Incretin hormone especially GLP-1 has potency as anti-diabetes. However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively to become inactive forms. GLP-1 have a short half-life, approximately 2–5 min due to DPP-4 activity. The inhibition of DPP-4 is effective to treat T2DM because GLP-1 bioavailability can be retained more than compared to the diabetic group whereas blood glucose level decreased about 30%, 35%, and 40% respectively (p<0.05). Extract at doses of 500 and 1000 mg/kg bw also increased insulin level by 4-fold and 8-fold respectively compared to the diabetic and the islet β-cells were repaired. The active compound in U. lobata leaves extract are suggested to prevent degradation of GLP-1 by inhibition of DPP-4 activity. Aqueous extract of U. lobata also improved the structure and function of islet β-cells by increasing of GLP-1 bioavailability.

1. Introduction

Modulation of incretins in the treatment of type 2 diabetes mellitus (T2DM) has received attention in the recent search for potent anti-diabetes. Glucagon-Like Peptide-1 (GLP-1) and Glucose-Dependent Insulinotropic Polypeptide (GIP) are major incretin hormone secreted by intestinal due to induction of oral nutrition. GLP-1 plays a role in maintaining blood glucose level because of their biological activity such as stimulating insulin secretion, increasing β-cell proliferation, inhibiting glucagon secretion, reducing the rate of gastric emptying and inducing satiety. In a patient with T2DM, chronic hyperglycemia is caused by a decreasing GLP-1 bioavailability, therefore the secretion of insulin reduced. Significant incretin-like drug has also side effects such as flu-like symptoms, skin reaction, gastrointestinal problem, and this effect is able increase in long-term use of drugs. This phenomenon induces people to search a medicinal plant as an alternative therapy for T2DM through controlling of incretin bioavailability.
2. Material and methods

2.1. Preparation of U. lobata leaf extract

U. lobata leaf powder was obtained from Balai Materia Medika Batu Malang with certificate number 074/027/101.8/2015. In brief, 50 g U. lobata leaf powder was extracted according to decoction method in 250 ml hot water at 90 °C for 30 min therefore the extract was evaporated until resulting concentrated extract.

2.2. Animals and treatments

Male Sprague-Dawley (SD) rats (180–200 g) were obtained from Gajah Mada University Yogyakarta Indonesia. The study was conducted according to the ethical guidelines which were approved by the Commission of Ethical Research Brawijaya University Malang Indonesia with certificate number 245-KEP-UB. SD rats were housed in an individual cage and automatically controlled animal room at 25 ± 1 °C on a 12:12-h light–dark cycle. They were fed by standard food, water ad libitum and fasted overnight before the experiments. Normal diet (ND) and a high fructose diet (HFD) food were freshly mixed in every two days. Diabetic rats were induced by HFD (65% fructose and 35% ND food) and a single dose of streptozotocin 25 mg/kg BB intraperitoneal refer to Guo et al with minor modification. Rats were stated diabetic if the fasting blood glucose level more than 126 mg/dL. The experiment was assigned into five groups for five rats each. For eight weeks, the normal group (NG) received ND whereas the diabetic (DG) and treatment groups received HFD. The treatment groups were given an aqueous extract of U. lobata (AEU) at a dose of 250 mg/kg, 500 mg/kg, and 1000 mg/kg bw for four weeks after the rats were classified as diabetic according to Shirwaikar et al. Body weight and food consumption were monitored weekly. Blood samples were obtained 15 min after orally glucose stimulation in a dose of 2 g/kg body weight and taken from tail vein after overnight fasted. A blood sample was immediately centrifuged 4500 rpm. The serum was separated and saved under –20 °C.

2.3. GLP-1 assay

GLP-1 serum level was analyzed by rat GLP-1 ELISA kit (USCN CE804). 50 μl samples were added 50 μl detection reagent A and then incubated for 60 min at 37 °C. After aspirating and washing, samples were added 100 μl detection reagent B and incubated for 30 min at 37 °C. Added 90 μl substrate reagents then was added 50 μl stop solution. Samples were read with a microplate reader at λ = 450 nm.

2.4. Insulin assay

Insulin serum level was analyzed by rat insulin ELISA kit (Elabscience E-EL-R0023). 50 μl samples were added 50 μl Bio-tinylated detection Ab and incubated for 45 min at 37 °C. After aspirating and washing then samples were added 100 μl HRP conjugate and incubated for 30 min at 37 °C. Added 90 μl substrate reagents then incubated for 15 min at 37 °C. 50 μl stop solution was added then read with a microplate reader at detector wavelength.

