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Research Article

Activities Of Fermented Maman (*Cleome gynandra* L) On Blood Sugar Level in Hyperglycemic White Rats

Lily Restusari*, Muharni and Fitri

Department of Nutrition, Riau Health Polytechnic of Indonesian Republic Health Ministry, Indonesia

***Corresponding Author:** Lily Restusari, Department of Nutrition, Riau Health Polytechnic of Indonesian Republic Health Ministry, Indonesia.

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Abstract

Cleome gynandra L. (called as "Maman" in Riau) is a traditional medicine used for diabetes, anti-aging, anti-cancer and cardiovascular diseases prevention. This study was carried out to determine the activities of Fermented Maman on blood sugar levels in hyperglycemic male white rats. The research was an experimental research, using the Post Test Only Control Group Design, that carried out 5 months at Riau Ministry of Health Poltekkes Laboratory. The data analysis using Multivariate ANOVA and continued with Duncan multiple range test.The results of the study indicate that Fermented Maman can reduce blood sugar levels but, the metformin group had a higher reduction compared to the control group negative, positive and Fermented Maman. Metformin reduces blood sugar levels better than Fermented Maman. It is necessary to add the time of Fermented Maman and research used metformin with simultaneous fermentation in groups of male white rats with dosage variations.

Keywords: Hyperglycemia; Maman (*Cleome gynandra* L); Fermented Maman; Diabetes; Traditional Medicine

Abbreviations

IDF: International Diabetes Federation; DM: Diabetes Mellitus; RISKESDAS: Riset Kesehatan Dasar; LAB: Lactic Acid Bacteria; FSJ: Fermented S. Cumini Juice; ANOVA: Analysis of Variance

Introduction

Diabetes or diabetes mellitus is a disease which high levels of glucose in the blood exceed normal levels. The limit of normal glucose levels in the blood is 80 - <110 mg/dl at fasting and 110 - <160 mg/dl at meal time. According to WHO, Indonesia ranks 4th with the largest number of diabetics after India, China and the United States. It is estimated that in 2025 as many as 12.4 million Indonesians suffer from diabetes [1].

According to the International Diabetes Federation (IDF), in 2013 around 382 million people worldwide suffered from diabetes and by 2035 it was estimated that this number would increase to 592 million people [2,3].

Data in Indonesia taken from Riset Kesehatan Dasar Nasional 2013, showed that there were 6.9% of Indonesia's population aged ≥ 15 years with diabetes mellitus (DM). RISKEDAS data also shows, in Indonesia the prevalence of DM patients obtained based on interviews also increased by 1.1% in 2007 to 2.1% in 2013 [4]. Maman are plants from South Africa. Maman is used as a traditional medicine for diabetes, antiaging, anti-cancer, and prevention of cardiovascular diseases [5]. In Rokan Hulu and Rokan Hilir Regencies, Riau Province, this plant is commonly found as wild plants that grow anywhere, people in this village usually consume these plants as fermented vegetables [6]. Fermented Maman or Fermented Maman is a food product that has been known to contain crude fiber and lactic acid bacteria that function as probiotics. Food fiber has a very important function for health maintenance and prevention of various degenerative diseases such as diabetes, hypercholesterolemia, stroke, coronary heart disease, obesity and digestion problems such as constipation, hemorrhoids, colon cancer [7].

The total adequacy of dietary fiber in adolescents and adults is based on the IOM, the study of the total benefits of dietary fiber in controlling cholesterol associated with reducing the risk of coronary heart disease, which are 14 g/1000 kcal [8]. Based on theresearch of Muharni., *et al*, the highest crude fiber content was obtained in Fermented Maman with 2% salt addition and 15% rice treatment, which was 0.43 g per 100 g of Fermented Maman. In ad-

dition to containing crude fiber which can reduce cholesterol levels, Fermented Maman also contains lactic acid bacteria. The content of lactic acid bacteria in Fermented Maman with 5% salt addition and 10% rice has the highest yield of 2.40 x 108 cfu/g [9].

The fermentation process cause changes in the composition of phenolic compounds and flavonoids owned by vegetables [10]. Previous research stated that developed the Lactic Acid Bacteria (LAB) mediated fermented S. cumini juice (FSJ) can that the resulting fermented juice rich in phenolic compounds, and antioxidants [11]. Flavonoid compounds are part of phenolic compounds that are thought to have real antioxidant activity to capture free radical that is good for health [12]. These antioxidant compounds can inhibit oxidative stress which is considered as one of the causes of increased blood sugar levels in diabetics [13]. According to Saha., *et al*, The successive ethanolic extract A. caudatum was effective in normalizing the elevated levels of blood sugar and blood lipid-like cholesterol, triglycerides [14].

