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Antidiabetic Activity of *Bengkuang* (*Pachyrhizus erosus*) Extracts in Diabetes Mellitus-induced Rats

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ABSTRACT

Bengkuang (*Pachyrhizus erosus*) is in the pea family (Fabaceae) and is a native Mexican vine that produces an edible tuber called jicama or Mexican turnip in English. This study evaluated the antidiabetic activity of *bengkuang* extracts administered via oral gavage into rats at 4 days post-induction of streptozotocin-nicotinamide-induced diabetes mellitus. At 14 days post injection (daily) of extracts at 28 and 56 mg/200 g body weight (BW), blood glucose levels were significantly reduced ($p < 0.05$) from 277 ± 4 milligrams per deciliter (mg/dl) for the Diabetes Control Group to 182 ± 3 and 99 ± 55 mg/dl, respectively. The latter glucose level was comparable to that in the Antidiabetic Control Group rats (111 ± 63 mg/dl) injected with glibenclamide at 0.09 mg/200 g BW. The diabetes-induced rats also showed signs of cell recovery from diabetic-associated pancreatic tissue damage, supporting the efficacy of *bengkuang* treatment. According to phytochemical tests, the *bengkuang* extract contained various metabolites, mainly alkaloids and flavonoids, that may have been responsible for its antidiabetic activity. The results justify further studies on the underlying mechanisms of *bengkuang* antidiabetic activity.

Keywords: Antidiabetic, diabetes mellitus, *Pachyrhizus erosus*

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INTRODUCTION

According to Soelistijo (2015), diabetes mellitus is a group of metabolic disorders brought on by deficiencies in insulin

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secretion and/or function, which results in hyperglycemia. According to Dal Canto et al. (2019) and Majnarić et al. (2020), it is linked to coronary heart, cerebrovascular, and peripheral vascular disease, all of which can cause death or a lower quality of life. Chemical medication side effects in diabetic patients can include tremors, hypoglycemia, nausea, vomiting, and constipation (Putra et al., 2017). Additionally, it necessitates expensive and lengthy treatment. Due to its accessibility, safety, and tolerable side effects, traditional medicine is becoming increasingly popular (Khamees et al., 2020).

Bengkuang (*Pachyrhizus erosus*) is in the pea family (Fabaceae) and is a native Mexican vine that produces an edible tuber called jicama or Mexican turnip in English. Spanish introduced this plant to the Philippines in the 17th century, spreading to the rest of Asia. Nowadays, in Indonesia, *bengkuang* is well-known as a food and source of raw materials for beauty products (Li & Li, 2020). A recent preliminary study showed that administering a *bengkuang* extract dosage of 250 g (150 ml)/day for 7 consecutive days could lower blood glucose levels in diabetic patients (Safitri & Nurhayati, 2018). This initial finding served as the basis for this research. This study aims to evaluate the antidiabetic activity of *bengkuang* extracts in diabetes mellitus-inducible rats to confirm their efficacy as a possible justification for further research on the underlying mechanisms. It could lead to additional practical treatment for diabetes mellitus treatment in humans.

MATERIALS AND METHODS

Ethics Approval

This study obtained animal ethics approval from Dr. Moewardi General Hospital, Surakarta, Indonesia and has been declared to meet the ethical requirements for conducting research with certification number 943/VII/HREC/2019.

Animal Model and Chemicals

This study used a male, 2-month-old Wistar white rats ranging from 150–200 g. The animals were prepared from the laboratory of The Centre for Food and Nutrition Studies, University of Gadjah Mada, Yogyakarta, Indonesia. Rats were kept in small cages with good air circulation and lighting. Cages were differentiated for each experimental group, each containing 5 animals. Each cage had a drinking water container that the rats could easily access. Food was also supplied into the cage, which could be easily accessed. The cage bedding was cleaned and replaced daily so that the living environment of the rats was not mixed with their urine and feces.

Preparation and administered dosage determination of 0.5% sodium carboxymethyl cellulose (Na-CMC, Merck, Germany) was performed following Salma et al.'s (2013) as well as Saputri and Zahara's (2016) research. Streptozotocin-nicotinamide (STZ-NA, Merck, Germany) and glibenclamide (GBC, Merck, Germany) solutions were made according to the instructions provided by Arifin et al. (2011) and Ghasemi et al. (2014).

