Effectiveness of Combination of Moringa Leaf Extract (Moringa Oleifera Lamk.) and Papaya Seed Extract (Carica papaya L.) in Reducing Blood Sugar Levels of Diabetic Rats

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Submission date: 08-Aug-2022 08:16PM (UTC-0500)
Submission ID: 1880466887
File name: Effectiveness_of_Combination_of_Moringa_Leaf_Extract.pdf (124.1K)
Word count: 3536
Character count: 16878

Effectiveness of Combination of Moringa Leaf Extract (*Moringa Oleifera* Lamk.) and Papaya Seed Extract (*Carica papaya* L.) in Reducing Blood Sugar Levels of Diabetic Rats

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Abstract

Moringa leaf exract and papaya seed extracts contain flavonoids which can lower blood sugar levels. The purpose of this study was to determine the antidiabetic effectiveness of moringa leaf extract and papaya seed extract and to determine the effective dose of moringa leaf extract and papaya seed extract in reducing blood sugar levels.This research is an experimental research. This study used 24 diabetic rats and the diabetic rats were induced by glucose 10% for 4 days. Measurement of blood sugar levels was carried out on 0th, 5th, 8th, and 15th days. The pre-test (T1) and post-test (T2 and T3) blood sugar levels were measured, as well as their percentage reduction at T2 and T3.The result was that the treatment group with extract combination of 700:500 had antidiabetic effectiveness. It was indicated by the mean value of decreasing blood sugar levels on day 15 (57.24%).

Keywords: Moringa leaf extract, papaya seed extract, antidiabetic mellitus

Introduction

Diabetes mellitus (DM) is a chronic progressive disease characterized by an increase in blood glucose levels (hyperglycemia) due to decreased insulin secretion and/or activity caused by insulin resistance. This disease is a disease that causes increased risk of death and decreased quality of life due to various serious complications. The risk factors for diabetes mellitus are very diverse and currently the most common disease suffered by people is Diabetes Mellitus Type 2 (T2DM) (Yasin et al., 2016). According to the International Diabetes Federation (IDF), the latest estimate in 2013 was that 382 million people were living with diabetes. In 2035, this number is estimated to increase to 592 million people.

The use of plants as medicine derived from natural/plant materials has been widely used and two of the plants that are used to reduce blood glucose levels are Moringa leaf (Moringa oleifera Lamk.) and Papaya seed (Carica papaya L.). One of the ingredients of Moringa leaf that has an antihyperglycemic effect is flavonoids. Flavonoids can stimulate pancreatic β cells and increase insulin secretion (Ambarwati et al., 2014). Meanwhile, the dosage of papaya seed extract has a significant effect on GLUT4 expression. Glucose transporter 4 (GLUT4) is a specific protein that facilitates glucose transport. GLUT4 is a glucose transporter that is responsive to insulin in muscle and adipose tissue in both humans and rodents. Determination of the dose of papaya seed extract was to be given orally for 14 days. The results of this study showed that the most effective dose of Moringa leaf extract was 300 mg/Kg BW, so that in this study, 300 and 500 mg/Kg BW of papaya seed extract doses were used (Wulansari, et al., 2017).

Based on the urgency of the research, the effectiveness of single extract from each of Moringa leaf extract and Papaya seed extract has been widely used. So in this research, we wanted to find out how effective the combination of Moringa leaf extract and papaya seed extract was in decreasing blood sugar levels.

Material and Method

This research is experimental by looking at the comparison of blood glucose levels in male rats before and after the experiment. The method that was used to extract the chemical content in Moringa leaves and papaya seeds was maceration using 96% ethanol as a solvent. The antidiabetic effectiveness test was carried out by measuring blood glucose levels in male white rats that had previously been induced with glucose 10%. There were 6 groups of tested animals with each treatment, namely a negative control group, a positive control group, 5 mg glibenclamide administration group, and a single or combined extract treatment groups.

Working Procedure

Preparation of Moringa Leaf Extract

Moringa leaf samples (*M. oleifera*) were sorted wet. The wet sample of Moringa leaves was washed and then weighed 2 kg of wet weight.The simplicia of dried Moringa leaves was blended and sieved using a 40 Mesh sieve. The Moringa dried leaves that had been sifted were then weighed as much as 300 grams and extracted by the maceration method, namely by soaking the simplicia of Moringa leaves with 96% ethanol solvent in a ratio of 1:5 for 3 days while being stirred. After 3 days, the simplicia was filtered and the residues were soaked again with a new filter liquid, and this process was done 3 times. After the extraction was complete, the extract was filtered and then concentrated to get a thick extract with a rotary evaporator at a temperature of 40° C. The overall yield of the extract that had been evaporated was put together and weighed to get the yield (Dewiyeti et al., 2015; Jusnita et al., 2019).

