Inhibitory Effects of *Loranthus Parasiticus* Extract on Carbohydrate Digestive Enzymes and Postprandial Hyperglycemia

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This study was designed to investigate whether *Loranthus parasiticus* extract (LPE) could inhibit the activities of carbohydrate digestive enzymes and alleviate postprandial hyperglycemia in diabetic mice. Lyophilized *L. parasiticus* was extracted with 80% ethanol and concentrated. The inhibitory effects of LPE on carbohydrate digestive enzymes were evaluated by examining α-glucosidase and α-amylase, and it was seen to inhibit the activities of both enzymes in a dose-dependent manner. More specifically, the IC₅₀ values of LPE against α-glucosidase and α-amylase were 0.121±0.007 and 0.157±0.004 mg/ml, respectively, significantly lower than those of acarbose, showing that LPE has stronger inhibitory effects than the positive control. These results suggest that LPE strongly inhibits the activities of these digestive enzymes. Blood glucose levels in the control group of diabetic mice increased to 490.00±28.52 mg/dl and 474.60±25.30 mg/dl at 60 and 120 min after a meal, respectively. However, when LPE was added to starch, postprandial blood glucose levels were significantly reduced (463.0±23.73 and 418.5±24.50 mg/dl at 60 and 120 min, respectively; p<0.05). The area under the curve also significantly decreased following administration of LPE, with no cytotoxicity. These results therefore indicate that LPE could be used as an α-glucosidase and α-amylase inhibitor and delay carbohydrate digestion and, thus, glucose absorption after a meal.

**Key words**: α-amylase, diabetic mice, α-glucosidase, *Loranthus parasiticus* extract, postprandial hyperglycemia

**Introduction**

Diabetes is increasing the global health burden due to various complications [30]. An acute increase in postprandial blood glucose is a direct and indirect acute toxicity to the vasculature, which can lead to complications of diabetes. To achieve proper glycemic control, it is necessary to reduce postprandial hyperglycemia. Various epidemiological studies have suggested an association between postprandial blood sugar fluctuations and diabetes complications [4].

One of the treatments for suppressing postprandial hyperglycemia is to delay glucose absorption through inhibition of carbohydrate hydrolyzing enzymes [29]. α-Amylase and α-glucosidase are the two main hydrolytic enzymes [27]. α-Glucosidase breaks down the byproduct of starch into glucose. Inhibitors of this enzyme are widely used for the regulation of blood glucose levels in type 2 diabetes. α-Glucosidase inhibitors block the membrane-bound intestinal α-glucosidases which hydrolyzes carbohydrates into glucose in the small intestine. Pancreatic α-amylase breaks down carbohydrates, producing oligosaccharide, maltotriose and maltose. α-Amylase inhibitor is also considered as important factor in the development of antidiabetic drugs [34]. Saliva and pancreatic α-amylase inhibitors may inhibit postprandial hyperglycemia by reducing the rate of digestion of carbohydrates [31].

Recent studies indicated that modulation of postprandial blood glucose played an important role in the long term glycemic control and complication prevention [9]. The most effective oral glucose-lowering drug on the market is acarbose, which has been widely used in clinical practice as a drug to inhibit α-glycosidase activity [25, 36]. Although acarbose can effectively reduce the increase of postprandial blood glucose, the adverse side effects are appeared simultaneously, such as diarrhea, abdominal cramping, flatulence, and liver disorders [14, 37]. Thus, using natural products, such as plant extracts, to reduce hyperglycemia without causing side effects may be a promising approach.
**Loranthus parasiticus** is mistletoe parasitic on mulberry. Mistletoe, a semi-parasitic plant, is widely distributed throughout the world and has been used as an ingredient in Northeast Asian traditional medicine for centuries [16]. *Loranthus parasiticus* are mainly used for traditional medicine in Korea [12]. It has various beneficial effects, such as anticancer, antioxidant, anti-obesity, anti-inflammatory and neuroprotective activity [19]. These effects of *Loranthus parasiticus* are associated with various biologically active compounds, including lectins, bis-cotoxins, phenolic compounds, sesquiterpenes lactones, triterpenes and flavonoids [39]. Nonetheless, there are few studies on the inhibitory effect of *L. parasiticus* on α-amylase and α-glucosidase and the regulation of postprandial hyperglycemia in diabetes. Therefore, this study was conducted to determine whether *L. parasiticus* extract (LPE) inhibits α-amylase and α-glucosidase activities in vitro and suppresses postprandial hyperglycemia in diabetic mice in vivo.

