Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/
Research Article

Antidiabetic activity of matoa leaves (*Pometia pinnata* J.R.Forst & G. Forst) extract on hyperglycaemic alloxan-induced rats

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Submitted 08 June 2021; Revised 08 December 2022; Accepted 08 August 2022; Published 30 October 2023

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Abstract

Matoa (*Pometia pinnata* J.R.Forst & G. Forst) leaves contain polyphenols and flavonoids which are prospective for the development of the new antidiabetic agent. This research aimed to explore the potency of matoa leaves in reducing blood glucose level and increasing plasma insulin concentration in alloxan-induced rats. The experimental study was done in 6 treatment groups: the aquadest (normal), alloxan (Alx) 150 mg/kgBW (negative control), glibenclamide (Gli) 5 mg/kgBW (positive control), and matoa leaves extract (MLE) at the doses of 50, 100, and 200 mg/kgBW. The treatment was administered for 14 days orally post-induction of alloxan and fasting blood glucose (FBG) reached >200 mg/dL. Blood glucose levels were established by the GOD-PAP (Diasys) method while plasma insulin was measured using Rat insulin ELISA kit. Hematoxyline-eosin (HE) staining on tissues was performed to observe the population of pancreatic beta cells. The results showed that the MLE extract at a dose of 200 mg/kgBW could decrease the FBG level to 135.25±21.14 mg/dL and increase the plasma insulin level up to 0.14%. Pancreas histopathology indicated that the number of damaged cells were lower than negative control. In conclusion, our results affirm the promising potential of MLE as a candidate of the novel antidiabetic agent.

Keywords: alloxan, antidiabetic, matoa leaves, plasma insulin.

Aktivitas antidiabetes Ekstrak daun matoa (*Pometia pinnata* J.R.Forst & G. Forst) pada hiperglikemik *alloxan*-tikus yang diinduksi

Abstrak

Daun matoa (*Pometia pinnata* J.R.Forst & G. Forst) mengandung polifenol dan flavonoid yang dapat dimanfaatkan sebagai sumber alami pada pengembangan agen antidiabetes baru. Penelitian ini bertujuan untuk menetapkan potensi daun matoa dalam menurunkan kadar gula darah (KGD) dan peningkatan kadar insulin plasma pada tikus hiperglikemia yang diinduksi aloksan. Penelitian eksperimental dengan 6 kelompok hewan uji: yaitu aquadest (normal), aloksan (Alx) 150 mg/kgBB (kontrol negatif), glibenklamid (Gli) 5 mg/kgBB (kontrol positif), dan ekstrak daun matoa (MLE) dengan dosis 50, 100, dan 200 mg /kgBB. Perlakuan diberikan selama 14 hari secara oral pasca induksi aloksan dan kadar gula darah puasa (FBG) mencapai >200 mg/dL. Kadar glukosa darah ditentukan dengan metode GOD-PAP (Diasys) sedangkan insulin plasma diukur menggunakan kit ELISA insulin tikus. Pewarnaan hematoxyline-eosin (HE) pada jaringan dilakukan untuk mengamati populasi sel beta pankreas. Hasil penelitian menunjukkan bahwa ekstrak MLE dosis 200 mg/kgBB mampu menurunkan kadar FBG hingga 135,25±21,14 mg/dL dan meningkatkan kadar insulin plasma hingga 0,14%. Histopatologi pankreas menunjukkan jumlah sel yang rusak lebih sedikit dibandingkan kontrol negatif. Kesimpulannya, hasil kami menegaskan potensi MLE yang menjanjikan sebagai kandidat agen antidiabetik baru.

Kata Kunci: aloksan, antidiabetes, daun matoa, insulin plasma.