According to Restusari's research., *et al*, Lactic Acid Bacteria (LAB) contained in Fermented Maman, in addition to affecting total cholesterol levels can also affect blood triglyceride levels. The Fermented Maman dose given to the treatment showed no difference in lowering cholesterol compared to simvastatin. Therefore, further research needs to be done by increasing the number of Fermented Maman doses and seeing the activity of Fermented Maman consumption on other degenerative diseases such as diabetes [15].

Based on this background, this study will examine the activity of Fermented Maman consumption (*Cleome gynandra* L) on changes in blood sugar levels in male white rats after being given Fermented Maman with pathogenic hyperglycemia (diabetes) conditions.

Materials and Methods

Design, place and time of research

This is an experimental experiment with the Post Test Only Design Group Design. This research was carried out at the Riau Ministry of Health Poltekkes Laboratory. Research Conducted from April to August 2018.

Tools and materials

The tools used in this study were sonde, syringes, measuring cups, analytic scales, animal scales, measuring flasks, animal pens (size 30 x 20 cm per group), oral needles, glass beakers, drop pipettes, watch glass, mortar and pestle, scissors, cotton, handscoon, hooks, glass containers, Accutrend, and Accutrend Glucose strips.

The material used in this study was Maman leaf (*Cleome gynandra* L), metformin tablets produced by PT. Kimia Farma, streptozotosin production of PT. Sigma Aldrich, 70% ethanol, aquadest, 0.9% NaCl and standard food for cp ss2 mice (standard cp ss2 food contains water, amino acids and vitamins made from fish meal, shrimp meal, krill meal, soybean meal, wheat flour, wheat germ, spirulina, vitamins and minerals, color enhancers).

Research Sample

Sample that we used are 30 wistar male white mice, 3- months old, mean weight ± 200 grs. Characteristics of Wistar rats are wide rat heads, long ears, and have a tail length that is less than its body length, so Wistar rats are more active (aggressive) than other types such as Sprague-Dawley rats [16].

Stages of research

Preparation of experimental animals

Animals were acclimatized for 14 days to familiarize animals in experimental conditions and were given standard food and adequate drinks. During treatment, animal weights were weighed and observed. Animals are declared suitable for use if they do not lose more than 10%.

Making hyperglycemic male white mice

Diabetes-induced test animals were fasted for 18 hours (drinking water is still given), injected with streptozotocin solution (made new) intraperitonially at a dose of 65 mg/kgBB. Mice were given standard food and drinking water containing 10% glucose for 2 days after streptozotocin administration. Day 3 and so on 10% glucose was replaced with ordinary drinking water and the mice were transferred to the metabolic cage where each cage contained one mouse. On the 5th day induced rats were returned with the same treatment as the first treatment. If its positive diabetes, it's given Fermented Maman. Mice were made hyperglycemia with intraperitoneal streptozotocin injections at a dose of 65 mg/kgBB. STZ is dissolved in 0.9% NaCl. This injection solution is made 5-10 min before injection.

Making Fermented Maman Products

Maman plants (*Cleome gynandra* L) are cleaned from the roots and stems. Then weighed as much as 1 kg for each treatment. The leaves that have been weighed are washed and drained as raw material in making this fermentation product. Prepare other ingredients used, namely white rice as much (5%, 10% and 15%), coarse kitchen salt (2%, 5% and 7%) and warm sterile water with a ratio of 1: 4 (all percentages are calculated from the volume of boiled water used) against maman leaves. Mix Maman leaves (*Cleome gynandra* L) with salt, rice and water according to the treatment into a glass container/jar. Then stir until evenly distributed. After all the ingredients are mixed, cover the container tightly. Store the

container containing Maman and leaves at room temperature for 1 day [9].

Treatment in animals

The study was conducted using 4 groups of male white rats. The test preparation was carried out for 10 days. Before starting treatment, initial blood sampling was taken for each male white rat to determine blood sugar levels of male white rats. The animal group was given induction to get hyperglycemia, after 14 days of checking blood sugar levels in experimental animals that were induced by using Accutrend by taking blood on the tail. After the hyperglycemia mice were obtained, treatment was carried out by giving the test substance, namely Fermented Maman. On the 3rd, 6th, 9th and 12th days, blood was taken. Blood sugar levels were analyzed in the Riau Ministry of Health Poltekkes laboratory.

