ORIGINAL ARTICLE

Antidiabetes effects - Combination of cowpea juice (*Vigna sinensis* L.), tomato juice (*Solanum lycopersicum* L.), and green apple juice (*Malus sylvestris* Mill.) in white male mice

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Abstract

Aim: This study attempts to know the right combination of juice cowpea, tomatoes, and an green apple to a decrease in blood glucose levels in white male mice-induced alloxan 200 mg/kg BW. **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/Kg BW), JC I (1:1:1), JC II (0.5:0.5:0.5), and JC III (0.25:0.25:0.25). All groups except normal control induced alloxan dose of 200 mg/Kg BW on the day 0, and treatment continued from day 1 to 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 with $\alpha = 0.01$. Results and **Discussion:** The results showed that there was a decrease in blood glucose level a combination of cowpea juice, tomato juice, and green apple juice at $P < 0.01$. **Conclusion:** Combination of cowpea juice, tomatoes, and green apple more effectively lowers the blood glucose levels on white male mice compare from the use of plants singly.

Key words: Antidiabetic, *Malus sylvestris, Solanum lycopersicum, Vigna sinensis*

INTRODUCTION

Diabetes mellitus is a disease characterized by an increase in blood sugar levels from the normal limit due to disruption of carbohydrate metabolism. Disruption of carbohydrate metabolism is caused by reduced insulin hormone production and insulin resistance caused by the disruption of pancreatic function.[1] Common symptoms experienced by diabetics include fatigue, thirst (polydipsia), frequent hunger (polifagi), frequent urination (polyuria), and reduced weight.[2]

Various treatments are done to treat diabetes mellitus and usually use synthetic drugs such as metformin. Metformin has side effects of nausea, vomiting, and gastrointestinal disease.[3] To avoid side effects caused by the use of synthetic drugs, then the community turns to traditional medicine. Various herbal medicines have been widely used to treat diabetes mellitus. However, not all herbal ingredients have been scientifically proven. Empirical data show that cowpea, green apples, and tomatoes are able to overcome and reduce blood glucose levels.[4]

The results of the study stated that the combination of cowpea juice (*Vigna sinensis* L.) with tomato juice (*Solanum lycopersicum* L.) was able to reduce blood sugar levels from 201.67 mg/dl to 85.33 mg/dl.[5] Khotimah study in 2016 stated that green apple juice alone is better for lowering blood sugar levels and efficacious as antidiabetic than red

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apple juice.[6] To find the antidiabetic activity in accordance with empirical data and the results of a single study of each plant, so in this study, cowpea juice was used in combination with tomatoes and green apples which were thought to have antidiabetic activity *in vivo*.

MATERIALS AND METHODS

Plant Material

Plant materials used in this study are cowpea, tomato, and green apple obtained in Bukittinggi city, West Sumatera, and were authenticated by a botanist of the Department of Pharmacognosy, Dwi Farma Academy of Pharmaceutical, Bukittinggi, Indonesia. A voucher specimen (ADF) is kept in our laboratory for future reference.

Chemicals and Drug

Chemicals used include Metformin HCl (Phapros), Aquabidest (Ikapharmindo Putramas), and CMC-Na 0.5% (Phapros). Alloxan monohydrate was purchased from Sigma Chemicals through Medisia Sainsindo. All other chemicals used for this study were of analytical grade. Equipment used include analytical scales, filter paper, juicer, glass tools (pirex), 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 Sumatera. 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.: 0210/KEP/FK/2018).

Sample Preparation

Each fruit weighs 50 g, 50 g, and 25 g added with 20 mL of distilled water, blended at low speed, and filtered. The filtrate is then concentrated by comparison JC I (1:1:1), JC II (0.5:0.5:0.5), and JC III (0.25: 0.25:0.25) for further activity testing.

EXPERIMENTAL PROCEDURE

Antidiabetic Activity Test

The method used to examine the antidiabetic effect of alloxan-induced mice is 30 mice which 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/kg BW, Group IV JC I was given a combination juice of cowpea, tomato, and green apple with a ratio 1:1:1, Group V JC II was given a combination juice of cowpea, tomato, and green apple with a ratio 0.5:0.5:0.5, and Group VI JC III was given a combination juice of cowpea, tomato, and green apple with a ratio 0.25:0.25:0.25.