Preparation of Papaya Seed Extract

Papaya seed samples (*C. Papaya* L) were sorted wet and then washed under running water. Clean papaya seeds were wet weighed 2 kg. The weight of the simplicia after drying was as

much as 690 grams. Papaya seeds that had been dried were mashed using a blender to become powder, and then the sieving process was carried out using a 100 Mesh sieve. The sieved papaya seeds were then weighed as much as 425 grams and extracted by maceration method, namely by soaking the simplicia of papaya seeds with 96% ethanol solvent in a ratio of 1:3 for 3 days while being stirred. After 3 days, the simplicia was filtered and the dregs were soaked again with a new filter liquid. After the extraction was complete, the extract was filtered and then concentrated to get the extract with a rotary evaporator at a temperature of 50° C. The overall yield of the extract that had been evaporated was put together and weighed to get the yield (Ariani et al., 2019).

Acclimatization of Test Animals

Acclimatization aimed to give the animals time to try to adjust to their surroundings. Acclimatization of test animals was carried out for 21 days. During acclimatization, the rats were given regular feed in the form of pellets and drinking water ad libitum. 6 gram food was given per day. Bottles of drinking water were cleaned every 3 days, the cage was cleaned, and sawdust was changed every 3 days. The rats were weighed during adaptation or at day (-21) (Dewiyeti et al., 2015;Theresia et al., 2017).

Diabetic Rat

The test animals used in this research were male white rats (*Rattus novergicus*). The rats were induced with 10% glucose (w/v) solution for 4 days to trigger high blood sugar levels (diabetes mellitus). Before induction, the rats were satisfied for 12 hours, and then the rats' body weight was weighed, and blood glucose levels were measured as initial blood glucose levels on day 0 (T0). The rats were given glucose 10% for 4 days and before measuring blood sugar levels on the 5th day, the rats were fasted again for 12 hours and weighed again and then measured for their fasting blood sugar levels after induction (T1). The rats were declared diabetes mellitus if

the blood glucose levels were satisfied after glucose 10% induction, which were 132 mg/dl (Ambarwati et al., 2014).

Test Animal Treatment Group

The test animals used in this research were male white rats (*Rattus Novergicus*) which were two months old. The average body weight of the tested animals used was 150-250 grams, consisting of 24 animals, divided into 6 treatment groups with 3 repetitions each.

The treatment was carried out for 10 days after diabetes from 5th to15th day. For 10 days, the rats' body weights and fasting blood glucose levels were measured twice, on the 9th day and the 16th day. Measurement of blood glucose levels was done by taking the blood of the test animal through the tail end and dropping it on the glucometer strip as the final blood sugar level (T2).

Preparation of 10% Glucose Solution

A total of 10 grams of anhydrous glucose were dissolved with 100 ml of distilled water in a 50 ml beaker. Then, the solution was transferred to a 100 ml volumetric flask and the distilled water was added to the limit. Then, it was shaken until it became homogeneous.

Preparation of Negative Control

Weighing 1% Na-CMC as much as 1.5 grams developed in hot water then adding 150 ml of aquadest.

Preparation of Positive Control

Weighing 1% Na-CMC as much as 0.5 grams developed in 5 ml hot water (10 x CMC Na) while being stirred homogeneously, adding 194.4 mg of glibenclamide powder and then adding 50 ml of aquadest.

Preparation of Combination of Moringa Leaf Extract and Papaya Seed Extract

For the combination of Moringa Leaf Extract 350 mg/kg BW: Papaya Seed Extract 250 mg/kg BW, make the stock solution for each 350 mg/kg BW of moringa leaf extract as much as 350 mg/10 ml and a stock solution for each 250 mg/kg BW of papaya seed extract as much as 250 mg/10 ml. Meanwhile, for the combination of Moringa Leaf Extract 700 mg/kg BW: Papaya Seed Extract 500 mg/kg BWmake the stock solution of 700 mg/kg BW of Moringa leaf extract as much as 700 mg/10 ml and a stock solution for each 500 mg/kg BW of papaya seed extract as much as 500 mg/10 ml.