Measurement of blood sugar levels

Blood sugar measurement is done using random blood sugar. This examination is done to find out how big the insulin response is in balancing blood sugar. Measurements were made using an Accutrend digital device to determine initial blood sugar levels after the animal was induced. The tool is calibrated first with a code number that is adjusted to the test strip used. After the code matches and on the left the dash appears flashing, enter the test strip. After a long beep and indicator to open the cover appears on the display screen, open the cover and drop blood on it. But if not a long beep, open the cover, pull the strip back and reinsert it. Mice were initially anesthetized using ether, after fainting then the tail of the mouse was disinfected with 70% ethanol, the tail end was cut off, the first drop of blood was removed, and the next drop was dripped on a test strip tucked into the device. A certain amount of blood will be absorbed according to the absorption capacity of the test strip until beep sounds, after which the rat bleeding is stopped. The results will be seen on the screen after 14 seconds for blood sugar in mg/dL. Tests were carried out on each mouse of all groups.

Measurement of Advanced blood sugar Levels

Measurement of advanced hyperglycemia levels was carried out for 12 days. Mice that have been induced and have hyperglycemia then treated for 12 days. After that, the blood of rats was taken on the 3rd, 6th, 9th and 12th days. The blood of the rats was taken as follows: The rats were initially anesthetized using ether, after fainting then the mouse tail was disinfected with 70% ethanol, the tail end was cut off, blood droplets first discarded, the next drop is dripped on a test strip tucked into the Accoutered device. A certain amount of blood will be absorbed according to the absorption capacity of the test strip until a beep sounds, after which the rat bleeding is stopped. The results will be seen on the screen after 14 seconds for blood sugar in mg/dL.

Processing and analysis of data

The data of rats blood glucose levels were measured on days 0, 3, 6, 9 and 12 days. Based on Restusari's study, blood sampling was carried out so that the blood sugar levels gradually decreased based on the time of administration. Results are displayed in tables and graphics [17]. The average decrease in blood sugar in each group was processed in Multivariate ANOVA and continued with Duncan multiple range test to see differences and interactions with significance ($p < 0.05$).

Results and Discussion

Results

Based on the results of research that has been carried out on the decrease in blood sugarlevels of hyperglycemic male white rats given Fermented Maman can be seen in table 1.

No	Groups	Blood Sugar Levels Change (%)			
		Day-6	Day-9	Day-12	Day-3
1	Negative Control	-0.52	-2.70	-5.78	-2.52
2	Positive Control	-11.12	-0.76	-33.00	-42.96
3	Metformin	-15.00	-36.38	-53.74	-81.92
4	Fermented Maman	17.82	-3.48	-42.06	-75.46

Table 1: Percentage of Decrease in Blood Sugar Levels of All Groups on Day 3, 6, 9and 12.

Notes: Minus (-) marks means decreased blood sugar levels.

Graph percentage of decrease in blood sugar levels in white rats Hyperglycemia givenFermented Maman can be seen in figure 1.

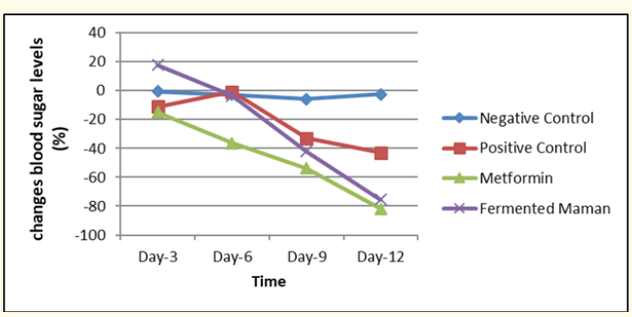

Figure 1: Graph of Percentage of Decrease in Blood Sugar Levels of All Groups on Day 3, 6, 9 and 12.

The results of normality testing to decrease blood sugar levels in this study were normallydistributed. So it continued with the Anova test. Anova test results in a decrease in blood sugar levels can be seen in table 2.

Due Group	Mean ± SD	p-value
Negative Control	2.88 ± 5.00	0.002*
Positive Control	21.96 ± 25.49	
Metformin	46.76 ± 32.26	
Fermented Maman	25.8 ± 54.61	

Table 2: Results of Anova Analysis on the Activity of Maman (Cleome gynandra l.) Consumption on the blood sugar levels in hyperglycemic male white rats ($p < 0.05$).