**Materials and Methods**

**Material and preparation of L. parasiticus extract**

*L. parasiticus* was collected from Yeongcheon, Gyeongbuk, Korea. The sample was washed with fresh water, and then freeze-dried. The lyophilized sample was homogenized with a grinder prior to extraction. The sample was extracted three times with ten volumes of 80% ethanol for 12 hr at room temperature. The *L. parasiticus* extract (LPE) was then evaporated at 40°C using a rotary evaporator (N-1300VW, EYELA, Tokyo, Japan). After the solvent had been completely removed from the LPE, it was stored in a deep freezer (-80°C).

**Inhibition assay for α-glucosidase activity in vitro**

The α-glucosidase inhibitory activity assays were carried out using readily available yeast enzymes, using the method of Watanabe et al. [35]. Yeast α-glucosidase (0.7 U, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/l of bovine serum albumin and 0.2 g/l of NaCl, and used as the enzyme test solution. Five mM p-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) was used as the substrate solution. 10 μl of LPE [5 mg/ml in dimethyl sulfoxide (DMSO)] and 50 μl of enzyme solution were mixed in a well, and the absorbance at 405 nm was measured as time zero using a microplate reader. After incubation for 5 min, the substrate solution (50 μl) was added, and the incubation continued for another 5 min at room temperature. The increase in absorbance from the zero time point was then measured. The inhibitory activities of varying concentrations of *L. parasiticus* were expressed as 100 minus the absorbance difference (%) of the test compounds relative to the absorbance change of the negative control (i.e., DMSO used as the test solution). The measurements were performed in triplicate, and the IC50 value (i.e., the concentration of LPE that results in 50% inhibition of maximal activity) was determined.

**Inhibition assay for α-amylase activity in vitro**

The α-amylase inhibitory activity was analyzed in the same manner as α-glucosidase inhibition measuring method [35], except that porcine pancreatic amylase (100 U, Sigma-Aldrich, St. Louis, MO, USA) and p-nitrophenyl-α-D-maltopenta-glycoside (Sigma-Aldrich Co.) were used as the enzyme and substrate, respectively.

**Measurement of cytotoxicity**

Cytotoxic cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and 3T3-L1 cells were purchased from the Korean Cell Line Bank (Seoul, Korea). 3T3-L1 cells were seeded at 1×10^4 cells/well in 96-well plates and pre-incubated in a humidified atmosphere containing 5% CO2 at 37°C for 24 hr. Afterward, the cells were treated with various concentrations (0.1, 0.5, 1, and 2 mg/ml) of LPE, and incubated for 20 hr. After completion of the treatment, the cells were incubated for 3 h at 37°C with filtered MTT (Sigma-Aldrich, St. Louis, MO, USA) solution, which was added to each well at a final concentration of 0.5 mg of MTT/ml. The supernatants were carefully aspirated, 200 μl of DMSO was added to each well, and the plates were agitated to dissolve the crystal product. The absorbance of DMSO solution was measured spectrophotometrically at 540 nm.

**Experimental animals**

Four-week-old male mice (ICR, Orient, Inc., Seoul, Korea) were individually housed in a temperature control room (25-30°C) with 45-55% relative humidity. Animals were randomly given pellet food and tap water. After two weeks adjustment period, streptozotocin [STZ; 60 mg/kg body weight (b.w)] and freshly dissolved in citrate buffer (0.1M, pH 4.5) [38]. And after 7 days, tail bleeds were performed and animals with a blood glucose concentration above 250 mg/dL were considered to be diabetic. Mouse handling and
care procedures have complied with the guidelines (NIH Guide for the Care and Use of Laboratory Animals) in compliance with current international laws and policies, and all procedures have been approved by the Pusan National University Animal Ethics Committee.-2018-1823.

Measurement of blood glucose levels
Both normal and STZ-induced diabetic mice were fasted overnight and randomly divided into three groups of 7 mice. Before testing blood glucose levels, the animals were kept on an empty stomach for at least 12 hr but had free access to water. Mice were orally administered as follows: control group, mice were orally administered with starch (2 g/kg b.w); LPE, orally administered starch LPE to mice (300 mg/kg b.w); acarbose, mice received acarbose orally administered with starch (100 mg/kg b.w). Blood samples were taken from the tail vein at 0, 30, 60, and 120 min. Blood glucose was measured using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany). The areas under the curve (AUC) were calculated using the trapezoidal rule [17].