1. Introduction

Diabetes mellitus (DM) is one of metabolic disease identified as a disruption of insulin function which in long-term could cause detrimental effects in the organs[1]. This disease has affected 463 million people worldwide in 2019 and forecasted to increase up to 578 million people in 2030[2]. High level of blood glucose caused by insulin insensitivity or an absolute loss of insulin are the main hallmarks of diabetes mellitus disease[3]. Type 1 diabetes is mainly caused by the damage of pancreatic beta cells from autoimmune reaction, while type-2 diabetes is precedented by insulin receptor resistance that prevents glucose molecule entry into the cells leading to the accumulation of glucose molecules in the blood stream[4]. The damage of pancreatic beta cells can also be caused by chemical substance such as alloxan. Alloxan acts as a toxic glucose analogue which accounts for reactive oxygen species formation that could destroy the pancreatic beta cells resulting in an elevated level of blood glucose level[5]. Low-sugar diet is highly recommended for diabetic patients, in addition, oral antidiabetic drugs (OADs) and insulin are the main clinical therapy against diabetes. Several mechanisms of OADs including reduce hepatic glycogen catabolism to glucose molecules, increasing insulin secretion as well as insulin sensitivity[3]. In fact, not only chemical substances are beneficial in lowering blood glucose level, people throughout centuries have been empirically practiced traditional medicine using plants as a solution to maintain their blood glucose level, one of the many is matoa plant.

Matoa (*Pometia pinnata* J.R.Forst & G. Forst) is a very well-known plant in tropical hemispheres such as Southeast Asian countries and widespread especially in Indonesia. Several parts of the plant namely seed, fruit, leaf, and stembark have been previously researched denoting the presence of various secondary metabolites especially tannins, steroids, flavonoids, and polyphenols[6] Previous research has shown extensive effects from matoa plant such as diuretic from its fruit and seed on rats at 100 mg/kgBW[7], antihypertensive[8], and antioxidant[9]. In addition, matoa's seed and stembark were proven capable to inhibit α-glucosidase thus implying its benefit in diabetic management[10,11]. Furthermore, a research of Tandi et.al, (2021) reported a decrease of the blood glucose levels up to 133 mg/dL in the diabetic animals given ethanolic extract of matoa leaves with the dose of 100 mg/kg for 21 days[12]. All this data ascertained the high potential of matoa plant to counter the severity of diabetes mellitus disease. However, the effect of matoa plant in diabetic is yet unknown, thus, the goal of this research was to investigate the ability of MLE with the dose of 50, 100, and 200 mg/dL in reducing blood glucose level, increasing serum insulin concentration, and describing the pancreatic cell damage in diabetic animal model.

2. Method

2.1. Materials

Matoa leaves, 50% ethanol, distilled water, cabosil, lactose, alloxan (Alx) (SigmaAldrich), saline solution (WIDA), glibenclamide (Gli) (Indofarma), Glucose GOD FS (Diasys), urine strip test (Uriscan), and ELISA Rat INS (Insulin) ELISA Kit (FineTest, ER1113).

2.2. Instruments

Analytical balances (OHAUS Pioneer; 0,0001 g), drying cabinet, maceration apparatus, rotary evaporator, syringe (OneMed), oral probe, vortex, glassware, spectrophotometer (Star Dust MC15), and ELISA reader (Biotek).

2.3. Procedures

2.3.1. Extraction

The matoa leaves used in this research were obtained from Jatinom, Klaten, Central Java. The plant was then authenticated by Biology Laboratory of the Faculty of Teacher Training and Education at Universitas Muhammadiyah Surakarta (008/A.E-I/LAB. BIO/I/2019). The extraction was performed using 50% ethanol by maceration method and followed by solvent vaporization using rotary evaporator and water bath until viscous

consistency, approximately for three days. One gram of the mixture of lactose: cabosil (4:1) was then incorporated into nine portions of the viscous extract and mixed until dry extract was[12] formed[13].

