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Anti-diabetic effects of *Protaetia brevitarsis* in pancreatic islets and a murine diabetic model

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Abstract. – OBJECTIVE: In this study, the antidiabetic efficacy of *Protaetia brevitarsis* in alloxan-treated pancreatic islets and db/db mice was investigated. *P. brevitarsis* was tested for alloxan-mediated cytotoxicity and nitric oxide production in mice pancreatic islets.

MATERIALS AND METHODS: The anti-diabetic effect of *P. brevitarsis* was also evaluated in db/db mice after 4 weeks of administration. Biochemical analysis, oral glucose tolerance test (OGTT), and pancreatic histological analysis were performed.

RESULTS: *P. brevitarsis* displayed hypoglycemic activity in alloxan-treated mice pancreatic islets. Our results showed that *P. brevitarsis* protects pancreatic islets from cytotoxicity. Moreover, daily oral supplementation with *P. brevitarsis* for 4 weeks reduced plasma glucose levels without affecting body weight and food intake, elevated glucose tolerance in OGTT, improved blood lipid parameters, inhibited fat accumulation, and restored islet structure of *db/db* mice.

CONCLUSIONS: The present study provided evidence for the anti-diabetic effect of *P. brevitarsis* in alloxan-treated pancreatic islets and db/db mice. These results suggest that *P. brevitarsis* may be used as an adjunctive anti-diabetic agent or as a functional food.

Key Words:
Protaetia brevitarsis, Diabetes, Pancreatic islets, Edible insect.

Introduction

Type 2 diabetes mellitus is currently one of the most prevalent chronic diseases, and its prevalence is rapidly increasing[1]. Without appropriate treatment, diabetes ultimately leads to long-term complications that reduce the quality of life and increase treatment cost; it may also increase mortality[2-5]. The early management of diabetes involves dietary and lifestyle interventions; however, in most cases, early treatment is not provided[6]. Recommended treatment goals by current medical treatment standards are not achieved in about 50% of diabetes patients[7,8]. Therefore, the development of alternative therapies is of prime importance, and the discovery of novel natural components that can manage blood glucose levels in patients without exerting significant adverse effects are gaining considerable attention.

Alloxan is a commonly used diabetogenic agent[9]. It has been used for a long time in diabetes research and has been reported to be specifically toxic to pancreatic beta cells. Alloxan is thought to produce oxygen free radicals and nitric oxide (NO) during its metabolism[10-12].

Insects have been considered as alternative food and drug resources. Bioactive compounds, such as norepinephrine derivatives, lactams, and sesquiterpenes, have been found in the edible insect, *Aspongopus chinensis* Dallas; these compounds have been reported to be effective in treating pain, indigestion, and kidney diseases[14]. *Protaetia brevitarsis*, belonging to the Cetoniinae family, is a white-spotted flower beetle, and native to Eastern Asia and Europe[14]. It has been used as a traditional medicine and functional food to treat liver cirrhosis, inflammatory diseases, breast cancer, hepatic cancer, and hepatitis[15]. Its

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antioxidant effects have been reported at different tumor growth stages[14], and antimicrobial peptides have been purified from *P. brevitarsis* larvae[16]. The compositional analysis of *P. brevitarsis* larvae revealed the presence of fatty acids, volatile constituents, and nutrients[17]. Its fatty acids have been shown to induce apoptosis in tumor cells[15]. In addition, most of the previous studies on *P. brevitarsis* larvae focused primarily on the biochemistry and physiology of the insect itself[18,19]. However, the anti-diabetic effect of *P. brevitarsis* has not been reported yet.

In this study, the antidiabetic effect of *P. brevitarsis* in alloxan-treated pancreatic islets and *db/db* mice was investigated. Furthermore, we have identified the fundamental mechanism of the anti-diabetic effect of *P. brevitarsis,* with emphasis on its NO production inhibition and the protection of the pancreatic islets.