Data analysis carried out in this research was to calculate the average percentage of decreased blood sugar levels on the 8th and 15th days and compare the decreased blood sugar levels on the 8th day (T2) and 15th day (T3) with blood sugar levels on the 5th day (T1) in all groups of tested animals with one way ANOVA statistical analysis SPSS test version 24.0.

Results

Identification of flavonoid compound was needed to find out that the Moringa Leaf Extract and Papaya Seed Extract used contained flavonoid compound by means of adding a few drops of 10% NaOH solution to the 0.5 grams thick extract until a color change occurred (Rahayu et al., 2015). The color change that occurred in the Moringa Leaf Extract was yellowish and Papaya Seed Extract was yellow-orange. The ethanol-free test was carried out on the Moringa Leaf Extraxt and Papaya Seed Extract to find out that the Moringa Leaf Extract and Papaya Seed Extract were completely free of ethanol. The extract was added with acetic acid solution and concentrated sulfuric acid solution, then it was heated until there was no smell of ester. Based on the results of the ethanol-free test, the Moringa Leaf Extract and Papaya Seed Extract did not contain ethanol.

Measurement of blood sugar levels was carried out on day 0 as an initial blood sugar level (T0). For 4 days, white male rats were given glucose 10% to be induced to become

diabetic rats, then after induction of glucose 10%, the blood of the rat was taken as blood sugar levels after induction on the 5th day (T1), on the 8th day (T2), and on the 15th day (T3) after treatment. The rat blood was taken through the tip of the tail which had been fasted for 12 hours before. A glucometer test strip was used in the measurement of blood sugar levels.

The percentage of average decrease in blood sugar levels on the 8th day (T2) was calculated by comparing the decrease in the blood sugar levels before test (pre-test) on T1 and blood sugar levels after test (post-test) on T2 with blood sugar levels before test (pre-test) on T1 multiplied by 100%, while the percentage of average decrease in blood sugar levels on the 15th day (T3) was calculated by comparing the decrease in blood sugar levels before test (pre-test) on T1 and blood sugar levels after test (post-test) on T3 with blood sugar levels before test (pre-test) on T1 multiplied by 100%.

Table 1. Percentage of Average Decrease in Blood Sugar levels on the 8th Day (T2)

Treatment Group*	Blood Sugar Level Decreased (%)				Average (%) ± SD	*P*
	1	2	3	4		
Group1	4.44	9.42	6.66	4.62	6.29 ± 2.32	0.000
Group 2	52.94	54.44	54.28	52.17	53.46 ± 1.09	
Group 3	48.85	47.81	48.07	50.53	48.82 ± 1.23	
Group 4	31.51	34.86	37.54	37.9	35.45 ± 2.96	
Group 5	31.23	27.13	33.21	30.62	30.55 ± 2.53	
Group 6	48.22	46.56	48.85	45.74	47.34 ± 1.44	

*Group 1: Negative control (Na-CMC 1%), Group 2: Positive control (Glibenclamid 5 mg), Group 3: Moringa Leaf Extract 700 mg/kg BW, Group 4: Papaya Seed Extract 500 mg/kg BW, Goup 5: Combination of Moringa Leaf Extract 350 mg/kg BW: Papaya Seed Extract 250 mg/kg BW, Group 6: Combination of Moringa Leaf Extract 700 mg/kg BW: Papaya Seed Extract 500 mg/kg BW

The results of the percentage of average decrease in blood sugar levels in rats for 10 days showed that the best value of blood sugar levels was the treatment group 3 with a dose of 700 mg/kg BW of Moringa leaf extract (48.82%) in reducing blood sugar levels which was close to Blood sugar levels in treatment group 2 or positive control group (53.46%). The dose of 700 mg/kg BW of Moringa leaf extract could be said to be effective because at this dose, it showed the results of blood sugar levels that were close to normal. The decrease in blood sugar levels was thought to occur as a result of repairing pancreatic β cells by ethanol extract of Moringa leaves because Moringa leaves contained flavonoids.The flavonoid content found in Moringa leaves can also function as antioxidant that can reduce oxidative stress in cells, so that it can reduce the process of pancreatic β cell damage,and accelerate the regeneration process of pancreatic β cells (Ambarwati et al., 2014).

