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Original Article

Incretin effect of *Urena lobata* leaves extract on structure and function of rats islet β-cells

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ABSTRACT

This study aims to determine the incretin effects of *Urena lobata* leaves extract on the structure and function of rats islet β-cells. This study utilizes male Sprague-Dawley rats divided into 2 control group and 3 test group ($n = 5$). Diabetic rats were induced with High Fructose Diet (HFD) and single dose intraperitoneal streptozotocin 25 mg/kg bw. Aqueous leaves extract of *U. lobata* was prepared by decoction methods and administrated orally with doses of 250, 500, and 1000 mg/kg bw for 4 weeks then incretin effect was evaluated by measuring serum GLP-1, insulin, and blood glucose levels. Histology of islet β-cells was evaluated using photomicroscopy by analyzing size, shape, and number. Data were analyzed using ANOVA test followed by LSD test and $p \leq 0.05$ is considered significant. Oral administration of aqueous extract *U. lobata* leaves at doses of 250, 500, and 1000 mg/kg body weight were able to prolong GLP-1 bioavailability by 3-fold, 5-fold, and 7-fold respectively when compared to the diabetic group whereas blood glucose level were 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 group 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.[1] 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.[2,3] In a patient with T2DM, chronic hyperglycemia is caused by a decreasing of GLP-1 bioavailability, therefore the secretion of insulin reduced.[1,2]

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.[3] GLP-1 have a short half-life, approximately 2–5 min due to DPP-4 activity.[1,3] The inhibition of DPP-4 is effective to treat T2DM because GLP-1 bioavailability can be retained moreover it was able to regulate blood glucose level.[3,4]

Therapy T2DM through inhibition of DPP-4 show less side effect[6] although the data of drugs safety in long-term use is still limited.[7] Adverse reaction of Oral Anti-Diabetic (OAD) such as body weight gain and hypoglycemia are seldom in using of incretin-like drug.[4] The less side effect of drugs is affected by GLP-1 activity that could suppress appetite and it does not have insulin secretory effect.[3,5] However, 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 trough controlling of incretin bioavailability.[7]

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Herbs are becoming popular medications of choices in the managements of diseases due to their perceived less side effect and holistic care property. One of the traditional plants which have anti-diabetes effect is Caesar weed (*Urena lobata*). The root and leaf extract of *U. lobata* have been used empirically by Nigeria people to treat diabetes mellitus.[8,28] Preclinical study of *U. lobata* root extract demonstrates the anti-hyperglycemic effect on streptozotocin-induced rat.[8,32] Bioactivity of *U. lobata* is regulated by its active substances such as a sterol, alkaloid, and flavonoid.[9,32] In Indonesia, *U. lobata* is known by Pulutan and this plant showed the anti-bacterial effect based on preliminary study.[25,33] Some study showed the anti-diabetic effect of *U. lobata* extract[8,9] however the mechanism of *U. lobata* on incretin activity has not been investigated. Therefore, this study aims to examine the anti-diabetes effect of *U. lobata* leaf extract trough incretin activity focus on structure and function of rats islet β-cells.

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.[10] 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 CEA804). 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 Biotinylated 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 λ = 450 nm.

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 $p \leq 0.05$ 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 ($p \leq 0.05$) (Table 1). The oral administration of *U. lobata* leaf extract decrease fasting blood glucose level compared to diabetic group ($p \leq 0.05$) whereas insulin level was increased ($p \leq 0.05$).

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 ($p \leq 0.05$) 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 ($p \leq 0.05$). 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) 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.

Table 1
Body weight, food consumption, blood glucose, and insulin level of diabetic rats.

	Normal group	Diabetic group	AEU-250	AEU-500	AEU-1000
Body weight (g)	298.0 ± 13[b]	239.5 ± 19[a]	223.0 ± 11[a]	222.0 ± 16[a]	229.0 ± 12[a]
Food consumption (g)	25.0 ± 0	24.1 ± 3	15.4 ± 2[b]	14.8 ± 2[b]	20.2 ± 3[b]
Food consumption (%)	100.0 ± 0	96.0 ± 11	61.6 ± 7[b]	59.0 ± 6[b]	80.0 ± 8[b]
Fasting blood glucose (mg/dL)	101.0 ± 8[b]	129.0 ± 6[a]	96.0 ± 10[b]	87.0 ± 5[b]	92.0 ± 9[b]
Fasting serum insulin (pg/ml)	1242.9 ± 47[b]	226.9 ± 30[a]	350.8 ± 30[b]	536.2 ± 39[b]	699.2 ± 24[b]

Result is expressed as means ± SD, (n = 5).
[a] Significant different compared to normal group ($p \leq 0.05$, LSD test).
[b] Significant different compared to diabetic group ($p \leq 0.05$, LSD test).