Materials and Methods

Preparation and Gas Chromatography (GC) of P. Brevitarsis

P. brevitarsis larvae were supplied by Congmaeul. Ltd. (Imsil, Jeonbuk, Korea). Briefly, the larvae were collected and washed with distilled water. After freeze drying at −20°C, the sample was ground using a laboratory pulverizer and passed through a 30-mesh sieve. The powder of *P. brevitarsis* larvae was kept in airtight containers at −70°C prior to the experiments.

The compositional analysis of *P. brevitarsis* powder was performed by GC (GC 7890B, Agilent technologies, INC., Santa Clara, CA, USA) equipped with a flame ionization detector. SP-2560 (100 m × 0.25 mm × 0.2 m) was used for column separation; the flow rate was 0.75 mL/min and the split ratio was 200:1. Temperatures of the inlet and the detector were 225°C and 285°C, respectively, and the temperature of the column was elevated to 240°C for 15 min at a rate of 3°C/min and then maintained at 100°C for 4 min.

Animals and Experimental Design

7week-old male C57BL/KsJ*db/db* (*db/db*) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and fed ordinary chow. After a week of acclimatization, mice that developed diabetes were treated with *P. brevitarsis* (100, 300, or 1,000 mg/kg) by daily oral gavage for 4 weeks. Food intake and body weight were recorded every week. The blood glucose level was measured from the tail vein at 0, 30, 60, 90, and 120 min following glucose administration. The mice were anesthetized by CO_2, sacrificed by decapitation, and blood samples were collected. The protocols used for the animal studies were approved by the Committee on Care and Use of Laboratory Animals of the Wonkwang University (Iksan, Jeollabuk-do, Korea; approval no. WKU17-34).

Islet Isolation

Pancreatic islets were isolated from 10-week-old mice by standard collagenase digestion as previously described[20]. After isolation, islets were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 1% antibiotics, and 2 mM L-glutamine at 37°C in a 5% CO_2 incubator.

Cell Viability

Cell viability assays were performed using a WST-1 assay kit (ITSBio, INC., Seoul, Korea) according to the manufacturer's instructions. Briefly, isolated pancreatic islets (1×10^5 cells/well) were seeded into 96-well plates and incubated at 37°C for 4 h to allow for cell stabilization. Next, the cells were treated with *P. brevitarsis* (0, 10, 30, 50, 100, or 300 μg/mL) and alloxan (4 mM), and incubated for 24 h in a 5% CO_2 incubator. After incubation, the visible absorbance at 560 nm of each well was quantified using a microplate reader (Sunrise™; Tecan Group Ltd., Männedorf, Switzerland). Each experiment was performed in triplicate.

NO Measurement

NO is rapidly oxidized to nitrate and nitrite in aqueous solutions[21]. Therefore, NO production was measured based on nitrite level in cell culture supernatant using a colorimetric assay. After treatment with *P. brevitarsis* (0, 10, 30, 50, 100, or 300 μg/mL) and alloxan (4 mM), NO in the cell culture supernatants was measured after the addition of 100 μL of Griess reagent to 100 μL of each sample and incubation at room temperature for 5 min. NO concentration was determined based on the absorbance at 540 nm; sodium nitrite was used as a standard.

Lactate Dehydrogenase (LDH) Activity

LDH activity was measured in an aliquot of the culture medium using the optimized LDH/LD procedure. This reaction was based on the LDH-mediated conversion of lactate to pyruvic acid and NADH. LDH activity was measured using an LDH cytotoxicity kit (Takara Bio Inc., Shiga, Japan; Cat. No. MK401) and the absorbance

was measured (Ex/Em = 490/600 nm) using a microplate reader (Sunrise™, Tecan Group Ltd.).

Biochemical Analysis

Whole blood glucose levels were determined using an Accu-Chek Aviva glucose monitoring system (Roche Diagnostics, Indianapolis, IN, USA), and plasma insulin was measured by a mouse insulin ELISA kit (Alpco, Maryland, MA, USA). The plasma concentrations of triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL)-cholesterol were measured by commercially available kits (Asan Pharmaceutical, Seoul, Korea).