Preparation of *Bengkuang* Extract and Phytochemical Test

Bengkuang tubers were obtained from Teluk Keramat, Kubu Raya District's local farmer gardens in West Kalimantan, Indonesia. A total of 6,737.82 g of fresh *bengkuang* tubers were peeled, chopped, and blended, followed by macerating with 96% ethanol (Merck, Germany), as described previously (Valentina, 2013). The maceration process was carried out three times within 24 hr. The filtrate was collected at 235.35 g after being condensed in a rotary evaporator at 40°C. The quantitative phytochemical analysis was conducted at the Laboratory of Chemistry, The Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Pontianak, Indonesia.

Antidiabetic Activity Test of *Bengkuang* Extract

The number of test animals was determined based on the Fereder formula, namely (t-1) (n-1) ≥15, where t is the number of treatments while n is the number of repetitions in each treatment. A total of 25 experimental rats were randomly divided into 5 groups (i.e., 5 in each): (1) A: Normal Group (without any treatment), (2) B: Diabetes Control (administered with 0.5% Na-CMC (Merck, Germany) with a dosage of 2 ml/200 g BW), (3) C: Antidiabetic Control (GBC (Merck, Germany) administered at 0.09 mg/200 g BW) (10), (4) D: Treatment Group I (administrated with *bengkuang* extract at 28 mg/200 g BW as suggested by Park & Han [2015]), and (5) E: Treatment Group II administrated with *bengkuang* extract at 56 mg/200 g BW.

Blood was withdrawn from the rats via sinus orbitalis. The blood was measured using a glucose oxidase-peroxidase aminoantypirin diagnostic system (GOD-PAP DiaSys). Blood glucose levels of all groups were measured at the start of the experiment (Day 0) following the method of GOD-PAP (Subiyono et al., 2016). On the same day, diabetes mellitus was induced in Groups B, C, D, and E by intraperitoneal injection of STZ (Merck, Germany) (45 mg/kg BW) and NA (Merck, Germany) (110 mg/kg BW). Three days later (Day 3), blood glucose levels were measured to confirm success in diabetes mellitus induction. Subsequently, the animals were injected daily with either GBC (Merck, Germany) or our plant extracts for 14 days, starting from Day 4 and ending on Day 17. On Day 18, their blood was again withdrawn to measure glucose levels.

Histological Examination

All rats were euthanized by cervical dislocation before the organs were collected. On Day 18, the pancreatic organs of all rats were collected and then subject to paraffin methods followed by hematoxylin (Merck, Germany) and eosin (Merck, Germany) (H&E) staining according to the procedures at the Laboratory of Pathology, the Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia. All organs were cut approximately 1 cm × 1 cm in size, followed by histological preparations, including washing, dehydration, clearing, infiltration, embedding, trimming, sectioning, affixing, and staining. The

H&E staining was used to differentiate nuclei from their surroundings (Suvarna et al., 2019). Observations were made under a microscope with 40× magnification to observe the normal cell, necrotic cell, and degenerated Langerhans of insular cells in the Normal Group, Diabetic Control, Antidiabetic Control, and Treated Group (Extract I and II).

Statistical Analysis

The data on blood glucose levels were analyzed using the program Statistical Product and Service Solutions (SPSS, version 24) with one-way analysis of variance (ANOVA) and continued with Tukey's test at a 5% confidence. Histopathological images of the pancreatic tissue were analyzed descriptively.

RESULTS

Phytochemical Test of *Bengkuang* Extract

The *bengkuang* extract tested strongly positive for alkaloids (Dragendroff's reagent, Merck, Germany) and weakly positive for flavonoids (10% sodium hydroxide [NaOH], Merck, Germany), saponins, and steroids (Chloroform [Merck, Germany] and sulfuric acid [H_2SO_4, Merck, Germany]), but negative for alkaloids (Mayer's reagent, Merck, Germany), alkaloids (Wagner's reagent, Ottokemie, India), flavonoids (Magnesium powder [Mg, Laboratorium Discounter, Netherland] and hydrochloric acid [HCl, Merck, Germany]), flavonoids (2N H_2SO_4, Merck, Germany), terpenoids (Salkowski reagent, which was a mixture of 0.5 M of iron (III) chloride [$FeCl_3$, Eisen-Golden Laboratories, Netherland] and 35% perchloric acid solution [$HClO_4$, Merck, Germany]), tannins ($FeCl_3$, Kuhlmann, France), and phenolics (Folin Ciocalteu's phenol reagent, Merck, Germany) (Table 1).