2.3.2. Animals

24 Wistar-male rats aged 2-3 months and weighed 150-300 grams were obtained from a local breeding farm "Rumah Tipu" in Klaten. Animals were housed in a standardized cage in the animal facility at the Pharmacology Laboratory of the Faculty of Pharmacy, Universitas Muhammadiyah Surakarta. The animals were divided into six cages with four animals each. Acclimatization was done for seven days once the animals arrived at the facility. During acclimatization, the animals were fed daily and given water ad lib. Ethical clearance was obtained from the Ethical Committee of Health Research, the Faculty of Medicine, Universitas Muhammadiyah Surakarta (1741/A.2/KEPK-FKUMS/I/2019)

2.3.3. In-vivo antidiabetic study

Experimental design: This research was performed according to the Completely Randomized Design method. The animals were divided into six groups of categorized based on the given treatments, namely; group 1 as normal group, group 2 as negative control group, group 3 as positive control group, while group 4, 5, and 6 received MLE with the dose of 50, 100, and 200 mg/kgBW respectively. The doses of MLE used in this research were based on previous work of Tandi et.al, (2021)[12]. Diabetogenic agent used in this experiment was intraperitoneally administered Alx with the dose of 150 mg/kgBW)[14]. Hyperglycaemic condition occurred at day 5 post induction (FBG >200 mg/dL). Gli 5 mg/kgBW as an oral antidiabetic agent was given to the animal in the group 2 as a positive control[15].

2.3.4. Animal modelling

Group 2 to 6 were given with 150 mg/kgBW alloxan via intraperitoneal injection to induce diabetes condition[16]. Prior to induction, the animals were fasted for 24 hours and only allowed access to water. Every day, the animals were given 1 mL 20% glucose orally. Hyperglycaemic condition was achieved when the blood glucose level reached >200 mg/dL[17].

2.3.5. Animal treatments

Once the animals reached hyperglycaemic condition, treatments with MLE were started. Group 1 and 2 were given aquadest, while group 3, 4, 5, and 6 were given Gli 5 mg/kgBW, MLE with the dose of 50, 100, and 200 mg/kgBW respectively. Body weight, blood glucose, and insulin level were measured three times as baseline, at day 0 and day 14. Baseline was determined right after the acclimatization prior any intervention, day 0 was established four days after alloxan induction for group 2 to 6 when hyperglycaemic condition was reached, and day 14 was set after 14 days of MLE treatment.

2.3.6. Blood glucose level

The blood glucose level was determined by GOD-PAP method using Glucose GOD FS kit (Diasys). The samples, blank reagent, and standard mixtures were prepared as per manufacturer's instruction and measured under λ 546 nm light using spectrophotometer (Star Dust MC15).

2.3.7. Serum insulin level

Insulin levels are established by the Sandwich ELISA method with Double Antibody. Serum samples were reacted with monoclonal anti body anti-mouse insulin (FineTest, ER1113), and measured with a microplate reader at a wavelength of 450 nm. The insulin concentration was quantified based on the standard curve equation between concentration and optical density.

2.3.8. Hematoxylin-Eosin (HE) staining of pancreatic cells

Histopathological preparations were worked out according to the Kiernan method: tissue fixation in a 10% normal formalin buffer for 24 hours. Tissues that have been blocked with paraffin, were cut with microtoms with a thickness of 4-5 μm.

Table 1. Measurements of the blood glucose level (mg/dL), plasma insulin concentration (pg/dL), body weight (g), and urine glucose level (mg/dL)