Histological Analysis

Pancreases were isolated, weighed, and fixed in 10% neutral buffered formalin. The tissues were then embedded in paraffin and sectioned (4-7 μm) using a microtome (Thermo Scientific, Waltham, MA, USA). The sectioned tissues were then immunostained. The sections were incubated with anti-insulin antibody (Santa Cruz Biotechnology, Dallas, TX, USA) for 12 h at 4˚C and the peroxidase activity was detected using 3-amino-9-ethyl carbazole substrate. Tissue damage was assessed under an optical microscope (Olympus, Fukuoka, Japan).

Statistical Analysis

All data were expressed as mean±SEM. We performed by one-way analysis of variance and Tukey test using SPSS ver. 10.0 (SPSS Inc., an IBM Company, Chicago, IL, USA). Each value was the mean of at least three separate experiments in each group. p-values < 0.05 were considered statistically significant.

Results

GC Analysis of P. Brevitarsis

The larvae powder was subjected to GC profiling to analyze the constituents present in *P. brevitarsis*. The *P. brevitarsis* used in this study contained a large amount of oleic acid (Figure 1).

Effects of P. Brevitarsis on Alloxan-Mediated Cell Cytotoxicity and NO Production in Mice Pancreatic Islets

To determine the protective effects of *P. brevitarsis* on pancreatic islets, we examined the efficacy of this compound in protecting pancreatic islets from alloxan toxicity. The results showed that alloxan significantly reduces cell viability and increases LDH activity compared with control (Figure 2A and B). Pre-treatment with *P. brevitarsis* increased the viability and decreased the LDH activity of alloxan-treated pancreatic islets in a concentration-dependent manner. As shown in Figure 2C, the incubation of pancreatic islets with alloxan for 24 h resulted in significant nitrite formation by pancreatic islets. The presence of *P. brevitarsis* inhibited alloxan-mediated nitrite formation by pancreatic islets in a concentration-dependent manner.

P. Brevitarsis Prevents Hyperglycemia and Dyslipidemia in db/db Mice

In this study, the oral administration of *P. brevitarsis* for 4 weeks improved glucose and lipid metabolism in *db/db* mice. Food intake and body weight were not significantly influenced by *P. brevitarsis* treatment (Figure 3A and B). The decrease in blood glucose levels by *P. brevitarsis* appeared to be dose-dependent (Figure 3C). *P. brevitarsis* significantly reduced the AUC of glucose in *db/db* mice. We also confirmed that the plasma insulin levels in *P. brevitarsis*-treated mice were significantly lower than those in PBS-administered *db/db* mice (Figure 3D). In our study, *P. brevitarsis* improved glucose utilization by significantly reducing blood glucose levels in *db*/*db* mice (Figure 3E). These results indicated that *P. brevitarsis* significantly suppressed the increase of blood glucose in diabetic model.

In addition, we tested the plasma lipid levels. As shown in Figure 4A, *P. brevitarsis* significantly lowered plasma TG levels compared with PBS treated group. Plasma TC and HDL-cholesterol levels were not significantly different among the groups (Figure 4B and 4C).

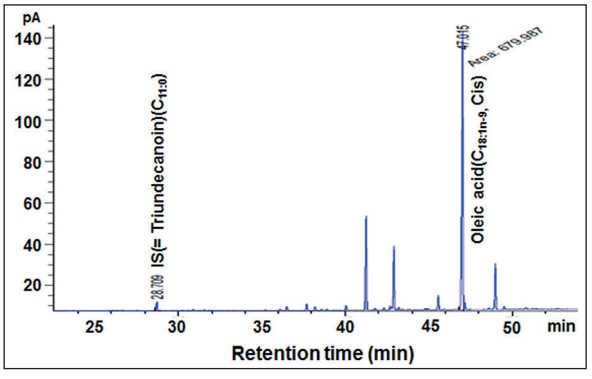

Figure 1. Gas chromatography profile of *Protaetia brevitarsis*. Oleic acid was detected.

