used.

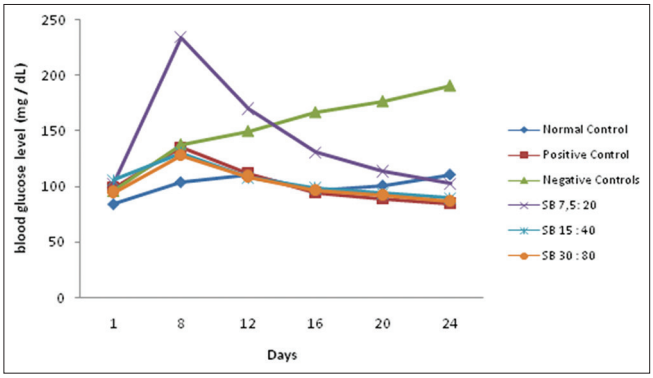

Figure 1: Graph of blood glucose level (mg/dL) of animal test each treatment group

Table 1: Mean blood glucose levels

Group	Blood glucose level (mg/dL)				
	8	12	16	20	24
Normal control	90	111	96	101.33	111.33
Positive control	135	112.33	95.67	89	85
Negative controls	138.67	150.33	167.67	177.33	191
SB 7.5:20	234.67	170.33	131.33	114	103.33
SB 15:40	130	108	99.33	94	90
SB 30:80	128.33	109	97.33	92.33	87

Blood glucose level measurement was used by GOD-PAP method. The principle of examination in this method is glucose oxidase catalyzes the oxidation reaction of glucose into gluconic acid and hydrogen peroxide. Hydrogen peroxide formed reacts with phenol and 4-aminophenazone with the help of peroxidase enzymes to produce quinoneimine in pink and can be measured with a wavelength of 546 nm. The intensity of the color formed is equivalent to the blood glucose levels present in the sample.[14,15]

To increase blood glucose levels, animals induced with the alloxan 200 mg/kg BW intraperitoneally. The purpose of alloxan is to damage pancreatic cells so that insulin cannot be produced and mice become diabetes. Normal control group is not given alloxan but still given a drink. Alloxan-induced animals were given 10% of glucose solution for 2 days to accelerate the rise in blood glucose levels. Measurements of fasting blood glucose levels on the 4th day aim to classify test animals for subsequent treatment. White male mice that already have diabetes on the transfer of individual cages, while test animals that have not been diabetic are reinduced with alloxan.

From the measurement of blood glucose levels obtained data that blood glucose levels of normal group or without given test solution were still normal, i.e., 90 mg/dL–111.33 mg/dL. Blood glucose levels that have been given a suspension of metformin HCl decreased from 135 mg/dL to 85 mg/dL. Meanwhile, blood glucose levels in the negative control group given aquadest or without tested solution increased from 138 mg/dL to 191 mg/dL. Blood glucose levels in the group given the extract combination test solution also continued to decline as in metformin HCl. Complete data of test result and measurement of blood glucose level can be shown in Table 1.

The data in Table 1 show that in the normal group, blood glucose levels on day 8–day 24 remain in normal circumstances ranging from 90 mg/dL to 111.33 mg/dL. This situation is caused by the test animals not induced by alloxan as a trigger increased blood glucose levels so that blood glucose levels remain in normal condition. The positive control group given metformin HCl had a decrease in blood sugar levels from 135 mg/dL on day 8 decreased to 85 mg/dL on day 24. This decrease is caused by the non-glycemic effect of metformin HCl which decreases glucose in blood and the adverse effects of metformin HCl such as gastrointestinal.

The combination of dosage I (SB 7,5: 20) decreased average blood glucose level from 234 mg/dL to 103 mg/dL. Combination solution of dose II (SB 15:40), the average blood glucose level decreased from 130 mg/dL to 90 mg/dL on day 24. Combination of dose III (SB 30:80), the highest decrease from 128 mg/dL on day 8 to 87 mg/dL on day 24. Diabetic group (negative control) increased blood sugar level from 138.67 mg/dL on day 8 increased to 191 mg/dL on day 24. Increased blood glucose levels caused by mice are only given alloxan as an inducing agent so that blood glucose levels continue to increase. Based on statistical test that is with ANOVA test result, there is no significant difference between solution of combination test at $P = 0.01$.

Research on antidiabetes activity of salam leaves in single dose states that infusa of salam leaves at a dose of 175 mg/kg BW may decrease rabbit glucose levels (Limawan, 1998). The ethanol extract of salam leaves at dose of 5.24 mg/20 g can also decrease the blood glucose level of alloxan-induced mice.[16] Patients who drank 2 g of salam leaves powder for 4 weeks experienced decreased levels of glucose.[17] A single study of bitter leaf has also been performed, where ethanol extract of bitter leaves at a dose of 20.5 mg/KgBB can lower blood sugar levels in rats.[10] Caused by high doses in a single use to treat diabetes, then try to combine the two plants to get a lower dose. In this study, it was found that at a lower dose of 1.5 mg salam leaves and 4 mg bitter leaves has been able to give effect almost the same with single use. From the economic point of view, it can also be useful because the use of materials is also relatively less.

Extract salam leaves and bitter leaves can lower blood glucose levels because the salam leaves contain flavonoids and terpenoids, while bitter leaves contain andrographolide. Some studies show *in vivo* andrografolida found in bitter leaves, flavonoids, and steroids on salam leaves can lower blood glucose in male rats induced alloxan. The mechanism of action is by stimulating insulin secretion through direct action on pancreatic beta cells and can repair damage to pancreatic beta cells that, if not repaired, can have a further effect of insulin reduction.[18] Bitter contains active andrografolid substances that are thought to reduce blood glucose levels and has a working mechanism as antidiabetic. There are several allegations of bitter's work mechanism as antidiabetes such as inhibiting gluconeogenesis[19] and inhibit alpha-glucosidase in the gut.[20,21]

CONCLUSION

Combination of aqueous extracts of salam leaves (*Stevia rebaudiana* Bert.) and bitter leaves (*Andrographis folium*) more effectively lower blood glucose levels in white male mice induced alloxan from the use of both plants singly.

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