ABSTRACT: This study was designed to investigate the inhibitory effects of *Polyopes lancifolia* extract (PLE) on α-glucosidase activity, α-amylase activity, and postprandial hyperglycemia in streptozotocin (STZ)-induced diabetic mice. The results of this study revealed a marked inhibitory effect of PLE on α-glucosidase and α-amylase activities. The IC50s of PLE against α-glucosidase and α-amylase were 0.20 mg/mL and 0.35 mg/mL, respectively. PLE was a more effective inhibitor of α-glucosidase and α-amylase activities than acarbose, the positive control. The postprandial blood glucose levels of STZ-induced diabetic mice were significantly lower in the PLE treated group than in the control group. Moreover, PLE administration was associated with a decreased area under the curve for the glucose response in diabetic mice. These results indicate that PLE may be a potent inhibitor of α-glucosidase and α-amylase activities and may suppress postprandial hyperglycemia.

Keywords: *Polyopes lancifolia*, α-glucosidase, α-amylase, postprandial hyperglycemia, diabetic mice
The samples were washed three times with tap water to remove salt, epiphytes, and sand attached to the surface, then carefully rinsed with fresh water and freeze-dried. The dried sample was ground and sifted through a 50-mesh standard testing sieve. The sample was extracted with ten volumes of 80% methanol for 12 h three times at room temperature. The filtrate was then vacuum-evaporated to obtain the extract. After the PLE was thoroughly dried, the extract was stored in a deep freezer (−80°C).

**Inhibition assay for in vitro α-glucosidase activity**
The α-glucosidase inhibition assay was conducted by the chromogenic method described by Watanabe et al. (18) using a readily available yeast enzyme. Briefly, yeast α-glucosidase (0.7 units, Sigma, St. Louis, MO, USA) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L NaN₃ to form the enzyme solution. Five millimolar p-nitrophenyl-α-D-glucopyranoside was dissolved in the same buffer (pH 7.0) to form the substrate solution. Next, 50 μL of enzyme solution and 10 μL of sample dissolved in dimethylsulfoxide (5 mg/mL) were mixed in a well of a microtiter plate and the absorbance at 405 nm was measured with a microplate reader (zero time point). After incubation for 5 min, the substrate solution (50 μL) was added and the mixture was incubated for another 5 min at room temperature. Then, the increase in absorbance from the zero time point was measured. The inhibitory activities of varying concentrations of PLE were expressed as 100 minus the absorbance change of PLE relative to the absorbance change of the carrier solvent. The measurements were performed in triplicate and the IC₅₀ value (i.e., the concentration of carrier solvent. The statistical analysis was performed using SAS software ver. 9.1 (SAS Institute Inc., Cary, NC, USA). Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range tests. P-values of less than 0.05 were considered statistically significant.

**Induction of diabetes**
To induce diabetes, mice were fasted for 18 h and then given a single intraperitoneal (i.p.) injection of 60 mg/kg STZ prepared in 0.1 M sodium citrate buffer (pH 4.5). Beginning one week after injection of STZ, fasting blood glucose levels were periodically measured using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Blood was obtained via tail bleed. Mice with fasting blood glucose values of 250 mg/dL or higher were included in the diabetic groups.

**Measurement of blood glucose level**
Normal mice and STZ-induced diabetic mice were fasted overnight (i.e., deprived of food for at least 12 h but allowed free access to water). After overnight fasting, normal and STZ-induced diabetic mice were each randomly divided into 3 groups of 7 mice (i.e., a total of 6 groups) and treated as follows: 1) control: mice received oral administration of soluble starch (2 g/kg body weight [BW]) alone; 2) PLE: mice received oral administration of starch with PLE (300 mg/kg BW); 3) acarbose: mice received oral administration of starch with acarbose (100 mg/kg BW). The PLE and acarbose doses were determined based on previous research (19,20). Blood samples were taken from the tail vein at 0 min, 30 min, 60 min, and 120 min after oral administration. Blood glucose was measured using a glucometer (Roche Diagnostics GmbH). Areas under the curve (AUC) of the glucose response were calculated using the trapezoidal rule (21).