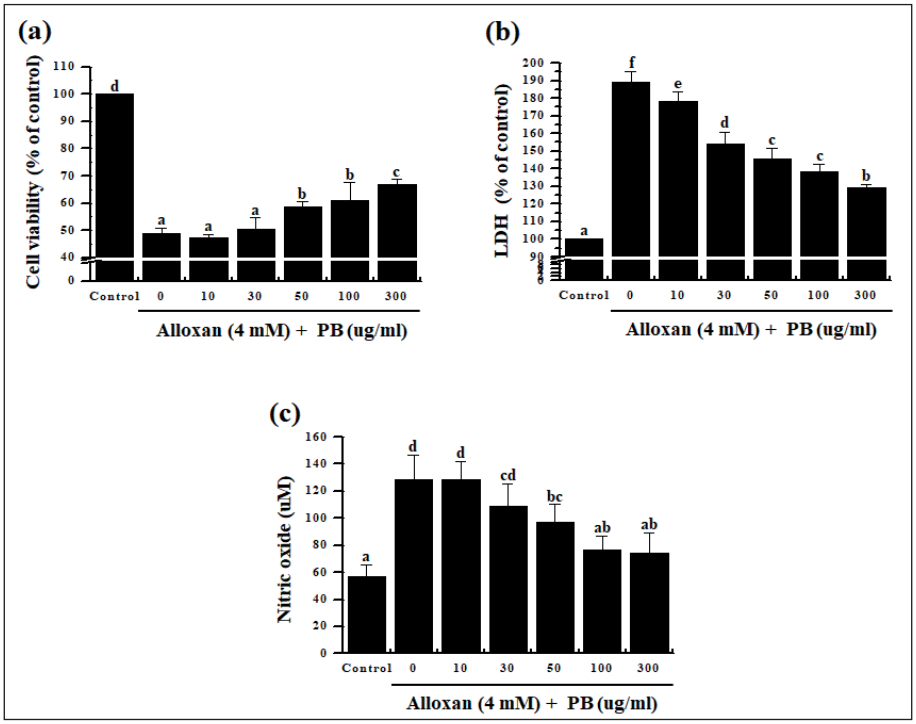

Figure 2. *Protaetia brevitarsis* (PB) prevents alloxan-mediated cytotoxicity and nitric oxide production in mice pancreatic islets. Pancreatic islets were pre-treated with the indicated concentrations of PB, and alloxan was added. **(a)** Following a 24 h incubation, cell viability was determined using a WST-1 assay kit. **(b)** lactate dehydrogenase (LDH) activity was determined using an LDH cytotoxicity kit. **(c)** Nitrite level was measured using a nitric oxide assay kit. Bars labeled with different superscripted numerals indicate $p < 0.05$. Data are presented as the mean ± standard error (n = 3).

P. Brevitarsis Protects Pancreatic β-cells in db/db mice

Immunostaining with an insulin antibody revealed that pancreatic islets of diabetic *db/db* mice showed degeneration and incomplete margins (Figure 5), whereas *P. brevitarsis*-supplemented *db/db* mice showed near-normal islets. Our results show that *P. brevitarsis* prevents mouse pancreatic damage by diabetes.

Discussion

Type 2 diabetes is one of the chronic diseases with rapidly increasing prevalence and, without adequate treatment, can reduce quality of life, increase treatment costs, and lead to long-term complications and death[1-5]. However, approximately 50% of people with diabetes do not meet their recommended treatment goals, according to current medical standards[7,8]. Therefore, it is of utmost importance to find and use natural ingredients that do not have serious side effects. Currently, insects have been considered as an alternative food and pharmaceutical resource.

Protaetia brevitarsis, belonging to the Cetoniinae family, is a white-spotted flower beetle, and native to Eastern Asia and Europe[14]. It has been used as an herbal medicine and functional food for the treatment of liver cirrhosis, inflammatory diseases, breast cancer, liver cancer, hepatitis, and has been reported to show antioxidant effects in various cancers[14,15]. And antimicrobial peptides have been purified from *P. brevitarsis* larvae[16]. A previous compositional analysis of *P. brevitarsis* larvae has indicated the presence of oleic acid (64%), palmitic acid (16%), palmitoleic acid (10%), and linoleic acid (5%), constituting more than 95% of fatty acids[17]. The *P. brevitarsis* used in this study also contained a large amount of oleic acid as in the previous study. Omega Fatty acids (including oleic acid) have been reported to have a variety of biological activities[22-24].

