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RESEARCH ARTICLE

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

Introduction: Moringa leaf extract and papaya seed extract contain flavonoids that can lower blood sugar levels. **Objectives:** The purpose of this study was to determine the antidiabetic effectiveness of moringa leaf extract and papaya seed extract and the effective dose of moringa leaf extract and papaya seed extract in reducing blood sugar levels. **Methods:** This study used 24 diabetic rats. Diabetes was induced by giving rats glucose 10% for four days. Measurement of blood sugar levels was carried out on the 0th, 5th, 8th, and 15th days. The pre-test (T1) and post-test (T2 and T3) blood sugar levels were measured, as well as the percentage of reduction at T2 and T3. **Results:** The result was that the treatment group with an extract combination of 700:500 showed decreased blood sugar levels on day 15 (57.24%), indicating antidiabetic effectiveness of the extract combination.

Introduction

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

The use of plants has been widely used in natural medicine to reduce blood glucose levels, particularly Moringa leaves (*Moringa oleifera Lamk.*) and Papaya seeds (*Carica papaya L.*). Flavonoids are the ingredients of Moringa leaves that have an antihyperglycemic effect. They stimulate pancreatic β cells and increase insulin secretion (Ambarwati *et al.*, 2014). Moreover, the papaya seed extract significantly affects the expression of Glucose Transporter 4 (GLUT4). GLUT4 is a protein that facilitates glucose transport, responsive to insulin in muscle and adipose tissue in both humans and rodents. A study showed that the most effective dose of Moringa leaf extract was 300 mg/Kg body weight (bw), and that of papaya seed extract was 500 mg/Kg; thus, in this study, doses of 300 and 500 mg/Kg bw were used (Wulansari *et al.*, 2017).

Based on the urgency of research, a single extract from each of Moringa leaf and Papaya seed has been used. So this research explored 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 and compares blood glucose levels in male rats before and after the experiment. The method to extract the chemical contents 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 white male rats that had previously been induced with glucose 10%. The tested animals were divided into six treatment groups: negative control, a positive control, glibenclamide 5 mg, and single extract or combined extract treatment groups.

Preparation of the Moringa Leaf Extract

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

Preparation of the Papaya Seed Extract

Papaya seed samples (*C. Papaya* L) were sorted wet and 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. The dried Papaya seeds 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, soaking the Simplicia of papaya seeds with 96% ethanol solvent in a ratio of 1:3 for three days while stirring, then 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 concentrated with a rotary evaporator at 50°C. The overall thick extract obtained was put together and weighed to get the yield (Ariani *et al.*, 2019).

Acclimatisation of test animals

Acclimatisation aimed to give the test animals time to try to adapt to their surroundings. It was carried out for 21 days, during which the rats were given a total of six grams of regular food per day in the form of pellets and drinking water *ad libitum*. Bottles of drinking water and the cage were cleaned every three days, and sawdust was changed every three days. The rats were weighed during adaptation or at day (-21) (Dewiyeti *et al.*, 2015; Theresia *et al.*, 2017).

Diabetic rats

The test animals used in this research were white male rats (*Rattus novergicus*). Before diabetes induction, the rats were satisfied for 12 hours, then weighed, and blood glucose levels were measured as initial blood glucose levels on day zero (T0). The rats were given glucose 10% glucose (w/v) solution for four days to trigger high blood sugar levels (diabetes mellitus). Before measuring blood sugar levels on the fifth day, rats fasted again for 12 hours, then weighed; their fasting blood sugar levels were measured after induction (T1). The rats were declared diabetic if the blood glucose levels after induction were 132 mg/dl (Ambarwati *et al.*, 2014).

Test animal treatment group

The 24 test rats used in this research were two months old with an average body weight of 150-250 grams. They were divided into six treatment groups with three repetitions each.

The treatment was carried out for ten days after diabetes from the fifth to the fifteenth day, during which weight and fasting blood glucose levels were measured twice, on the 9th day and the 16th day. Blood samples were taken from the test animal tails then dropped on the glucometer strip to get the final blood sugar level (T2).

Preparation of 10% glucose solution

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

Preparation of negative control

The negative control was prepared by preparing 1.5 grams of 1% Na-CMC in hot water then adding 150 ml of aqua dest.

Preparation of positive control

The positive control was prepared by dissolving 0.5 grams 1% Na-CMC in 5 ml hot water (10 x CMC Na)

while stirring, then adding 194.4 mg of glibenclamide powder and 50 ml of aqua dest.