**Data and statistical analysis**
The data are represented as the mean±standard deviation of triplicate experiments. The statistical analysis was performed using SAS software ver. 9.1 (SAS Institute Inc., Cary, NC, USA). Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range tests. P-values of less than 0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**
The treatment goal for diabetic patients is to maintain a normal blood glucose level in both the fasting and the postprandial states. Postprandial hyperglycemia is the first metabolic abnormality to occur in diabetes mellitus (22). Thus, inhibition of pancreatic α-amylase activity or intestinal α-glucosidase activity is an effective strategy for the management of diabetes mellitus, as it retards the absorption of carbohydrates thereby controlling postprandial hyperglycemia (23). Several synthetic compounds have been tested in efforts to develop therapeutic agents for diabetes. However, these compounds generally are
Inhibitory effect of PLE on in vitro α-glucosidase and α-amylase activities

The inhibitory effect of PLE against α-glucosidase is shown in Fig. 1. PLE inhibited α-glucosidase activity in a dose-dependent manner by 24.67%, 38.41%, 55.56%, and 74.99% at PLE concentrations of 0.05 mg/mL, 0.10 mg/mL, 0.25 mg/mL, and 0.50 mg/mL, respectively. A 0.50 mg/mL concentration of acarbose, an α-glucosidase inhibitor used as an oral hypoglycemic agent, inhibited α-glucosidase activity by 62.03%. The α-glucosidase inhibitory activity of PLE was higher than that of the same concentration (i.e., 0.50 mg/mL) of acarbose.

The inhibitory effect of PLE against α-amylase is shown in Fig. 2. PLE inhibited α-amylase activity by 18.97%, 33.17%, 42.70%, and 61.02% at PLE concentrations of 0.05 mg/mL, 0.10 mg/mL, 0.25 mg/mL, and 0.50 mg/mL, respectively. A 0.50 mg/mL concentration of acarbose, an α-glucosidase inhibitor used as an oral hypoglycemic agent, inhibited α-glucosidase activity by 53.40%. The α-amylase inhibitory activity of PLE was higher than that of the same concentration (i.e., 0.50 mg/mL) of acarbose.

The IC₅₀ values of PLE against α-glucosidase and α-amylase were 0.20 and 0.35 mg/mL, respectively. The IC₅₀ values of PLE against α-glucosidase and α-amylase were lower than that of acarbose, suggesting that PLE has stronger inhibitory effects than the positive control (i.e., acarbose) (Table 1).

α-amylase and α-glucosidase are key carbohydrate digestion enzymes. α-amylase catalyzes the hydrolysis of α-1,4-glycosidic linkages of starch, glycogen, and various oligosaccharides (24). α-glucosidase is located on the brush-border surface membrane of intestinal cells. α-glucosidase catalyzes the hydrolysis of disaccharides and oligosaccharides present in the lumen of the intestine; as a result, the glucose generated by α-glucosidase activity is readily available for intestinal absorption (25). The inhibition of α-amylase and α-glucosidase activities prevents the release of glucose from starch, thus reducing the absorption of glucose by the intestine (26). For this reason, the inhibition of these enzymes is considered to be an effective strategy for the management of post-prandial blood glucose levels in diabetic patients, and scientists continue to seek effective and non-toxic inhibitors of α-glucosidase and α-amylase.

In this study, we investigated the inhibitory effect of PLE against α-glucosidase and α-amylase to elucidate the possible use of PLE as an anti-hyperglycemic agent. PLE had greater inhibitory effects against α-glucosidase and α-amylase than the commercial carbohydrate digestive enzyme inhibitor, acarbose. Previous work has revealed that Polypotes lancifolia contains bromophenol compounds (12). These polyphenolic compounds are known to form complexes with a variety of proteins (27). Notably, previous studies indicate that the hydroxyl groups of

### Table 1. IC₅₀ values of PLE on α-glucosidase and α-amylase

| Sample     | IC₅₀ (mg/mL) | α-Glucosidase | α-Amylase |
|------------|-------------|---------------|-----------|
| PLE        | 0.20±0.02*  | 0.35±0.02*    |           |
| Acarbose   | 0.34±0.02   | 0.45±0.04     |           |

1IC₅₀ is the concentration of sample required for 50% inhibition. Each value is expressed as mean±SD (n=3).