Alloxan is commonly used as a diabetogenic agent. This compound has long been used in diabetes research and is known to be particularly toxic to pancreatic cells. Alloxan induces β-cell dysfunction and destruction by increasing oxygen free radicals and NO[11,25]. Excess NO produced in cells inhibits mitochondrial metabolism and in-

duces protein changes and DNA cleavage, and these functions may ultimately hamper insulin secretion and cause beta cell death[25-27].

We investigated the efficacy of this compound in protecting pancreatic islets from alloxan toxicity to confirm the protective effect of *P. brevitarsis* on pancreatic islets. As a result, it was confirmed that alloxan significantly reduced cell viability and increased LDH activity compared to the control. However, in the *P. brevitarsis* pre-treated group, it was confirmed that the survival rate of alloxan-treated pancreatic islets increased and LDH activity decreased in a concentration-dependent manner. Incubation of pancreatic islets with alloxan for 24 h resulted in significant nitrite formation by pancreatic islets, but we confirmed that the presence of *P. brevitarsis* inhibited alloxan-mediated nitrite formation by pancreatic islets in a concentration-dependent manner.

Natural compounds have been identified through meta-analysis studies to be effective against acute and chronic effects of diabetes on glucose and lipid metabolism[28-30]. In this study, the decrease in blood glucose levels by *P. brevitarsis* appeared to be dose-dependent. *P. brevitarsis* significantly reduced the AUC of glucose in *db/db* mice. Based on this result, it is reasonable to expect *P. brevitarsis* to exhibit acute and chronic anti-diabetic effects. This result implies that *P. brevitarsis* improves glucose tolerance to some extent in hyperglycemic conditions. Insulin plays an important role in maintaining postprandial blood glucose levels within the normal range by regulating glucose metabolism[31]. In general, *db/db* mice exhibit early stages of hyperinsulinemia to compensate for insulin resistance and progressively show insulinopenia according to age; this is a commonly observed phenomenon in patients with late-stage type 2 diabetes mellitus[32]. In our results, we confirmed that the plasma insulin levels in *P. brevitarsis*-treated mice were significantly lower than those in PBS-administered *db/db* mice. The OGTT is an important tool for assessing the effects of materials for preclinical tests[33]. OGTT is useful as an indicator of the relative role of insulin secretion and insulin resistance in the progression of glucose intolerance. In our study, *P. brevitarsis* improved glucose utilization by