Table 1
Phytochemical test results of bengkuang extract

Parameters	Levels
Alkaloids (Mayer's reagent)	-
Alkaloids (Wagner' reagent)	-
Alkaloids (Dragendroff's reagent)	+++
Flavonoids (Mg + HCl)	-
Flavonoids (2 N H_2SO_4)	-
Flavonoids (10% NaOH)	+
Saponins	+
Terpenoids (Salkowski reagent)	-
Steroids (Chloroform + H_2SO_4)	+
Tannins ($FeCl_3$)	-
Phenolics (Folin Ciocalteu's phenol reagent)	-

Note. - = Not detected; + = Low level; ++ = Sufficient level; +++ = High level; Mg = Magnesium; HCl = Hydrochloric acid; H_2SO_4 = Sulfuric acid; NaOH = Sodium hydroxide; $FeCl_3$ = Iron (III) chloride

Antidiabetic Activity Test of *Bengkuang* Extract

Clearly shown in Table 2, blood glucose levels of all groups were normal on Day 0, then increased to > 250 mg/dl on Day 3 post-STZ-NA injection, indicating that diabetes mellitus had developed in the rats (Ghasemi et al., 2014). However, after administering *bengkuang* extracts, the glucose levels

decreased significantly ($p < 0.05$) to 111 ± 63 mg/dl with 28 mg treatment Group D and 99 ± 55 mg/dl with 56 mg treatment Group E when compared to the Diabetes Control Group B showing elevated glucose levels (Day 18).

Table 2
The average blood glucose levels of streptozotocin-nicotinamide (STZ-NA) injected rats on pre- and post-treatments with glibenclamide (GBC) or plant extracts

Treated group	Average levels of blood glucose in rats (mg/dl)		
	Day 0	Day 3	Day 18
A (Normal Group)	67± 2	69 ± 2	70 ± 2
B (Diabetes Control)	70 ± 2[a]	271 ±13[b]	277 ± 9
C (Antidiabetic Control)	69 ± 3[a]	269 ± 9[c]	111 ± 63
D (Extract I)	70 ± 1[a]	278 ± 4[d]	182 ± 3
E (Extract II)	72 ± 2[a]	277 ± 4[e]	99 ± 55

Note. [a]STZ-NA injected at Day 0 to induce diabetes mellitus; [b,c,d,e] injected with sodium carboxymethyl cellulose, GBC, Extract I (28 mg/200 g BW), and Extract II (56 mg/200 g BW) on Day 4, respectively. Day 0 shows the initial blood glucose levels, while Day 18 shows the blood glucose levels 14 days after the start of treatments. Superscripts indicate it is statistically significant compared to other groups

Histological Examination of the Rat Pancreas

Figure 1 shows the Normal Group (A), the Diabetes Control (B), the Antidiabetic Control (C), the Treated Group with Extract I (D), and the Treated Group with Extract II (E). Histopathological examination of rat pancreatic tissue revealed gross variations in Langerhans pancreatic cells. To illustrate, Normal Group A did not exhibit any specific changes leading to necrosis (i.e., absence of necrotic cells and degeneration of Langerhans islands as indicated by very dense cell nuclei and no cells with edema [swelling]. Islets composition Langerhans appear compact and contain many endocrine cells Figure 1A). In contrast, the Diabetes Control Group B (STZ treatment) displayed damage in the form of necrotic cells on Langerhans islands as indicated by the size of Langerhans islands getting smaller, the