Parameter	Animal Groups (mg/kg BW)	Baseline[a]	Day 0[b]	Day 14[c]
Blood glucose (mg/dL)	Normal	119.00 ± 3.16	125.75 ± 10.72	109.75 ± 13.30*
	Alx 150	108.50 ± 18.63	648.75 ± 57.03	323.75 ± 107.69
	Gli 5	94.50 ± 14.89	358.50 ± 97.79	158.00 ± 18.02*
	MLE 50	99.75 ± 22.26	298.25 ± 75.58	143.25 ± 24.86*
	MLE 100	106.75 ± 26.09	457.25 ± 118.13	137.75 ± 20.81*
	MLE 200	115.25 ± 5.62	334.50 ± 112.75	135.25 ± 21.14*
Plasma insulin (pg/mL)	Normal	16.42 ± 1.20	16.25 ± 3.78	16.66 ± 2.60
	Alx 150	21.28 ± 3.20	21.98 ± 3.45	21.80 ± 1.98
	Gli 5	17.57 ± 8.16	19.95 ± 6.81	20.46 ± 4.51
	MLE 50	22.54 ± 8.36	24.89 ± 11.11	24.62 ± 8.91
	MLE 100	29.52 ± 11.53	23.95 ± 7.36	28.55 ± 10.75
	MLE 200	18.33 ± 8.14	18.09 ± 7.38	20.74 ± 13.36
Body weight (g)	Normal	222.50 ± 51.16	216.75 ± 58.66	211.00 ± 65.35
	Alx 150	208.50 ± 17.52	192.00 ± 21.74	178.75 ± 18.73
	Gli 5	181.50 ± 36.19	169.75 ± 39.00	168.00 ± 30.92
	MLE 50	188.75 ± 33.72	191.75 ± 43.22	190.00 ±50.29
	MLE 100	223.00 ± 43.76	208.50 ± 37.90	215.00 ± 48.49
	MLE 200	167.50 ± 13.48	160.50 ± 24.69	166.25 ± 31.79

[a] Baseline, prior any intervention
[b] Four days after alloxan 150 mg/kgBW induction, animals reached hyperglycemic condition
[c] fourteen days after treatment with MLE
* P<0.05; means difference compared to negative control by One-way ANOVA

The prepared preparations were stained with Hematoxylin Eosin (HE). The tissues were then examined under a microscope against each at 5 microscopic field of view with 1000x magnification. HE staining and microscopic observation of pancreatic cells were performed at the Faculty of Medicine, Universitas Sebelas Maret, Surakarta.

2.3.9. Data analysis

Data from blood glucose and pancreatic cells number were statistically analysed using One-Way ANOVA followed by post hoc with LSD test, while Wilcoxon test was used to analysed the plasma insulin measurement data. The statistical analysis was performed using IBM SPSS Statistics 24 (United Kingdom) at 95% confidence interval (P value <0.05 is considered significant).

3. Result

FBG level was monitored at the time prior Alx induction (baseline), days 0, and 14 of the treatment, with the results of all MLE treated groups showing that FBG level were returned to normal on day 14 as did in the Gli group (P>0.05) in contrast to the Alx group which still showed hyperglycemic condition (Table 1). Table 1 also indicates an increase of insulin concentration post treatment at day 14 compared to day 0, with the highest was found at 100 mg/kg BW MLE treated group.

The weight of animals was measured twice at the day 0 and 14, which showed a decreasing trend at day 0 of treatment. Up to day 14, a steady decrease in the average body weight of the Alx group from day 0 to day 14 at 192.00 ± 21.74 and 178.75 ± 18.73 g respectively, was found; while a slight increase was observed in Gli and MLE treated groups.

Figure 1 presents the microphotographs of the pancreatic beta cells visualized with HE staining from the representatives of each group, while (a) and (b) representing damaged cell respectively.

Figure 2 shows the average number of damaged cells in all groups. 200 mg/kg BW MLE treated group exhibited similar number of damaged cells as normal and Gli treated