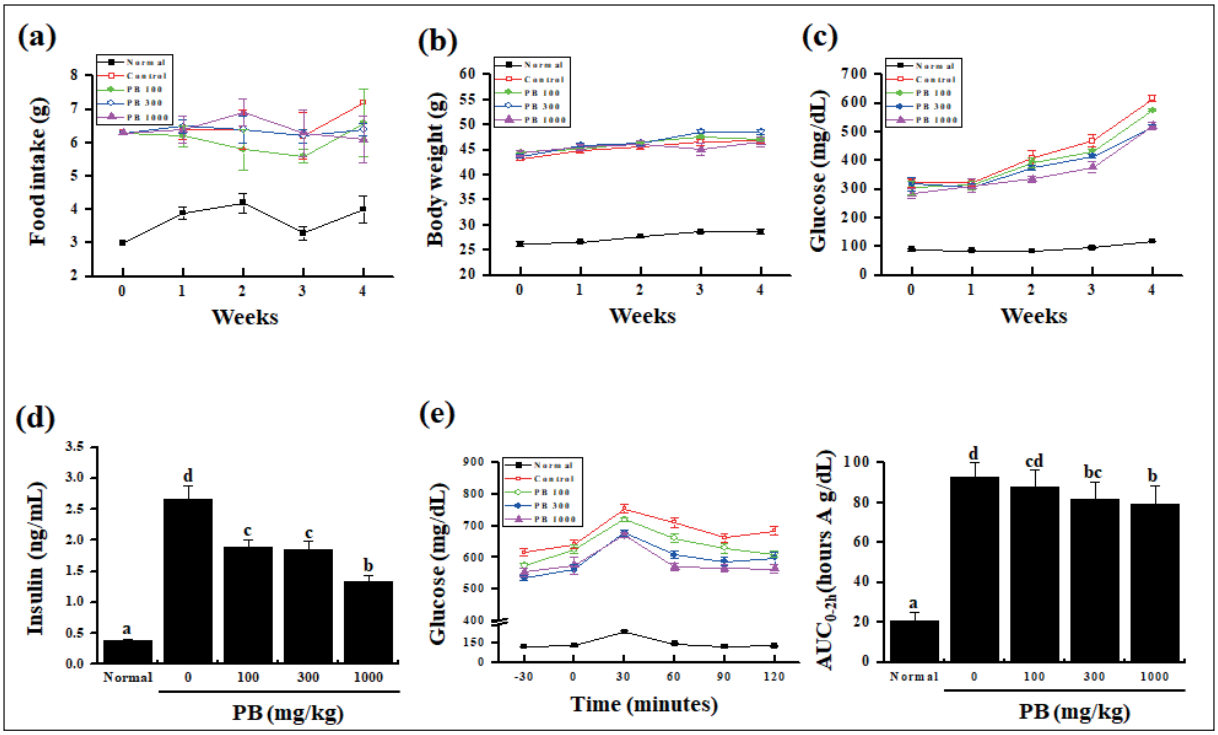

Figure 3. *Protaetia brevitarsis* (PB) improves glycemic control in *db/db* mice. Mice were orally supplemented daily with PB ad doses of 100 mg/kg, 300 mg/kg, or 1,000 mg/kg for 4 weeks. **(a)** Food intake and **(b)** body weight change were recorded at the indicated times. **(c)** Fasting blood glucose levels. **(d)** insulin levels, **(e)** Plasma glucose level during oral glucose tolerance test in overnight-fasted mice. The bar graph represents areas under the curve. Bars labeled with different superscripted numerals indicate $p < 0.05$. Data are presented as the mean ± standard error (n = 7).