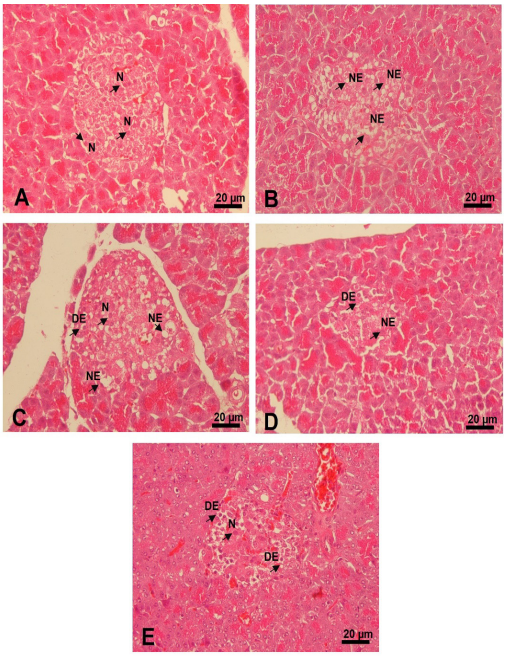

Figure 1. Histopathological images of rat pancreas: (A) Normal Group; (B) Diabetic Control; (C) Antidiabetic Control; (D) Treated Group with Extract I; (E) Treated Group with Extract II

Note. N, NE, and DE indicate the normal cell, necrosis cell, and degenerated Langerhans of insular cells, respectively

number of β-cell masses decreasing, and the empty spaces in the middle of the island Langerhans.

Necrosis is cell death due to fatal damage characterized by damage to the structure and function of the cell followed by cell lysis and tissue inflammation, in Figure 1B, marked with NE in Figure 1B. Interestingly, the pancreatic tissue of the Antidiabetic Group C treated with GBC and Extract Treated Groups D and E showed signs of recovery. These signs included normal starting cells and degeneration of islets of Langerhans as indicated by colonizing cells, characterized by monomorphic β-pancreatic cell shapes, β-pancreatic cells with enlarged nuclei, and visible nucleoli in Figures 1C, 1D, and 1E. Extract II (56 mg/200 g BW) gave the best recovery (Figure 1E). These recoveries were associated with the reduced glucose levels shown in Table 2.

DISCUSSION

This study aims to evaluate the efficiency of *bengkuang* extract in treating diabetes mellitus using a rat model. Both plant extract concentrates (28 and 56 mg/200 g BW) significantly reduced blood sugar levels after 14 days of treatment (Day 18, Table 2), indicating recovery from diabetes as previously reported (Ghasemi et al., 2014). According to Nubatonis et al. (2015), the leading causes of damage to β pancreatic cells are genetics, infections, nutrition, diabetogenesis, and free radicals. In this study, the damage occurring in the Langerhans cells was due to STZ-NA, a cytotoxic hazard for β pancreatic cells (Nubatonis et al., 2015). Histopathology examination of the white rat pancreas shown in Figure 1B, the Diabetes Control Group demonstrated necrotic cells, distinguished by smaller Langerhans islands, fewer β-cell masses, and empty spaces in the middle of Langerhans islands. It demonstrates how STZ-NA administration could cause damage to Langerhans cells. The Normal Group in Figure 1A demonstrates that Langerhans' islets appear normal, characterized by the absence of necrosis or degeneration of the Langerhans islets, a very dense cell nucleus, and the absence of any edema cells (swelling).

The islets of Langerhans have a dense appearance and are dense with endocrine cells. The Antidiabetic Control Group in Figure 1C shows that, despite necrotic cells, the islets of Langerhans have degenerated and are moving toward normal conditions, characterized by the presence of colonizing cells. Meanwhile, the histopathology of the white rat pancreas in Figure 1E showed that the group receiving Extract II experienced degeneration in the islets of Langerhans, and the cells started to appear normal as indicated by the presence of colonizing cells, pancreatic β-cells with enlarged nuclei, and visible nucleoli. Due to the presence of various metabolites, primarily alkaloids, the plant extracts were able to mitigate this damage in a dose-dependent manner (Table 1). This conclusion is supported by earlier research showing that alkaloids, saponins, flavonoids, and steroid groups are effective in treating hyperglycemia because they can stimulate insulin secretion and regenerate β

pancreatic cells (Barky et al., 2017; Kumar et al., 2019; Sandhar et al., 2011; Sediarso et al., 2012).

CONCLUSION

Bengkuang extract has antidiabetic activity, as indicated by a significant decrease in blood sugar levels and recovery of damaged pancreatic cells in treated diabetic rats. Its activity is dose-dependent, with 56 mg/200 g BW showing a comparable efficacy to the GBC (0.09 mg/200 g BW). The results justify further investigations into the underlying antidiabetic mechanisms to assess the potential of using *bengkuang* extracts or an extract component(s) for diabetes treatment.

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