Data statistical analysis
Statistical analysis was performed using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). Student’s t-test was used for comparison between control and treatment groups. Differences were assessed with one-way ANOVA followed by Duncan’s multi-range test ($p<0.05$). Data are displayed as mean ± standard deviation (SD).

Results and Discussion
Inhibitory effect of LPE on α-glucosidase and α-amylase in vitro
The inhibitory effect of LPE on α-glucosidase activity was measured using p-nitrophenyl-α-D-glucopyranoside as a substrate and compared with the effect of acarbose, a commercial α-glucosidase inhibitor used as an hyperglycemic agent. LPE inhibited α-glucosidase activity in a dose-dependent manner by 38.11±1.09, 45.87±2.98, 55.60±2.84, and 61.12±2.15% at concentrations of 0.05, 0.10, 0.15, and 0.20 mg/mL, respectively (Fig. 1). LPE inhibited the enzyme activity by 40.38±1.81% at a concentration of 0.10 mg/dl. The α-glucosidase inhibitory activity of LPE was significantly higher than that of acarbose at the same concentration.

As shown in Fig. 2, the inhibitory effects of LPE on α-amylase were increased in a dose-dependent manner by 25.69±1.40, 38.42±2.74, 47.38±2.70, and 65.97±3.21% at concentrations of 0.05, 0.10, 0.15, and 0.20 mg/mL, respectively. LPE also inhibited α-amylase activity more effectively than acarbose. The IC$_{50}$ values of LPE against α-glucosidase and α-amylase were 0.121±0.007 and 0.157±0.004 mg/mL, respectively. Its IC$_{50}$ values against α-glucosidase and α-amylase were significantly lower than those of acarbose, indicating that LPE has stronger inhibitory effects than the positive control (Table 1).

Inhibitions of α-amylase and α-glucosidase were important factors for managing postprandial blood glucose in patients with type 2 diabetes [8]. This study investigated the inhibitory effects of the natural product, LPE, against α-amylase and α-glucosidase to uncover potential as a postprandial hyperglycemic inhibitor. LPE provided significantly
Table 1. IC\textsubscript{50} values of the inhibitory effect of \textit{L. parasiticus} extract (LPE) against \(\alpha\)-glucosidase and \(\alpha\)-amylase activities

<table>
<thead>
<tr>
<th></th>
<th>IC\textsubscript{50} (mg/ml)\textsuperscript{1)\textsuperscript{a)}</th>
<th>(\alpha)-glucosidase</th>
<th>(\alpha)-amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPE</td>
<td>0.121±0.007*</td>
<td>0.157±0.004*</td>
<td>0.165±0.006</td>
</tr>
<tr>
<td>Acarbose</td>
<td>0.130±0.008</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD in triplicate experiments. \*Significantly different from acarbose at \(p<0.05\).
\textsuperscript{1)}IC\textsubscript{50} value is the concentration of sample required for 50\% inhibition.

higher inhibitory activities against both \(\alpha\)-amylase and \(\alpha\)-glucosidase than acarbose, the commercial inhibitor. It also did not show any cytotoxicity (Fig. 3). The inhibitory effects of LPE on these enzymes would be attributed to the active ingredients in \textit{Loranthus parasiticus}.

\textit{Loranthus parasiticus} contained total phenolic compounds, flavonoids, triterpene and sesquiterpene lactones, etc. [19]. Several studies have reported the anti-diabetic effects of triterpenes and triterpenes-containing plant extracts [23]. Flavonoids, especially quercetin and camphorol, have been also shown to exhibit \(\alpha\)-glucosidase inhibitory activity [18, 26]. Several beneficial flavonoids exhibited impressive hypoglycemic effects, with significant improvement, without producing health hazards [21]. These flavonoids showed \(\alpha\)-glucosidase inhibitory effect due to galloyl group and phenolic hydroxyl group, which was caused by the formation of complex with the enzyme [11]. The flavonoids exerted their \(\alpha\)-glucosidase inhibitory activities by forming complex with the enzyme through non-covalent interactions in the intestine [36]. As a result of this study, LPE had inhibitory effect on \(\alpha\)-glucosidase, which might be due to the active ingredients contained in LP, such as flavonoids and triterpenes.