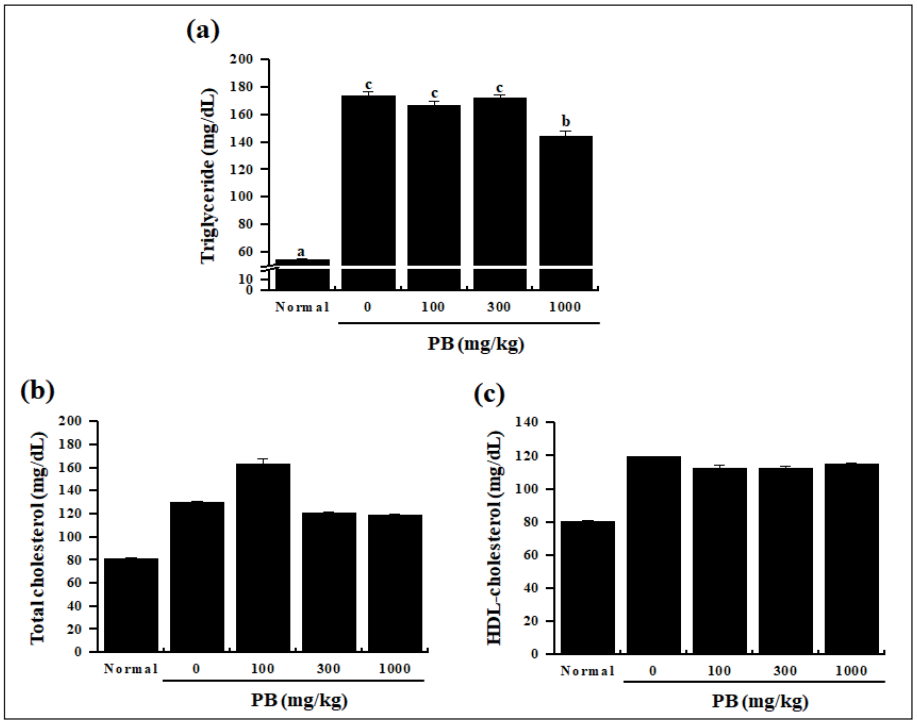

Figure 4. *Protaetia brevitarsis* (PB) improves biochemical parameters in *db/db* mice. Mice were orally supplemented daily with PB ad doses of 100 mg/kg, 300 mg/kg, or 1,000 mg/kg for 4 weeks. At the end of the study, plasma levels of **(a)** triglyceride (TG), **(b)** total cholesterol (TC), and **(c)** high-density lipoprotein (HDL)-cholesterol were determined. Bars labeled with different superscripted numerals indicate $p < 0.05$. Data are presented as the mean ± standard error (n = 7).

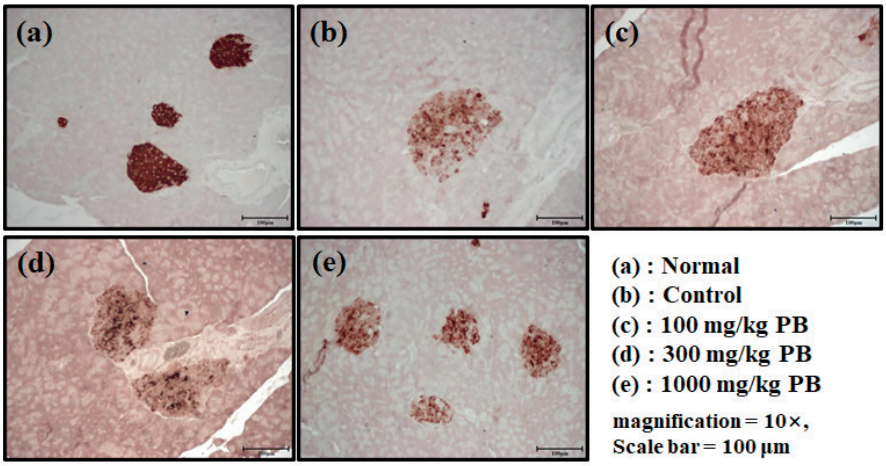

Figure 5. *Protaetia brevitarsis* (PB) prevents pancreatic islet destruction in *db/db* mice. Mice were orally supplemented daily with 100 (PB100), 300 (PB300), or 1,000 mg/kg (PB1000) for 4 weeks. Pancreas were isolated from surviving mice after 4 weeks, and the tissue sections were stained with insulin antibody (magnification, ×10).

significantly reducing blood glucose levels in *db/db* mice. This results indicated that *P. brevitarsis* significantly suppressed the increase of blood glucose in diabetic model. These results show that *P. brevitarsis* improves insulin sensitivity by increasing insulin secretion of pancreatic β-cells to improve glucose tolerance. In addition, the increase in serum lipid levels in diabetic patients is well known[34]. Therefore, we tested the plasma lipid levels. As shown results, *P. brevitarsis* significantly lowered plasma TG levels compared with PBS treated group. Overall, these results suggest

that *P. brevitarsis* exhibits glucose- and lipid-lowering effects.

Taken together, this study provided evidence for the anti-diabetic effect of *P. brevitarsis* in alloxan-treated pancreatic islets and *db/db* mice.

Conclusions

This study provides additional evidence for the anti-diabetic efficacy of *P. brevitarsis* in alloxan-treated pancreatic islets and *db/db* mice. *P. brevitarsis* improved glucose tolerance during OGTT and lipid parameters, and inhibited fat accumulation in the liver. This beneficial effect is at least partially mediated through the inhibition of the restoration of the function of preserved pancreatic cells. These effects decreased blood sugar levels and increased insulin levels. Therefore, the results of this study suggest that *P. brevitarsis* may be used as an adjunct to restore blood glucose level in diabetes patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability

The data used to support the findings of this study are included within the article.

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