2.5. Blood glucose assay

The blood samples were collected from the tail vein after overnight fasted and at 15 min after oral glucose administration. They were measured immediately using a commercially available glucometer (AccuCheck).

2.6. Histopathology of islet β-cells

Pancreas tissue was taken by section methods and continued by Hematoxylin–Eosin (H–E) staining. Mostly islet cells containing β-cells were observed including shape, size, number each view under the microscope with magnification 400 times.

2.7. Statistical analysis

The data were expressed as means ± SD. Statistical analysis was performed by one-way ANOVA. The least significant difference (LSD) test and Dunnet C were used for mean comparisons and then p < 0.05 was considered to be statistically significant.

3. Results

3.1. The effect of U. lobata leaf extract on body weight, food consumption, glucose, and insulin level of diabetic rats

In the end of the treatment, there is not a significant decrease of body weight on test group compared to diabetic group (p > 0.05) meanwhile food consumption is decreased (p < 0.05) (Table 1). The oral administration of U. lobata leaf extract decrease fasting blood glucose level compared to diabetic group (p < 0.05) whereas insulin level was increased (p < 0.05).

3.2. The effect of U. lobata leaf extract on GLP-1 serum level of diabetic rats

There is a significant decrease of GLP-1 levels on the diabetic group about 8-fold compared to normal group observed (p < 0.05) (Fig. 1). Aqueous extract of U. lobata at doses 250 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw can prevent degradation of GLP-1 respectively about 3-fold, 5-fold, and 7-fold compared to diabetic group (p < 0.05). An increased dose of U. lobata leaves extract prolong and enhance GLP-1 bioavailability.

3.3. The effect of U. lobata leaf extract on insulin serum level of diabetic rats

There is a significant decrease of insulin levels on diabetic group approximately 14-fold compared to normal group observed (p < 0.05) refer to Fig. 2. The administration of aqueous extract U. lobata 500, and 1000 mg/kg bw increase insulin level 4-fold, 8-fold respectively compared to diabetic group (p < 0.05) whereas the dose of 250 mg/kg bw cannot increase insulin level. The more increase dose of water extract U. lobata, the more insulin level escalated.
3.4. The effect of U. lobata leaf extract on blood glucose level of diabetic rats

Based on these results at Fig. 3, there is a significant increase at blood glucose level on a diabetic group up to 70% compared to normal group observed (p < 0.05). The administration of aqueous extract U. lobata at dose of 250 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw can decrease glucose level respectively 30%, 35%, and 40% compared to the diabetic group (p < 0.05) after glucose stimulation. Blood glucose level is not different significantly on an increase of dose U. lobata (p > 0.05).

3.5. The effect of U. lobata leaf extract on islet β-cells of diabetic rats

The normal group (Fig. 4A) shows the shape of cells are round, nucleated, and in a huge number, whereas the diabetic groups (Fig. 4B) cells show longer, not nucleated, and in a small number. Administration of aqueous extract U. lobata at dose of 500 and 1000 mg/kg bw could inhibit cells damage which shown as round cells, nucleated, and in a huge number (Fig. 4C-D). Test groups have islet β-cells in slightly bigger size than normal group, except aqueous extract at dose of 1000 mg/kg bw. The bigger size of cells shows a swelling cells and injury indications. The administration of aqueous extract U. lobata at dose of 1000 mg/kg bw are able to inhibit cells damage therefore the shape, size, and number are similar to islet cells at the normal group.

4. Discussion

4.1. The effect of U. lobata leaf extract on GLP-1 serum level of diabetic rats

Oral administration of aqueous extract U. lobata significantly maintains GLP-1 bioavailability of diabetic rats. Based on our previous study, active compounds in U. lobata such as mangiferin, stigmasterol, and β-sitosterol are able to prevent degradation of GLP-1 by inhibition of DPP-4.13 DPP-4 inhibitor substances prevent the degradation of active GLP-1 even though it does not increase the levels of total circulating GLP-1 and does not prevent the kidney from rapidly clearing GLP-1.12