\(\alpha\)-Amylase hydrolyses \(\alpha\)-linked polysaccharides such as starch and glycogen. \(\alpha\)-Amylase inhibitors block the hydrolysis of complex starch into oligosaccharides, reducing the rate of digestion of carbohydrates and consequently less glucose absorption [33]. The flavonoids such as luteolin, myricetin, and quercetin were potent inhibitors of \(\alpha\)-amylase, their inhibition activities on the enzyme were related to the functional group, such as 2,3-double bond, 5-OH and the linkage of the B ring at the 3-position in the compounds [32]. LPE was known to possess a high quantity of flavonoids [5]. It exhibited \(\alpha\)-amylase inhibitory effect and especially the higher inhibitory effect than acarbose. Thus, the high inhibitory effects of LPE on \(\alpha\)-glucosidase and \(\alpha\)-amylase activities might be attributable to the high content of flavonoids in it.

**Effects of LPE on blood glucose levels in vivo**

The effects of LPE on blood glucose levels after a meal were investigated in normal and STZ-induced diabetic mice. The postprandial blood glucose levels of the LPE administered mice were significantly lower than those of the diabetic mice (Fig. 4). Blood glucose levels in the diabetic mice in-

Fig. 3. Cytotoxic effects of \textit{L. parasiticus} extract (LPE) in 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0.1, 0.2, 0.5, 1.0 and 2.0 mg/ml) of LPE for 20 hr, and cell viability was measured by MTT assay. Each value is expressed as the mean ± standard deviation (SD) of three experiments. NS: Not-significant.

Fig. 4. Blood glucose levels after the administration of \textit{L. parasiticus} extract (LPE) in streptozotocin-induced diabetic mice. Each value is expressed as mean ± SD of seven mice. Values with different letters (a-c) are significantly different at each time (\(p<0.05\)) as analyzed by Duncan’s multiple range test. Control, mice received starch orally (2 g/kg); LPE, mice received starch with \textit{Loranthus parasiticus} extract orally (300 mg/kg); Acarbose, mice received starch with acarbose orally (100 mg/kg).
Postprandial hyperglycemia and fasting blood glucose control are very important in patients with type 2 diabetes. Postprandial hyperglycemia was reported to have a stronger correlation with morbidity of diabetes complications such as cardiovascular disease than fasting hyperglycemia [22]. It was associated with glycemic variability and has been suggested that postprandial hyperglycemic fluctuations could contribute to the development of diabetes complications [7]. Reducing postprandial hyperglycemia is one of the important diabetic treatments, which delays the absorption of glucose through the inhibition of carbohydrate hydrolase, such as α-amylase and α-glucosidase in the digestive organs. Because α-amylase is involved in the breakdown of long chain carbohydrates, and α-glucosidase breaks down disaccharides to glucose. In this study, LPE showed significantly higher inhibitory activities against both α-amylase and α-glucosidase than acarbose, the commercial inhibitor. Reduction in postprandial hyperglycemia of diabetic mice treated with LPE might be due to inhibition of these enzymes. The reduction effect on postprandial hyperglycemia of LPE was also observed in normal mice. These confirmed that LPE could inhibit the action of carbohydrate digestive enzymes and delay the absorption of glucose.

*Loranthus parasiticus* contained bioactive ingredients such as flavonoids, phenolic compounds, triterpene and sesquiterpene lactones [19]. Flavonoids were demonstrated to act on biological target of type 2 diabetes such as α-glucosidase [24]. Administration of naringenin, a kind of flavonoids, prevented a sharp rise in blood glucose levels of diabetic rats loaded with maltose and sucrose compared to control rats. This showed that the mechanism of action of flavonoid was associated with α-glucosidase inhibition in the intestine, thereby delaying glucose release [28]. Flavonoids also improved hyperglycemia in patients with type 2 diabetes [11]. Natural sesquiterpene lactones have also been reported to attenuate hyperglycemia in streptozotocin (STZ) induced diabetic rats [3]. *In vitro* α-amylase inhibition assay showed that sesquiterpene lactones had potent intestinal α-amylase inhibitory activity, which has the ability to reduce starch-induced postprandial blood glucose [1]. One of the anti-diabetic mechanisms of the triterpenes was their inhibitions against α-amylase and α-glucosidase [2, 10]. Additionally, some triterpenes significantly decreased the hyperglycemia in diabetic rats by inhibiting small intestinal α-amylase, sucrase and α-glucosidase activity [15]. The results of this