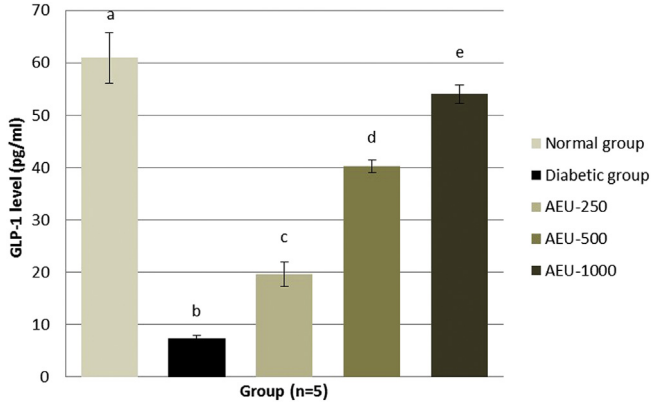

Fig. 1. GLP-1 level supplemented *U. lobata* extract. Means with different letters are significantly different ($p \leq 0.05$, Dunnet C test).

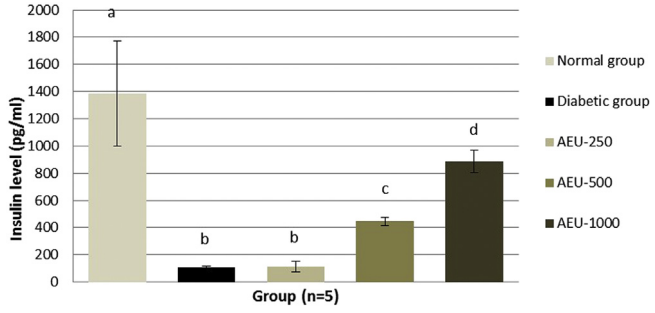

Fig. 2. Insulin level supplemented *U. lobata* extract. Means with different letters are significantly different ($p \leq 0.05$, LSD test).

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 ($p \leq 0.05$). 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% compare to the diabetic group ($p \leq 0.05$) 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.

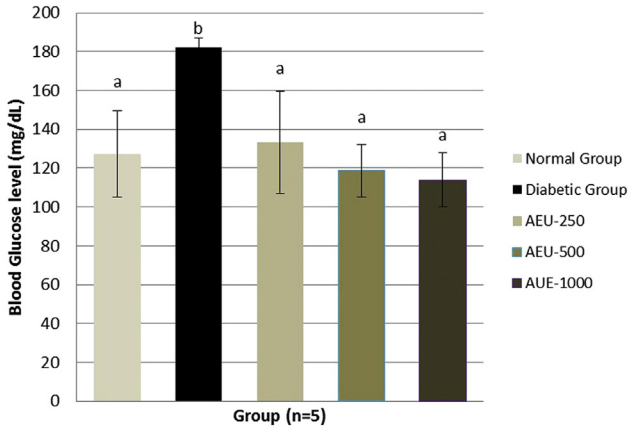

Fig. 3. Blood glucose level supplemented *U. lobata* extract. Means with different letters are significantly different ($p \leq 0.05$, LSD test).

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.[12,19] It produces an inactive form of GLP-1, they are GLP-1 (9–36) amide and GLP-1 (9–37) isopeptides.[6,7] 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]