GLP-1 is incretin hormone produced by L cell intestine and the secretion depends on oral nutrition. GLP-1 has a potency for T2DM therapy but it is metabolized excessively by DPP-4 into inactive form.7 GLP-1 has a short half-life, approximately for 2–5 min, it is caused of DPP-4 activity.3,6 The active form of GLP-1 is GLP-1 (7–36) amides and GLP-1 (7–37) which are rapidly inactivated by DPP-4 through cleave N-terminal dipeptide His-Ala.1,12 It produces an inactive form of GLP-1, they are GLP-1 (9–36) amide and GLP-1 (9–37) isopeptides.5,7 A number study showed that the importance of DPP-4 mediated inactivation of GLP-1 as a key determinant of GLP-1 and GIP bioactivity.1,12
GLP-1 is a superfAMILY peptide of glucagon which has a similarity degree approximately 48%. The similarity of amino acid sequence between GLP-1 and glucagon become one of this cause. Proglucagon gene is located at chromosome 2q36-q37 and only found in some tissues whereas the messenger RNA (mRNA) of proglucagon is met at α-cells pancreas, L cells intestine, and brain. Proglucagon production is started from transcription of preproglucagon gene and then is continued by translation process. The regulation of GLP-1 released from L cells intestine is a complex mechanism that involves combinations of nutrition, hormone, and neural stimuli. The GLP-1 receptor is classified in G protein-coupled receptor that is found in liver, muscle, and pancreas cells. This receptor has a specific character by activation of adenyl cyclase and results cAMP. After GLP-1 binding with the receptor, it will activate cAMP and Mitogen-Activated Protein Kinase (MAPK). The biological activities of GLP-1 are various and depend on the organ target. GLP-1 activity in the pancreas has functions in stimulating the insulin secretion by cAMP activation, increasing β-cell masses by MAPK pathway and inhibiting the secretion of glucagon. In the brain, it will reduce the rate of gastric emptying, induce satiety, and neuroprotection whereas in liver, fatty acid metabolism will be decreased and glucose utilization increased. All of them contribute to regulate blood glucose level in T2DM.

4.2. The effect of U. lobata leaf extract on insulin serum level of diabetic rats

Aqueous extract of U. lobata significantly increases insulin synthesis of diabetic rats. It is controlled by active compounds in the extract through the activity of GLP-1. The oral administration will maintain GLP-1 bioavailability moreover the insulin biosynthesis can be increased. GLP-1 has a potency to retain the insulinotropic activity for treating T2DM. In this study, the increase of insulin secretion is caused by the active compounds of U. lobata extract to maintain GLP-1 bioavailability trough inhibition of DPP-4 activity.

GLP-1 stimulates proinsulin biosynthesis and transcription of proinsulin gene. GLP-1 contributes to provide insulin deposition which loses from islet β-cells trough biosynthesis process. GLP-1 is different with oral anti-diabetic sulphonylurea in stimulating of insulin formation because the sulphfonylurea only stimulates insulin, not the biosynthesis of insulin. GLP-1 is incretin hormone which is potential to increase islet β-cells proliferation, and anti-apoptosis furthermore it is able to increase insulin secretion.

Hyperinsulinemia occurs in prediabetic condition or insulin resistance and then the secretion declines due to β-cell exhaustion or overwork. The biological effect of insulin is divided into two major groups, they are metabolic and mitogenic effect. The metabolic effect is glucose transport, lipid metabolism, protein, and glycogen synthesis whereas the mitogenic effect is cell growth and mitogenesis.

This study showed also that the administrations of U. lobata extract give a good description of islet β-cell. It is shown by the shape, size, and number of β-cell in better condition compared to diabetic groups. These conditions support the function of β-cell to produce insulin in order to maintain blood glucose level. However, the diabetic group shows β-cells destruction which is signaled by a decreasing number of islet β-cell and structure damage therefore it affect their performance to release insulin.

4.3. The effect of U. lobata leaf extract on blood glucose level of diabetic rats

Administration of aqueous extract U. lobata significantly decreases blood glucose level of diabetic rats. It is controlled by active compounds of U. lobata which has DPP-4 inhibitory activity like stigmasterol, mangiferin, and β-sitosterol furthermore GLP-1.
bioavailability can be retained for insulin biosynthesis when the blood glucose level increase after stimulating of oral nutrition. GLP-1 acts outside of metabolism purpose, that is inhibiting of gastric juices secretion, inhibiting of the GIT motility and inhibiting of the rate of gastric emptying. It is a benefit to prevent the increase of blood glucose level at postprandial.