![Fig. 5. Blood glucose levels after the administration of L. parasiticus extract (LPE) in normal mice. Each value is expressed as mean ± SD of seven mice. Values with different letters (a-c) are significantly different at each time (p<0.05) as analyzed by Duncan’s multiple range test. Control, mice received starch orally (2 g/kg); LPE, mice received starch with *Loranthus parasiticus* extract orally (300 mg/kg); Acarbose, mice received starch with acarbose orally (100 mg/kg).](image)

Table 2. Areas under the curve (AUC) of postprandial glucose responses in normal and streptozotocin-induced diabetic mice

<table>
<thead>
<tr>
<th>Group¹⁾</th>
<th>Normal mice</th>
<th>Diabetic mice</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>345.50±35.65^[g]</td>
<td>901.65±56.58^[g]</td>
</tr>
<tr>
<td>LPE</td>
<td>274.05±30.35^[b]</td>
<td>846.87±43.48^[b]</td>
</tr>
<tr>
<td>Acarbose</td>
<td>208.30±27.99^[f]</td>
<td>756.65±44.96^[f]</td>
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¹⁾Distilled water (Control), LPE (300 mg/kg), or acarbose (100 mg/kg) was coadministered orally with starch (2 g/kg). Each value is expressed as the mean ± SD of seven mice (n=42).

²⁾Values with different alphabets are significantly different at p<0.05, as analyzed by Duncan’s multiple-range test. LPE: *Loranthus parasiticus* extract.
study showed that LPE could delay the digestion and absorption of dietary carbohydrates in the intestine, which could suppress the rise in blood glucose levels after meals in diabetic mice. The suppression effect on postprandial hyperglycemia of LPE was thought to be due to the active ingredients contained in LPE, such as sesquiterpene lactones, triterpenes and flavonoids. In addition, various doses of animal toxicity studies on LPE showed no mortality or morbid symptoms when administered orally up to 1,500 mg/kg [20]. Studies on the same plant leaves administered orally up to and 827 mg/kg body weight, neither adverse biochemical changes nor mortality was detected [6]. While another study confirmed the safety of its up to 5,000 mg/kg [13]. In conclusion, LPE inhibited α-glucosidase and α-amylase activities and resulted in a reduction in postprandial hyperglycemia. LPE might delay the digestion and absorption of dietary carbohydrates in the intestine, resulting in suppression of increased blood glucose levels after a meal. Thus, this results suggest the possibility that LPE may be used as a natural anti-hyperglycemic food because of its inhibitory effects on α-glucosidase and α-amylase without side effects. However, if LPE is used clinically for medical purposes, a special permit procedure is required, and research on intake as a functional food should be conducted in the future.

Acknowledgement

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References


초록: 상기생(Loranthus parasiticus) 추출물의 탄수화물 소화 효소 및 식후 고혈당 저해 효과

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상기생(Loranthus parasiticus)은 뽕나무에 기생하는 겨우살이로 전세계에 널리 분포되어 있으며, 수 세기 동안 전통 의학의 성분으로 사용되어 왔고, 최근까지 항암, 항산화, 항비만, 항염증 효과 등이 연구되었으나, 탄수화물 효소나 식후 혈당 수치에 미치는 영향에 관한 연구는 부족한 실정이다. 본 연구는 Loranthus parasiticus 추출물 (LPE)이 탄수화물 분해 효소(α-glucosidase, α-amylase) 활성 억제와 streptozotocin (STZ)으로 유도된 당뇨병 마우스에서 식후 고혈당 완화 효과를 조사하였다. 그 결과 LPE는 농도에 비례하여 α-glucosidase와 α-amylase 활성 억제하였고, 각각의 IC₅₀ 값으로 0.121±0.007 및 0.157±0.004 mg/ml을 나타내어 양성 대조군인 acabose 보다 유의하게 강한 억제 효과가 있음을 보여주었다(p<0.05). 또한 STZ로 유도된 당뇨병 마우스에서는 대조군의 높은 혈당과 달리 LPE 첨가군에서는 혈당이 유의하게 감소하였다(p<0.05). 따라서, 이들 결과는 LPE가 α-glucosidase와 α-amylase 억제 효과로 식후 고혈당을 감소시킬 수 있는 천연 항고혈당 식품으로 사용 가능성을 시사한다.