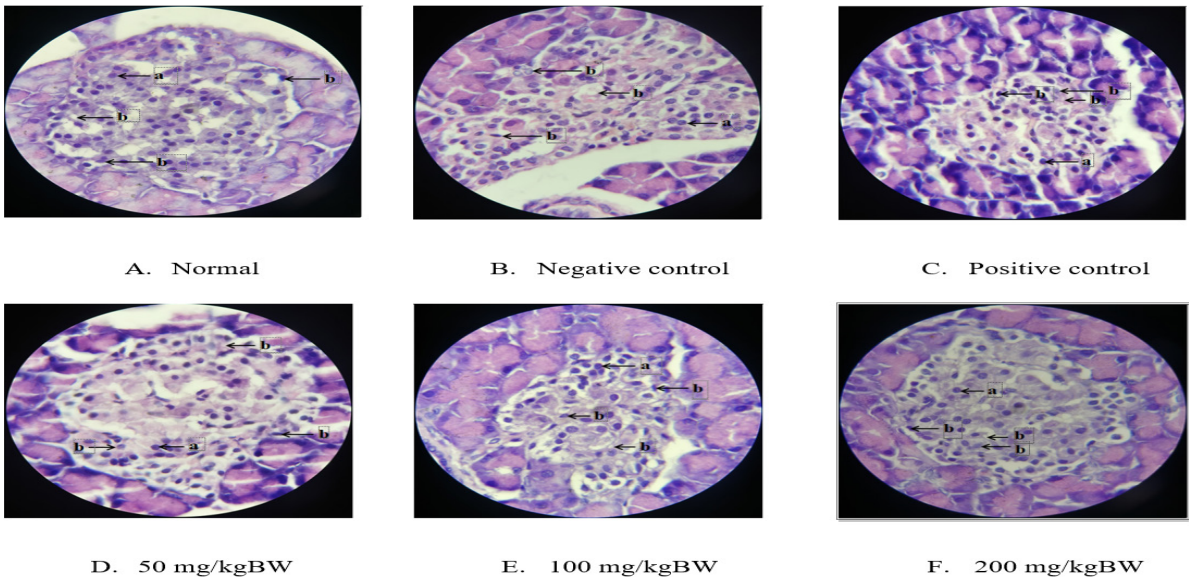

Figure 1. Representatives of the microphotographs of pancreatic cells collected from each group by HE staining. (a) Normal cells and (b) Damaged cells. Objects were observed under 1000x magnifications

groups, meanwhile, at 50 and 100 mg/kg BW MLE treated groups possessed the identical number of damaged cells as Alx group.

4. Discussion

Pometia pinnata has been widely researched for its pharmacological activity[7,8,18,19]. Previous reports have denoted its antidiabetic effects from several parts of the plant by inhibiting enzymatic activity of α-glucosidase[10,20]. Sukiman et al., (2018), found that the methanol, ethyl acetate, and n-hexane extracts of the seeds from the plant exhibited inhibition against α-glucosidase. In addition, the stembark of the plant showed 100% inhibition of α-glucosidase activity at the concentration of 50 µg/mL[20], indicating the promising activity as an antidiabetic agent. Thus, based on these data, we looked into another part of the plant for a screening of its antidiabetic activity. The leaves are the most abundant part of a plant of which made it easier to be used as raw materials, therefore it would have been more practical in

case of profound findings in our research. Prior references claimed the role of flavonoids in several pharmacological activities[21–23]; 50% ethanol was used to maintain the relatively polar solvent in order to obtain higher concentration of flavonoids in our extract[24]. Matoa leaf contains several active substances such as proanthocyanidin, epicatechin, quercetin, kaempferol, palmitoyl, stigmasterol, dan arabinofuranosyl[19], which most of them are structurally come from flavonoids family. Flavonoid is an infamous free radical scavenger which could act as a natural antioxidant[23]. Flavonoids were reported to have antidiabetic activity by contributing in pancreatic beta cells