Notes: *Significantly different in *Anova* Test ($\alpha < 0.05$).

Based on the results of the ANOVA statistical analysis, there is an effect on the decrease in rat blood sugar levels. Therefore it is continued by using a different test, which is usingthe Duncan Test which can be seen in table 3.

No	Due Group	Blood Sugar Levels Decreases
1	Negative Control	2.88a
2	Positive Control	21.96ab
3	Metformin	46.76c
4	Fermented Maman	25.79c

Table 3: Results of Duncan Test Analysis of the Activity of Maman (*Cleome gynandra* l.) Soil consumption on Blood Sugar Levels in Male Hyperglycemic White Mice ($p <0.05$).

Notes: The numbers followed by the same lowercase letters in one column show nosignificant difference in ($p < 0.05$).

Discussion

Plant Determination

The results of the identification of maman plants have been carried out stating that the plants obtained from Bunga Tanjung Village, Rokan Hulu Regency and determined at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University showed that the plants studied were maman plants (*Cleome gynandra* L).

Induction of hyperglycemia

The experimental animals used in this study were white rats with the same strain, sex and age and the environment and food that were relatively the same to reduce biological variation or other factors that influence the results of the study. The experimental animals used were male Wistar white rats, aged 2-3 months, average body weight of ± 200 grams, 24 rats divided into 4 groups. The choice of using male rats as experimental animals aims to reduce hormonal activity instability compared to female rats.

The initial stage of the study was done by inducing rats. Mice that will be induced by diabetes are fasted for 18 hours but still given drinking water and injected with intraperitonial streptozotocin solution at a dose of 65 mg/kgBB. Mice were given standard food and drinking water containing 10% glucose for 2 days after streptozotocin administration. Day 3 and so on 10% glucose was replaced with ordinary drinking water and on the 5th day induced rats were returned with the same treatment as the first treatment. If positive diabetes is given Fermented Maman.

According to research conducted by Lenzen, it was shown that induction with streptozotocin was better than alloxane induction [18]. Streptozotocin has a better safety limit than alloxan because of its wide dosage range and less frequent ketosis than alloxan. According to Szkudelski, also states that streptozotocin induction is better used in making diabetic animal models, because it is able to maintain hyperglycemia for a long time so as to facilitate observation of the pathophysiology and complications of diabetes [19].

Making fermented maman preparations

Maman plants (*Cleome gynandra* L) are separated from leaves and stems, weighing 1 kg of leaves, leaves washed and drained. Other ingredients used are white rice as much as 10%, kitchen salt 5% and sterile water in a ratio of 1: 4 (all percentages are calculated from the volume of boiled water used) against Maman leaves. 5% of kitchen salt is dissolved with boiled water. Maman Leaf (*Cleome gynandra* L) is soaked with salt solution and sprinkled with 10% white rice. Fermentation was carried out in a closed sterile glass jar for 1 day at room temperature. After fermentation is complete (24 hours), Fermented Maman is mashed, filtered and weighed according to dosage. Fermented Maman which has been mashed is given to rats that have been induced as much as 0.54 gr/200g of BB mice.

Results of Measurement of Blood Sugar Levels

Measurement of rat blood glucose levels in this study using digital devices. The advantage of this tool is more practical and the measurement time is relatively fast. The principle of measuring glucose is an enzymatic method wherein the end of the strip is equipped with a “reagent kit” which contains enzymes. Mouse blood will be applied to the end of the strip containing the enzyme. Before the blood is taken, the tip of the mouse tail is cleaned first with 70% ethanol which aims to avoid infection. After cutting the first drop is removed to avoid substances that will interfere with measurement [20].

Measurement of blood glucose levels in groups of rats 1, 2, 3, 4 and 5 was done 5 times, namely on day 0 (before induction of diabetes), day 3, day 6 and day 9 and day 12 after administration of the test. Blood collection is done so that the blood sugar levels

gradually decrease based on the time of administration. Data of blood sugar levels obtained were then processed statistically using the Anova test, and continued with Duncan's Test.