Male white mice (20–40 g) were made diabetic by a single intraperitoneal injection of alloxan monohydrate 200 mg/kg BW except Group 1 (normal control).[7] 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 and then scratched transversely with a scalpel to form a small wound. The blood used to measure blood glucose levels is blood that drips on the fourth drop. 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 the difference of blood sugar level of mice were analyzed using Shapiro–Wilk test followed by analysis of variance (ANOVA) test with $\alpha = 0.01$. The significant difference is indicated by the significance value <0.01.

RESULTS AND DISCUSSION

This research is an antidiabetic testing method for type II diabetes with test animals in the form of male, white Wistar strain 20–40 g. Animal tests were induced with alloxan dose of 200 mg/Kg BW until blood glucose levels of mice reached diabetes blood glucose levels. Treatment for animals was carried out for 24 days for all groups, and 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 an increase in blood glucose levels; meanwhile, for 7^{th}–14^{th} day, all groups experienced a decrease in blood glucose levels except for the negative control group that still experienced an increase

in blood glucose levels. The negative control group was not given drug treatment but only given 0.5% CMC-Na which had no effect as a drug. The administration of 0.5% CMC-Na is intended to determine the effect of giving alloxan as an inducer in increasing blood sugar levels in the animals tested.

Measurement of blood glucose levels on the 8th day after mice were induced with alloxan (0 h), showing that the positive control group (Metformin) had a significant difference ($P < 0.01$) with the negative control group, JC I, JC II, and JC III. Hoewver, between the positive control groups with JC I, JC II, and JC III, there were no significant differences ($P > 0.01$). Although there are differences, mice that have been induced by alloxan, on day 8, showed an increase in blood glucose levels.

To increase blood glucose levels, white male mice induced by alloxan on dose 200 mg/Kg BW with intraperitoneal. Alloxan is given for the purpose of damaging pancreatic cells so that insulin production is reduced and mice become diabetic. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas.[8,9] The results of the *in vitro* research also provide data that alloxan has a toxic effect selective on pancreatic beta cells.[10]

Male white mice that have been induced by alloxan were given a 10% glucose solution for 2 days to accelerate the increase in blood glucose levels in mice. Measurement of blood glucose levels on the 4th day aims to classify test animals for further treatment. Test animals that have experienced diabetes are transferred to individual cages, whereas test animals that have not been diabetes are induced again with alloxan.

From the results of measurements of blood glucose levels, obtained data that the blood glucose level of the normal control group was relatively stable with Blood glucose level between 82-83 mg/dL. Blood glucose levels that were given a Metformin HCl suspension decreased on average from 185.33 mg/dL to 83 mg/dL. Meanwhile, blood glucose levels in the diabetes group (negative control) without being given a test solution increased from 323 mg/dL to 144.33 mg/dL. Blood glucose levels in the group given a combination juice test solution JC I, JC II, and JC III also continued to decline such as Metformin HCl. Complete data on the results of testing and measurement of blood glucose levels can be seen in Table 1.

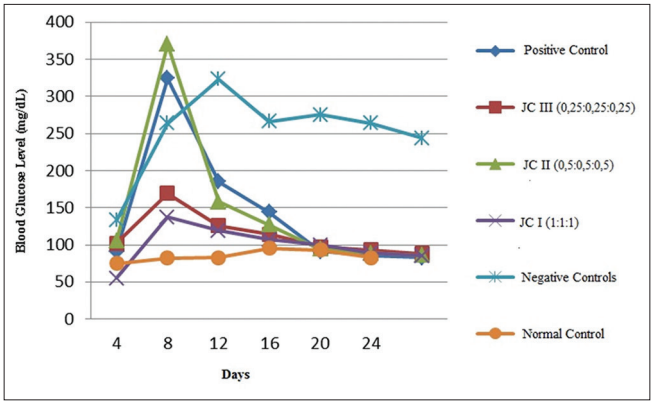

Figure 1: Graph of blood glucose levels (mg/dL) of test animals for each treatment group

Green apples combined with cowpea and tomatoes in each comparison can reduce blood sugar levels much better than a single apple green juice. The highest decrease in blood sugar levels was found in the combination of JC II with an average decrease in blood glucose levels of 158.67 mg/dl–86 mg/dL and can be said to have the best efficacy as antidiabetic.