Table 2. Percentage of Average Decrease in Blood Sugar Levels on the 15th Day (T3)

Treatment Group*	Blood Sugar Level Decreased (%)				Average (%) ± SD	*P*
	1	2	3	4		
Group 1	10.37	14.13	16.14	14.59	13.81± 2.45	0.000
Group 2	63.66	60	60.71	61.59	61.49± 1.59	
Group 3	56.1	56.2	54.61	60.07	56.75± 2.33	
Group 4	47.47	46.74	47.59	51.98	48.45± 2.38	
Group 5	52.17	48.99	51.15	48.84	50.29± 1.64	
Group 6	56.42	56.47	58.24	57.81	57.24± 0.93	

*Group 1: Negative control (Na-CMC 1%), Group 2: Positive control (Glibenclamid 5 mg), Group 3: Moringa Leaf Extract 700 mg/kg BW, Group 4: Papaya Seed Extract 500 mg/kg BW, Goup 5: Combination of Moringa Leaf Extract 350 mg/kg BW: Papaya Seed Extract 250 mg/kg BW, Group 6: Combination of Moringa Leaf Extract 700 mg/kg BW: Papaya Seed Extract 500 mg/kg BW

From the results of the average decrease in blood sugar levels percentage in rats on the 15th day, it was found that the best value of blood sugar levels was in the treatment group 6, namely a combination of 700 mg/kg BW of Moringa leaf extract: 500 mg/kg BW of Papaya seed extract (57.24%) in decreasing blood sugar that was close to the blood sugar level in the treatment group 2 or the positive control group (61.49%). Moringa leaf extract and Papaya seed extract both contain flavonoids which can be used as antioxidants, so that papaya seeds can reduce blood sugar levels by decreasing the rate of glucose absorption in the periphery and by stimulating the ability of pancreatic β cells to produce insulation and repair pancreatic β cells (Wulansari et al., 2017).

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Discussion

The identification test of flavonoid compound using NaOH 10% showed that the extract was positif for flavonoid with the presence of a yellow orange color change in the Moringa Leaf Extract and a yellow color change in the Papaya Seed Extract. The ethanol-free test used was the esterification method. The test aimed to determine that the extract used did not contain ethanol. The results of the ethanol-free test showed that the Moringa Leaf Extract and Papaya Seed Extract did not contain ethanol, which was indicated by the absence of an ester odor that came out of the Moringa Leaf Extract and Papaya Seed Extract.

Glibenclamide was used as a comparison because the short-term therapeutic effect of glibenclamide was almost the same as the hypoglycemic effect of flavonoids contained in Moringa Leaf Extract and Papaya Seed Extract, which increased insulin secretion from pancreatic β cells. Based on the decrease in blood sugar levels on T2 and T3, treatment 6 had a good decrease in blood sugar levels which was close to the positive control group 2 treatment. For the results of the decreases in blood sugar levels on T2 and T3, one way ANOVA test and Tuckey test were performed to see the difference in significance between groups.

The results of the one way ANOVA statistical test on the percentage of average decrease in blood sugar levels showed that there were significant differences between the treatment groups with a significance value of 0.000 < 0.05 on both the 8th day (T2) and the 15th day (T3). In the Tuckey test on T2, there was a value that did not have a significant difference (0.907 > 0.05) in treatment group 6, namely 700 mg/kg BW of Moringa leaf extract with treatment group 5, namely a combination of 700 mg/kg BW of Moringa leaf extract: 500 mg/kg BW of Papaya seed extract. Whereas in the Tuckey test on T3, there was a value that did not have a significant difference (0.063 > 0.05) in treatment group 2, namely a positive control with treatment group 6, which was a combination of 700 mg/kg BW of Moringa leaf extract: 500 mg/kgBW of Papaya seed extract, a value that did not have a significant difference (0.999 > 0.05) in treatment group 3, namely a Moringa Leaf Extract 700 mg/kg BW with treatment group 6, namely a combination of 700 mg/kg BW of Moringa leaf extract: 500 mg/kg BW of Papaya seed extract and a value that did not have a significant difference (0.768 > 0.05) in treatment group 4, namely a Papaya Seed Extract 500 mg/kg BW with treatment group 5, namely a combination Moringa Leaf Extract 350 mg/kg BW: Papaya Seed Extract 250 mg/kg BW.

Conclusion

The results of this research concluded that group 6 had antidiabetic effectiveness as indicated by the average value of decreased blood sugar levels on the 15th day (57.24%). This mean was almost close to the mean value of decline in the positive control group (61.49%). From the results of statistical tests, it could be concluded that the antidiabetic effectiveness of group 3, namely the single extract of 700 mg/kg BW of Moringa leaves was better than that in group 6, namely the combination of 700 mg/kg BW of Moringa leaf extract: 500 mg/kg BW of Papaya seed extract as indicated by the significance value of the LSD test on the 8th day (0.329) and on the 15th day (0.729).

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