Insulin works to maintain blood glucose level after induction of glucose by a metabolic pathway. This hormone transports glucose from blood to the tissue and then synthesizes it into glycogen in muscle in order to reduce blood glucose level. In diabetic groups, the insulin secretion is disrupted therefore they lose their control to maintain blood glucose level. This is showed by blood glucose level in the diabetic group which is higher than normal and also treatment groups.

4.4. Histopathology of islet β-cell supplemented U. lobata extract

Oral administration of aqueous extract U. lobata is able to prevent islet β-cells death of diabetic group. The effect of active compounds in U. lobata such as increasing β-cells proliferations and inhibiting β-cells apoptosis through GLP-1 activation. Bioavailability of GLP-1 could be retained due to DPP-4 inhibitor substances in the extract such as stigmasterol, mangiferin, and β-sitosterol. It affects the integrity of β-cells indirectly in the test group, it is shown in the shape of cells, size, and number which is close to normal groups. Some tests show swelling cells, it indicates cells damage at the first step even though the shape and number of cells are normal.

The active compounds of U. lobata leaves extract such as gossypetin, chrysoeriol, and mangiferin could protect cell damage leads to oxidative damage in tissue or organ and an increase of diabetic complication rate.

4.5. The effect of U. lobata leaf extract on body weight, food consumption, glucose level and insulin of diabetic rats

Aqueous extract of U. lobata reduces food consumption therefore it affects body weight gain of diabetic rats. It is related to active compound such as stigmasterol, mangiferin, and β-sitosterol in U. lobata that maintains bioavailability GLP-1 and their interaction with GLP-1 receptor in the brain could reduce the rate of gastric emptying and also induce satiety. The oral administration of U. lobata leaf extract decreases fasting blood glucose level and increase insulin level. GLP-1 activity in the pancreas has functions in stimulating the secretion of insulin by CAMP activation, increasing β cell masses by MAPK pathway and inhibiting the secretion of glucagon. In liver, it increases utilization of glucose and decrease fatty acid metabolism. In T2DM, all of them contribute to maintain blood glucose level.

Acknowledgements

This study was funded by Doctorate Research Grant of Directorate General of Higher Education Indonesia (No. 053/B/IULI/LPPM/2014).