The combination of cowpea juice, tomatoes, and green apples in this study can reduce blood glucose levels in white male mice. This decrease in blood glucose levels is caused by the content of flavonoids and polyphenols in each plant. Flavonoids have been known to have activity in lowering blood sugar levels by regenerating pancreatic beta cells, increase insulin regression, increase cell sensitivity to insulin, reduce glucose absorption, and regulate the activity of enzymes involved in carbohydrate metabolism.[11] One of the flavonoid compounds that have an influence in reducing blood sugar levels is quercetin; meanwhile, polyphenols contained by all three samples can help lower blood sugar levels by protecting the beta cells of the pancreas.[12]

The combination of cowpea juice, tomatoes, and green apples is thought to protect pancreatic β cell damage because of its function as an antioxidant. Antioxidants are known to diffuse the volatile toxic molecules of ROS and protect lung tissue from their toxic effects. Phytochemicals such as carotenoids, limonoids, tocopherols, ascorbates, lipoic acid, and polyphenols are strong natural antioxidants generally found in plants and foods that play an important role in human health.[13] Antioxidants will protect polyunsaturated fatty acid (PUFA), cell components, and protect cell membranes from oxidation of free radicals formed by toxic compounds (alloxan). The cowpea peptides can function in glucose metabolism through phosphorylation of Akt, which can potentially lead to the reduction in blood sugar levels. Akt (a form of protein kinase B [PKB]) is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis,

Table 1: Average blood glucose level

Group	Blood glucose level (mg/dL)				
	8	12	16	20	24
Normal control	82	83	95.67	93	83
Positive control	185.33	144.33	91.33	86.67	83
Negative controls	323	266	175.67	164	144.33
JC I (1:1:1)	119	107	100.67	89.67	85
JC II (0.5:0.5:0.5)	158	127.33	94.67**	89.33**	86**
JC III (0.25:0.25:0.25)	128.33	109	97.33*	92.33*	87*

Significance versus control group. ** $P<0.01$

cell proliferation, transcription, and cell migration. cAMP-elevating agents could activate PKB through protein kinase A in the presence of insulin.[14] Meanwhile, apple significantly inhibits α-glucosidase activity *in vitro*. We conclude that AKE exhibits antidiabetic properties by a dual mechanism, including the inhibition of α-glucosidase and sodium-dependent glucose transporter 1. Thus, AKE has the potential to serve as a natural plant bioactive compound for dietary prevention strategies against Type 2 diabetes mellitus.[15]

The combination of cowpea juice, tomatoes, and green apples also causes pancreatic β cells to run normally without the influence of alloxan induction. The combination of juice is also able to control fat peroxide by donating hydrogen into the reaction. Hydrogen donors by active substance components can convert peroxyl radicals from lipid peroxidation to less reactive radicals so that it cannot damage the fatty acid chain and further protect the pancreatic β cells from damage.[16] The combination of cowpea juice, tomatoes, and green apples in the JC II combination group for 21 days reduced the highest blood glucose levels compared to other test groups. This is because the ingredients contained in the combination of cowpea juice, tomatoes, and green apples are high antioxidants that are able to stimulate insulin production to be stable so that blood glucose levels decrease.[17] The effect of decreasing blood glucose levels is thought to be caused by compounds in a combination of juices that have insulin-like properties, where these compounds can stimulate the occurrence of glycogenesis, convert excess glucose into fat, and inhibit the process of gluconeogenesis.[18,19]

For testing data normality, Kolmogorov–Smirnov test was used, and the data obtained re normally distributed. Meanwhile, to test variance homogeneity, Bartlett test is used. In the ANOVA test, there was a significant difference at α 0.01. but at α 0.05 there was no significant difference between the combination of JC I, JC II, and JC III. From the results of this study, it can be concluded that the combination of cowpea juice, tomatoes, and green apples JC II can reduce blood glucose levels better than the combination of JC I and JC III at α 0.01.

CONCLUSION

In the present study, combination of cowpea juice, tomatoes, and apple green more effectively lowers the blood glucose levels in white male mice-induced alloxan from the use of both plants singly. This research study will be very useful for diabetic patients.

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