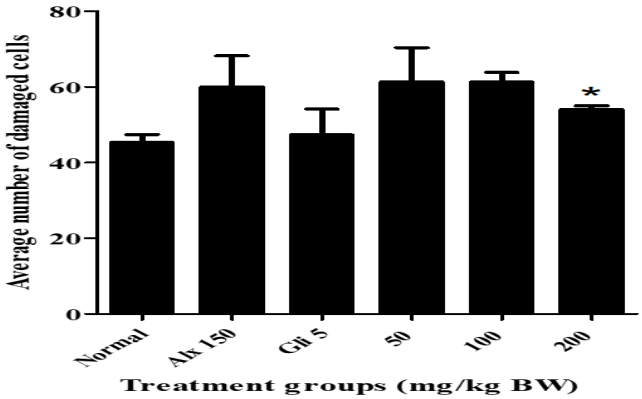

Figure 2. Comparative graphs depicting the average number of damaged cells across the animal groups. *P>0,05 against Normal and Glibenclamide 5 mg/kg BW treated groups.

regeneration[17] resulting in the recovery of insulin production. Therefore, it is likely that flavonoids were the primary active compound responsible in performing such effects.

Insulin secreted by pancreas β cells controls blood glucose levels by facilitating cellular glucose uptake, regulating carbohydrate, lipid, and protein metabolism[25]. Our experiments suggest that between the three doses, 200 mg/kgBW of MLE could maintain the number of normal cells the best and simultaneously had the lowest number of damaged cells (figure 2). The example of damaged cells could be manifested as necrosis marked by the loss of physical structure and function of the cells followed by cell lysis. The lysis of pancreatic beta cells could cause tissue inflammation which eventually result in the significant decrease of beta cells in islet of Langerhans[26]. This phenomenon can be seen in the negative control group at figure 1 (B) and figure 2, where profound empty spaces were spotted and the average number of damaged cells were significantly more than the normal cells. The damage of the cells led to a constant low level of insulin plasma since day 0 to 14 resulting in a notably high level of blood glucose in day 0 and 14 (table 1). However, a decrease of the blood glucose level was observed in the negative control group (table 1), denoting the ability of induced animals to recover from hyperglycaemic condition by pancreatic beta cell regeneration[27]. Other impacts caused by insulin disorder are the elevated urine glucose level and weight loss. High concentration of glucose molecules entering glomerulus filtration are failed to be reabsorbed resulting in the presence of glucose in urine[28].

Weight loss is one of the main hallmarks of hyperglycaemic caused by the increasing activity of gluconeogenesis as well as glycogenolysis resulting in a breakdown of glycogen and fat in muscle tissues and adipose cells into glucose molecules[29]. These data are supporting the precedent finding as people with diabetic condition could not utilize glucose as an energy source, instead, they over-catabolize fat and protein from adipose and muscle tissues[21] which could lead to significant weight loss.

However, despite of the positive result achieved from the treatment with 200 mg/kgBW MLE, this dose still could not protect the cell loss compared with the normal group, presumably because the extract needs longer duration to be significantly beneficial in therapy. Interestingly, the dose of 100 mg/kgBW possessed the most effective results, lowering the blood glucose level to 137.75 ± 20.81 mg/dL, eliminating glucose molecules in urine, increasing 0.16% of plasma insulin concentration, and maintaining body weight in the animals (table 1). Therefore, based on our experiments, MLE with the dose of 100 mg/kgBW could be the key of development for further research. Furthermore, our findings are also in line with the previous research on Pometia pinnata asserting the potential of the plant as a source of new candidates for antidiabetic agent.

However, it is necessary to perform in depth research regarding the extract in order to analyse other mechanism that could play role in the antidiabetic activity. Further assays may include α-glucosidase inhibition, reduction of hepatic glucose production, and improvement of peripheral insulin sensitivity.

5. Conclusion

Our findings strengthen previous works which once again emphasize the role of matoa plants to alleviate diabetes mellitus manifestations and as a potential source for a novel antidiabetic agent. Based on our data, it is clear that 14 days of daily MLE oral administration with the dose of 200 mg/kg BW against diabetic rats, resulted in a positive impact in three parameters, namely; blood glucose level, insulin level, and the average number of pancreatic damaged cells.

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