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Original Article

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

Y. Purnomo [a, *], D.W. Soeatmadji [b], S.B. Sumitro [c], M.A. Widodo [d]

[a] Department of Pharmacology, Faculty of Medicine, Islamic University of Malang, Indonesia
[b] Department of Internal Medicine, School of Medicine, University of Brawijaya, Indonesia
[c] Department of Biology, Faculty of Science, University of Brawijaya, Indonesia
[d] Department of Pharmacology, School of Medicine, University of Brawijaya, Indonesia

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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]

* Corresponding author. Pharmacology Department, Faculty of Medicine, Islamic University of Malang, MT. Haryono 193, Malang 65144, Indonesia.
E-mail address: y_purnomo92@yahoo.com (Y. Purnomo).
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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.