Preparation of combination of Moringa Leaf Extract and Papaya Seed Extract

For the combination Moringa Leaf Extract 350 mg/kg bw: Papaya Seed Extract 250 mg/kg bw, a stock solution of 350 mg/10 ml was prepared for the 350 mg/kg BW Moringa leaf extract and another one of 250 mg/10 ml for the 250 mg/kg bw Papaya seed extract.

For the combination Moringa Leaf Extract 700 mg/kg bw: Papaya Seed Extract 500 mg/kg bw, a stock solution of 700 mg/10 ml was prepared for the 700 mg/kg bw Moringa leaf extract and another one of 500 mg/10 ml for the 500 mg/kg bw Papaya seed extract.

Data analysis was carried out on SPSS version 24.0. One-way ANOVA was performed to calculate the average percentage of decreased blood sugar levels on the eighth (T2) and fifteenth (T3) days and compare these values to those of the 5th day (T1) in all groups of tested animals.

Results

A few drops of 10% NaOH solution were added to 0.5 grams of thick extracts until a colour change occurred to confirm the presence of flavonoid compounds in the Moringa Leaf Extract and Papaya Seed Extract (Rahayu *et al.*, 2015). The Moringa Leaf Extract turned yellowish, while the Papaya Seed Extract became yellow-orange. The ethanol-free test was carried out on both extracts to confirm they were completely free of ethanol by adding acetic acid solution and concentrated sulfuric acid solution; the mix was then 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.

The first measurement of blood sugar levels was carried out on day zero (T0). Then, diabetes was induced by giving white male rats glucose 10% for four days. After induction, blood sugar levels were measured on the fifth day (T1), the eighth day (T2), and the fifteenth day (T3) after treatment, using a glucometer test strip. The rat blood was taken from the tip of the tail after a 12-hour fasting period.

The average percentage of decrease in blood sugar levels at T2 (Day 8) was calculated by deducting blood sugar levels at T1 (pre-test) from T2 (post-test), dividing by blood sugar levels at T1, then multiplying by 100: ((T1-T2)/T1)*100. The average percentage of decrease in blood sugar levels at T3 (Day 15) was calculated using the same formula: ((T1-T3)/T1)*100.

This study showed that the best average percentage of decrease in blood sugar levels at T2 (Day 8) was 48.82%, seen with Group 3 (700 mg/kg bw of Moringa leaf extract), close to the percentage of the positive control treatment group (53.46%) (Table I). The dose of 700 mg/kg bw of Moringa leaf extract showed to be effective, with blood sugar levels close to normal values. The decrease in blood sugar levels was believed to be the result of the repair of pancreatic β cells by the flavonoids in Moringa leaves. These flavonoids can also function as an antioxidant that can reduce oxidative stress in cells, thus decreasing the pancreatic β cell damage process and accelerating the regeneration process of pancreatic β cells (Ambarwati *et al.*, 2014).

Table I: Percentage of average decrease in blood sugar levels on the eighth 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	<0.0001
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 (Glibenclamide 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 best average percentage of decrease in blood sugar levels at T3 (Day 15) found in this study was 57.24%, found in treatment group 6 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract), close to the percentage of the positive control treatment group (61.49%) (Table II). The combination Moringa leaf extract and Papaya seed extract contain flavonoids that can be used as antioxidants; thus, 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).

Table II: Percentage of average decrease in blood sugar levels on the fifteenth day (T3)

Treatment group*	Blood sugar level decreased (%)				Average (%) ± SD	*p*
	1	2	3	4		
Group1	10.37	14.13	16.14	14.59	13.81± 2.45	<0.0001
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 (Glibenclamide 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

Discussion

The identification test of flavonoid compound using NaOH 10% showed that the extract was positive for flavonoid, with the Moringa Leaf Extract turning yellowish and the Papaya Seed Extract yellow-orange. The ethanol-free test using the esterification method 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, indicated by the absence of an ester odour of the Moringa Leaf Extract and Papaya Seed Extract.