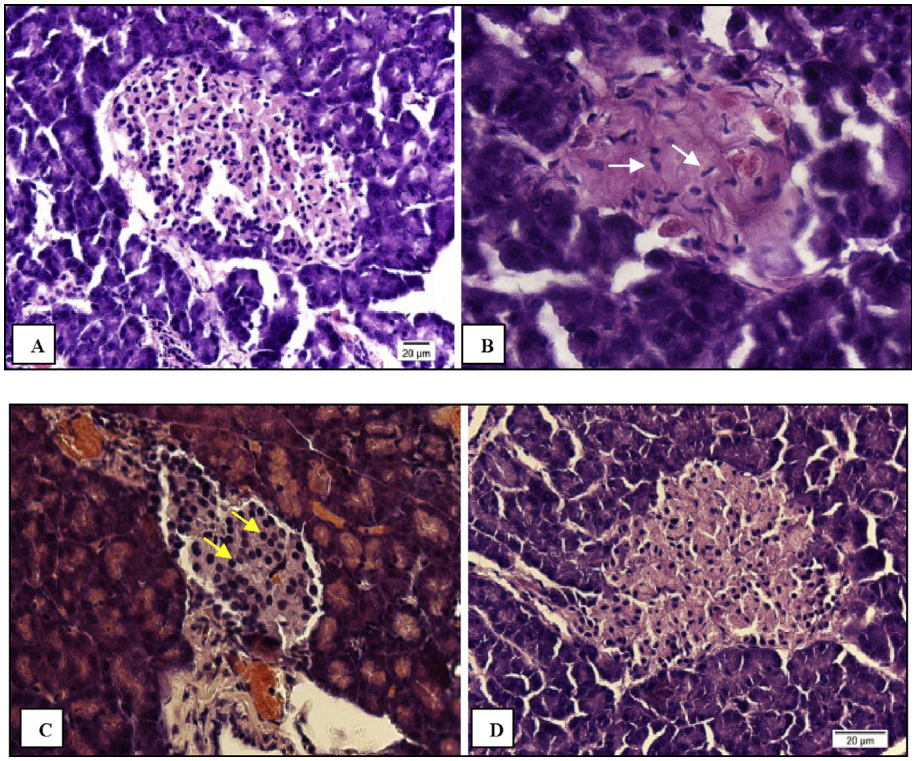

Fig. 4. Islet β-cells were stained by Hematoxylin–Eosin and observed under photomicroscope with magnitude 400×. (A). Normal group, (B). Diabetic group, (C). AEU 500 mg/kb bw, (D). AEU-1000 mg/kg bw. Magnification 400×. The white arrow shows unnucleated cells and longer shape whereas yellow arrow shows the swelling cells.

GLP-1 is a superfamily peptide of glucagon which has a similarity degree approximately 48%.[14] 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.[15] Proglucagon production is started from transcription of pre-proglucagon gene and then is continued by translation process.[3,14] The regulation of GLP-1 released from L cells intestine is a complex mechanism that involves combinations of nutrition, hormone, and neural stimuli.[14] The GLP-1 receptor is classified in *G protein-coupled* receptor that is found in liver, muscle, and pancreas cells.[2,3] This receptor has a specific character by activation of adenyl cyclase and results cAMP.[15] After GLP-1 binding with the receptor, it will activate cAMP and Mitogen-Activated Protein Kinase (MAPK).[3,7]

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.[3,5] 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.[14,15] All of them contribute to regulate blood glucose level in T2DM.[1,2]

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.[5,6] 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.[13]

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.[5] GLP-1 is different with oral anti-diabetic sulphonylurea in stimulating of insulin formation because the sulphonylurea only stimulates insulin, not the biosynthesis of insulin.[4,5] 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.[2,6]

Hyperinsulinemia occurs in prediabetic condition or insulin resistance and then the secretion decline due to β-cell exhaustion or overwork.[2] The biological effect of insulin is divided into two major groups, they are metabolic and mitogenic effect.[11] The metabolic effect is glucose transport, lipid metabolism, protein, and glycogen synthesis whereas the mitogenic effect is the cell growth and mitogenesis.[11]

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.[14,15] 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.[13,18,30,32] 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.[2,3] It is a benefit to prevent the increase of blood glucose level at postprandial.[5,6]

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 synthesize it into glycogen in muscle in order to reduce blood glucose level.[11,14] In diabetic groups, the insulin secretion is disrupted therefore they lose their's control to maintain blood glucose level.[5,11] 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* that has potency such as increasing β-cells proliferations and inhibiting β-cells apoptosis through GLP-1 activation.[5,9,32] Bioavailability of GLP-1 could be retained due to DPP-4 inhibitor substances in the extract such as stigmasterol, mangiferin, and β-sitosterol.[13,26,31] 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.[20,29]

The active compounds of *U. lobata* leaves extract such as gossypetin, chrysoeriol, and mangiferin could protect cell damage from free radical.[22,24,27,29] They work as an antioxidant by donating an electron to unstable compounds in order to stabilize it.[23] Besides it, mangiferin and gossypetin act also as scavenger free radical moreover it could decrease oxidant level causing oxidative damage.[16,22,27] Hyperglycemia in diabetes increases the production of free radical furthermore it occurs imbalance between oxidant and antioxidant.[21,23] This condition is caused by oxidative stress which leads to oxidative damage in tissue or organ and an increase of diabetic complication risk.[16,23]

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's interaction with GLP-1 receptor in the brain could reduce the rate of gastric emptying and also induce satiety.[13,16–18] 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.[3,5] In liver, it increases utilization of glucose and decrease fatty acid metabolism. In T2DM, all of them contribute to maintain blood glucose level.[1,2]

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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