References

1. Drucker DJ. Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes. Diabetes Care. 2007;30(6):1335–1343. http://dx.doi.org/10.2337/dc07-0228.
2. Chia CW, Egan JM. Incretin-based therapies in type 2 diabetes mellitus. J Clin Endocrinol Metab. 2008;93(10):3703–3716. http://dx.doi.org/10.1210/jc.2007-2109.
3. Drucker DJ. Biological actions and therapeutic potential of the glucagon like peptides. Gastroenterology. 2002;122(2):531–544. PMID: 11832466.
4. Brunton L, Chabner B, Knollman B. Goodman & Gilman’s The Pharmacological Basis of Therapeutics. New York: McGraw-Hill; 2006.
5. Holst JJ, Orskov C. The incretin approach for diabetes treatment. Modulation of islet hormone release by GLP-1 agonism. Diabetes. 2004;53(3):S197–S204.
6. Salehi M, Aulinger AB, D’ Alessio AD. Targeting -cell mass in type 2 diabetes: promise and limitation of new drugs based on incretins. Endocr Rev. 2008;29(3):367–379. http://dx.doi.org/10.1210/er.2007-0031.
7. Bailey C. Incretin-based therapies. Endocrin Abstr. 2008;15:541.
8. Onoaga IE, Negbenebor EO, Ogbeve BO, et al. A study of the anti-diabetic effects of Urena lobata and Sphenolobus stenomucra in streptozotocin-induced diabetic rats. Eur J Sci Res. 2010;43(6):6–14.
9. Awika JM, Rooney LW. Sorghum phytochemicals and their potential Impact on human health. Phytochemistry. 2004;65(9):1199–1221. http://dx.doi.org/10.1016/j.phytochem.2004.04.001.
10. Shirvankar A, Rajendran K, Barik R. Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol. 2006;107(2):285–290.
11. Rhodes CJ, White MF. Molecular insight into insulin action and secretion. Eur J Clin Invest. 2002;32(5):3–13. PMID: 12028370.
12. Rosenstock J, Zinman B. Dipeptidyl peptidase-4 inhibitors and the management of type 2 diabetes mellitus. Curr Opin Endocrinol Diabetes Obes. 2007;14(2):98–107.
13. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Anti-diabetic potential of Urena lobata leaf extract decreases fasting blood glucose level and in-streptozotocin-induced diabetic rats. J Ethnopharmacol. 2007;111(3):128–134. http://dx.doi.org/10.1016/j.jep.2006.05.018.
14. Brubacker PL, Drucker DJ. Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut and central nervous system. Endocrinology. 2004;145(6):2653–2659.
15. Aronoff SL, Berkowitz K, Shreiner B, et al. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectr. 2004;17(3):183–190. http://dx.doi.org/10.2337/diaspect.17.3.183.
16. Sosa A, Rosquete C. Flavonoid from Urena sinuata L. Av. Quim. 2010;5(2):95–98.
17. Ross MM, Sterk S, Verhagens H, Stalenhoef AF, De Jong N. Phytosterol consumption and the anabolic steroid boldenone in humans: a hypothesis piloted. Food Addit Contam. 2007;24(7):679–684.
18. Rudsikowska I, Aubuwess SS, Nicolle C, Jones PJ. Cholesterol-lowering efficacy of plant sterols in low-fat yogurt consumed as a snack or with a meal. J Am Coll Nutr. 2008;27(5):586–595.
19. Mentlein R, Gallwitz B. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem. 1993;214(3):829–835. http://dx.doi.org/10.1111/j.1432-1033.1993.tb17985.x.
20. Kumar Robbins. Pathologic Basic of Diseases. 6th ed. Philadelphia: WB Saunders Company; 2002:pp.12–21.
21. Miura T, Ichiki H, Iwamoto N, et al. Anti-diabetic activity of a xanthone compound, mangiferin. Phytomedicine. 2010;18(2):85–87.
22. Stolova I, Gargova S, Stoyanova A, Ho L. Antimicrobial and antioxidant activity of the polyphenol mangiferin. Herba Pol. 2005;51(1/2):37–44. ISSN 0038–0599.
23. Halliwel B, Gutteridge JM. Free Radic in Biology and Medicine. 3rd ed. Oxford: Oxford Science Publication; 1999.
24. Mattiwaska I, Sikorska M. Flavonoid compounds in the flowers of Abutilon indicum (L.) sweet (Malvaceae). Acta Pol Pharm. 2002;59(3):227–229.
25. Mazander UK, Gupta M, Malikandian L, Bhattacharya S. Antibacterial activity of Urena lobata root. Fitoterapia. 2001;72(8):927–929.
26. Panda S, Jafri M, Kar A, Maheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia. 2009;80(2):123–126.
27. De las Heras B, Slowing K, Benedi J, et al. Anti-inflammatory and antioxidant activity of plants used in traditional medicine in Ecuador. J Ethnopharmacol. 1998;61(2):161–166.
28. Omokhua AA, Onoagbe IO. Preliminary proximate and phytochemical analyses of some medicinal plants used to treat diabetes mellitus in Nigeria. Inven Impact Ethnopharmacol. 2010;1:68–70.

Conflict of interest statement

We declare that we have no conflict of interest.
29. Sellamuthu PS, Arulselvan P, Kamalraj S, Fakurazi S, Kandasamy M. Protective nature of Mangifera on oxidative stress and antioxidant status in tissues of streptozotocin-induced diabetic rats. ISRN Pharmacol. 2013:1–10. http://dx.doi.org/10.1155/2013/750109.

30. Saednia S, Manayi A, Gohari AR, Abdollahi M. The story of beta sitosterol—a review. Eur J Med Plants. 2014;4(5):590–609.

31. Chang CL, Lin Y, Bartolome AP, Chen YC, Chiu SC, Yang WC. Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. Evid Based Complement Altern Med. 2013:1–33. http://dx.doi.org/10.1155/2013/378657.

32. Islam MH, Rahman KMH, Rahman S, Rahmatullah M. Preliminary anti-hyperglycemic, antinociceptive activity, phytochemical analysis and toxicity studies on leaves of Urena lobata L. J Chem Pharm Res. 2015;7(4):559–563.

33. Nurfauziah C, Mulyani S. Anti-bacterial Potency of Urena lobata Leaf Extract on B. subtilis and E.Coli Also the Profile of Thin Layer Chromatography. Final paper. Faculty of Pharmacy Gajah Mada University Yogyakarta. 1999.