Glibenclamide was used as a comparison because its short-term therapeutic effects were almost the same as the hypoglycemic effect of flavonoids present in Moringa Leaf Extract and Papaya Seed Extract, which increased insulin secretion from pancreatic β cells. Based on the decrease in blood sugar levels at T2 and T3, treatment Group 6 had the best outcomes of blood sugar levels, close to the positive control treatment group. For the results of the decreases in blood sugar levels at T2 and T3, one-way ANOVA and Tuckey test were performed to examine 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 significant differences between the treatment groups with a significance value of $p < 0.0001$ at T2 (Day 8) and T3 (Day 15). The Tuckey test at T2 showed no significant difference (0.907 > 0.05) between treatment Group 6 (700 mg/kg bw of Moringa leaf extract) and treatment Group 5 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract). Whereas in the Tuckey test at T3, there was no significant difference (0.063 > 0.05) between treatment Group 2 (positive control group) and treatment Group 6 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract), no significant difference (0.999 > 0.05) between treatment Group 3 (Moringa Leaf Extract 700 mg/kg bw) and treatment group 6 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg BW of Papaya seed extract), and no significant difference (0.768 > 0.05) between treatment Group 4 (Papaya Seed Extract 500 mg/kg bw) and treatment Group 5 (combination Moringa Leaf Extract 350 mg/kg bw: Papaya Seed Extract 250 mg/kg bw).

Conclusion

This research found that the combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya

seed extract had the best antidiabetic effect, as indicated by the average value of decreased blood sugar levels on the fifteenth day, almost close to the mean value of the positive control group. Moreover, the single extract of Moringa leaves (700 mg/kg bw) showed better results than the combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract, as indicated by the values at eighth and fifteenth day.

References

Ambarwati., Sarjadi., Johan A., Djamiatun Kis. (2014). Efek Moringa oleifera Terhadap Gula Darah danKolagen Matrik Ekstraseluler Sel β Pankreas Diabetes Eksperimental. *Jurnal Kedokteran Brawijaya*, **28** (2),74-78

Ariani et al. (2019). Uji Aktivitas Antibakteri Ekstrak Biji Pepaya (Carica papaya L) Terhadap Pertumbuhan (*Escherichia coli*). *Journal of Current Pharmaceutical Sciences*, **2** (2): 160-166. Banjarmasin: Akademi Farmasi ISFI

Dewiyeti, Susi dan Hidayat, Saleh. (2015). Ekstrak Daun Kelor (Moringa oleiferaLamk) Sebagai Penurun Kadar Glukosa Darah Mencit Jantan (*Mus musculus*) Hiperglikemik, *Jurnal Penelitian Sains*, **17** (2), 72-77. Palembang: FKIP Universitas Muhammadiyah

Jusnita, Nina dan Syurya Wan. (2019). Karakterisasi Nanoemulsi Ekstrak DaunKelor (*Moringa oleifera Lamk*). *Jurnal Sains Farmasi & Klinis*, **6** (1), 16-24.Jakarta: Fakultas Farmasi Universitas 17 Agustus 1945

Kementerian Kesehatan RI. (2014). Situasi Dan Analisis Diabetes, Direktorat Jenderal Bina Kefarmasian dan Alat Kesehatan Departemen Kesehatan RI. Jakarta

Rahayu, Siti. Kurniasih, N., Amalia, V. (2015). Ekstraksi Dan Identifikasi Senyawa Flavonoid Dari Limbah Kulit Bawang Merah Sebagai Antioksidan Alami. *Jurnal al-Kimiya,* **2** (1), 1-8. Bandung: UIN Sunan Gunung Djati

Theresia, Rori., dkk. (2017). Aktivitas Antihiperglikemia Ekstrak Kulit Dan Daun Surian (*Toona sinensis*) Pada Tikus Diabetes (*Sprague-dawley*) Yang Diinduksi Streptozotocin. *Jurnal Gizi Pangan,* **12** (3), 187-194. Bogor: ITB

Wulansari, Diah, Devyani., Basori, Achmad., dan Suhartati. (2017). Pengaruh Pemberian Biji Pepaya (*Carica papaya Linn*) Terhadap Ekspresi GlucoseTransporter 4 (GLUT 4) Pada Jaringan Otot Rangka Tikus Model Diabetes Melitus Tipe II Yang Diinduksi Diet Tinggi Fruktosa. *Traditional Medicine Journal*, **22** (2), 131-138. Surabaya: Universitas Airlangga

Yasin, Y. K., Kartasurya, M. I., & RMD, R. K. (2016). Pengaruh kombinasi vitamin c dan vitamin e terhadap Kadar malondialdehid plasma pasien diabetes mellitus tipe 2. *Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition*), **4**(1), 1-8