Based on the test results on the decrease in blood sugar levels of white rats Hyperglycemia given Fermented Maman in table 1 can be seen that the metformin group has a higher reduction compared to the negative, positive and mamanic fermentation groups. Whereas in Figure 1 it can be seen that the decrease in blood sugar levels of mice given Fermented Maman has the same graphic pattern as the mice given metformin. On the 3rd to the 12th day the Fermented Maman group and metformin showed a lower decrease in blood sugar levels. Whereas in the positive control group, on the 6th day the blood sugar levels of rats increased and decreased again on days 9 to 12. In the negative control group there was no increase or decrease in blood sugar. The decrease in blood sugar levels on the graph shows that Fermented Maman can reduce blood sugar levels in hyperglycemic mice.

Judging based on the results of the percentage reduction in blood sugar, the Fermented Maman group had values similar to the positive control group. Decrease in blood sugar in positive control can occur naturally due to physical activity of mice and the length of time-consuming Fermented Maman. Barnes mentions that physical activity is directly related to the speed of recovery of muscle blood sugar. During physical activity, the muscle uses glucose stored so that the stored glucose will be reduced. At that time to fill the deficiency the muscle takes glucose in the blood so that glucose in the blood decreases which can improve blood sugar control. In this study, the rats used were male white wistar rats which were more active (aggressive). Decrease in blood sugar can occur due to non-pharmacological therapies, such as physical activity [21].

In addition to physical activists, the length of time-consuming Fermented Maman also affects the decrease in blood sugar levels. Fermented Maman requires a longer consumption time. Because Fermented Maman includes traditional medicine. One of the working principles of traditional medicine is the slow (but constructive) process (reaction), unlike chemical drugs that can react immediately (but are destructive/destructive). The benefits of herbal medicines generally can only be felt after a few weeks or months of use. That is because, the compounds efficacious in herbal medicine need time to blend into the body's metabolism. Unlike chemical drugs that work by reducing pain and symptoms, herbal medicines work by focusing on the source of the cause, namely by building and repairing the entire body system by repairing damaged cells and organs. Not surprisingly, it takes a relatively longer time to feel the effects of herbal medicines than when using chemical drugs. This is because traditional medicine is not an active compound. Traditional medicine comes from parts of medicinal plants that are sliced, dried, and destroyed. If you want to get compounds that can be used safely, medicinal plants must go through an extraction process, then be separated, purified physically and chemically (fractionated) [22].

The next test used is the Anova test. Anova analysis results of the influence of Fermented Maman (*Cleome gynandra* l.) On blood sugar levels in hyperglycemic male white rats can be seen in table 2. Based on the results of the ANOVA statistical test, there were significant differences ($p < 0.05$) between each treatment of changes blood sugar levels of male white rats (p 0.002 < 0.05).

Based on the results of the ANOVA statistical analysis, there is an effect on the decrease in rat blood sugar levels. Therefore, it was continued by using a different test, namely using the Duncan Test. Based on table 3 it can be seen from the Duncan test results that the negative control is significantly the smallest compared to other treatments and Metformin is the most significantly different compared to other treatments. Whereas Fermented Maman showed a significant difference in blood sugar levels compared to the negative control group, but not significantly different from positive control and metformin. The metformin group showed the highest percentage of blood sugar reduction (46.76%) compared to the Fermented Maman group (25.79%) and the positive control group (21.96%). It is proven that the Fermented Maman test substance has a lower activity in lowering blood sugar levels compared to metformin. So, it can be concluded that metformin decreases blood sugar levels of test animals better than Fermented Maman. This is because the work of herbal medicines in principle is slow (constructive), different from chemical drugs that can react immediately (but are destructive/destructive).

The results of this study are consistent with Zubaidah's study, that the highest decrease in blood glucose levels occurred in the diabetic group with metformin 9 mg/rat (P3) therapy compared to salak vinegar therapy [23]. The largest decrease occurred in the diabetic group treated with metformin due to the presence of a specific mechanism of metformin in reducing blood glucose levels. The mechanism of metformin in reducing blood glucose levels includes stimulation of glycolysis directly to peripheral tissues by increasing the release of glucose from the blood, reducing liver gluconeogenesis, slowing the absorption of glucose from the blood, reducing glucagon levels in plasma and increasing insulin increase in insulin receptors. The mechanism of action of metformin in reducing blood glucose levels does not depend on the presence of functioning pancreatic beta cells [24]. Because of the difference in the mechanism of action between metformin and Fermented Maman, research should be done by combining metformin and Fermented Maman.

Conclusion

Fermented Maman can reduce blood sugar levels and Metformin decreases blood sugar levels better than Fermented Maman.

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Conflict of Interest

All authors have none to declare no conflict of interests.

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