2Value is significantly different from the positive control, acarbose at P<0.05.

PLE, Polypotes lancifolia extract.
Table 2. Area under the curve (AUC) of postprandial glucose responses in normal and streptozotocin-induced diabetic mice

| Group          | Normal mice | Diabetic mice |
|----------------|-------------|---------------|
| Control        | 381.2±47.4  | 886.5±48.4a   |
| PLE            | 327.4±29.9  | 811.8±33.1ab  |
| Acarbose       | 304.8±37.8  | 790.5±32.5b   |

*Control (distilled water), PLE (300 mg/kg), and acarbose (100 mg/kg) were co-administered orally with starch (2 g/kg). Each value is expressed as mean±SD of seven mice (n=42). Values with different letters are significantly different at P<0.05 as analyzed by Duncan’s multiple range test. PLE, Polyopes lancifolia extract.

Polyphenolic compounds may bind to active binding sites of the enzymes, resulting in the inhibition of enzyme activity (13,28). Thus, we hypothesize that bromophenol compounds in PLE may have an important role in the inhibition of \( \alpha \)-glucosidase and \( \alpha \)-amylase activity.

Effect of PLE on in vivo blood glucose levels

The effect of PLE on postprandial blood glucose levels was investigated in STZ-induced diabetic and normal mice. In diabetic mice, postprandial blood glucose levels of the PLE administered group were lower than those of the control group (Fig. 3A). The blood glucose level of the control group increased to 483.8 mg/dL at 60 min after a meal, and decreased thereafter. However, postprandial blood glucose levels were significantly lower (P<0.05) when diabetic mice were fed with PLE (400.2 mg/dL, 433 mg/dL, and 394 mg/dL at 30 min, 60 min, and 120 min, respectively). In normal mice, PLE significantly alleviated (P<0.05) postprandial hyperglycemia caused by starch. The peak postprandial blood glucose level was significantly decreased when starch with PLE was orally administered in normal mice (Fig. 3B). In diabetic mice, the AUC for the glucose response was lower for the PLE administration group (811.8±33.1 mg/h/dL) than for the control group (886.5±48.4 mg/h/dL) (Table 2).

Postprandial hyperglycemia reduces insulin sensitivity (22,29) and insulin secretion due to the degradation of pancreas function (2), resulting in a deteriorated diabetic state. Also, postprandial hyperglycemia has been shown to increase the generation of free radicals, which stimulate thrombotic pathways and induce vasoconstriction, leading to an increased risk for cardiovascular disease, a major cause of premature death in patients with diabetes (30). Therefore, the regulation of postprandial hyperglycemia is considered important in the treatment of diabetes and the prevention of cardiovascular complications. In this study, we investigated the anti-hyperglycemic effects of PLE in STZ-induced diabetic mice after administration of starch. Following PLE administration, postprandial blood glucose levels were significantly decreased in STZ-induced diabetic mice and normal mice. These results indicate that PLE may delay the absorption of dietary carbohydrates, thus suppressing the typical increase in postprandial blood glucose levels. Inoue et al. (31) reported that medication that flattens peak of postprandial blood glucose reduces the AUC of the blood glucose response. In this study, PLE reduced both the peak blood glucose level and the AUC.

In conclusion, our study indicates that the \( \alpha \)-glucosidase and \( \alpha \)-amylase inhibitory effects of PLE are responsible for PLE’s anti-hyperglycemic activity. PLE had a noticeable inhibitory effect against these enzymes. Furthermore, PLE may delay the absorption of dietary carbohydrates by the intestine, thus suppressing post-meal increases in blood glucose. These findings support the use of PLE for the prevention of postprandial hyperglycemia in diabetic patients.
of PLE as a nutraceutical to control diabetes and alleviate postprandial hyperglycemia. Further studies are needed to reveal the active compounds in PLE that are responsible for its hypoglycemic effects.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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