

# *Oxya Chinensis Sinuosa* Mishchenko Extract: Potent Glycosidase Inhibitor Alleviates Postprandial Hyperglycemia in Diabetic Mice

Jae Eun Park and Ji Sook Han\*

Department of Food Science and Nutrition, Pusan National University, Busan 46241, Korea

Received October 12, 2020 / Revised November 2, 2020 / Accepted November 3, 2020

This study was designed to investigate whether extracts from *Oxya chinensis sinuosa* Mistshenk (an edible insect considered a grasshopper) could inhibit the activity of carbohydrate digestive enzymes and alleviate postprandial hyperglycemia in diabetic mice. *Oxya chinensis sinuosa* Mistshenk was extracted with 80% ethanol (OEE) or water (OWE) and then concentrated. The carbohydrate digestive enzyme-inhibiting activity of the resulting extracts was evaluated by examining  $\alpha$ -glucosidase and  $\alpha$ -amylase. The IC<sub>50</sub> values of OEE against  $\alpha$ -glucosidase and  $\alpha$ -amylase were 0.229 mg/ml and 0.106 mg/ml, respectively. This result indicated that OEE has stronger inhibitory effects than OWE and positive control. The blood glucose levels of the diabetic control mice increased after one meal. However, when OEE (300 mg/kg) was added to starch, this increase in postprandial blood glucose levels was significantly suppressed. The area under the curve also significantly decreased following the administration of OEE, which exhibited no cytotoxicity. These results indicate that OEE is more efficacious than OWE and may be used as a carbohydrate digestive enzyme inhibitor, delay carbohydrate digestion and glucose absorption, and thus alleviate postprandial hyperglycemia caused by dietary carbohydrates.

**Key words** :  $\alpha$ -amylase,  $\alpha$ -glucosidase, grasshopper, *Oxya chinensis sinuosa* Mishchenko, postprandial hyperglycemia

## Introduction

It has been estimated that the number of people with type 2 diabetes will increase globally from 405.6 million in 2018 to 510.8 million by 2030 [9]. Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose levels, due to changes in insulin resistance and blood insulin levels. Chronic hyperglycemia causes abnormalities in lipid and protein metabolism, leading to various complications such as decreased kidney function, arteriosclerosis, decreased vision due to retinal hemorrhage, foot ulcers, and peripheral neuropathy [33].

Fasting and postprandial hyperglycemia are the main features of type 2 diabetes [12]. Clinical studies show that postprandial hyperglycemia is an independent and direct risk factor for cardiovascular disease in diabetic patients [17]. Therefore, its early identification and effective control is important for the treatment of diabetes and prevention of dia-

betes-related complications [8, 34]. One way to control postprandial blood glucose level is to slow glucose absorption in the intestine, by inhibiting the action of certain carbohydrate hydrolases (glycosidases), namely pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase [2]. With this aim, synthetic inhibitors of these enzymes, such as acarbose and voglibose, have been developed to control hyperglycemia [25]. However, treatment of diabetes with drugs over the long term is associated with the manifestation of side effects. Efforts have been made, in recent years, to prevent excessive absorption of glucose and to treat diabetes by using functional foods that do not have any side effects [40].

Global interest in edible insects has also been increasing, and the market size of the global insect industry is expected to continue to grow. *Oxya chinensis sinuosa* Mishchenko (*O. Mishchenko*) has a long history of use as a medicine in several countries, and is traditionally used to treat diabetes, inflammation and liver disease [37]. *O. Mishchenko* is an edible insect belonging to the grasshopper family (Acrididae) and is widely distributed in Korea, Japan and China, where it is consumed as a nutritious insect, abundant in protein and unsaturated fatty acids such as linoleic (C18:2) and oleic acids (C18:1) [31]. Recent studies have reported that *O. Mishchenko* extract has various bioactivities, including anti-oxidant, anti-inflammatory, anti-microbial and cellular pro-

### \*Corresponding author

Tel : +82-51-510-2836, Fax : +82-51-583-3648

E-mail : hanjs@pusan.ac.kr

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

tective effects [27, 38, 44].

However, to our knowledge, none of the previous studies have investigated the use of *O. Mishchenko* extract in the treatment of postprandial hyperglycemia, *in vitro* or *in vivo*. This study investigated its effect on  $\alpha$ -glucosidase and  $\alpha$ -amylase *in vitro* and verified the same in a diabetic animal model.

## Materials and Methods

### Preparation of *O. Mishchenko* extract

A sample of *O. Mishchenko* was collected from Gangwon, Korea and washed with water to remove foreign substances. Wings and legs were carefully removed, lyophilized, and pulverized into a powder form using a grinder (Shinhan Science & Technology Co., Kyunggi, Korea). The sample was extracted three-times with 10x volume of either 80% ethanol or water, for 12 hr at room temperature. The filtered samples were vaporized by vacuum (BUCHI Co., Flawil, Switzerland) to obtain *O. Mishchenko* ethanol (OEE) and water (OWE) extracts, and were stored in a deep freezer.

### Measurement of cytotoxicity

Cell viability in response to the extracts was analyzed using 3T3-L1 cells (Korean Cell Line Bank, Seoul, Korea) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich Chemical Co., St. Louis, Missouri, USA). 3T3-L1 cells were seeded in 96 well plates at a density of  $1 \times 10^4$  cells/well and preincubated at 37°C under an atmosphere containing 5% CO<sub>2</sub> for 24 hr. After 24 hr, the cells were treated independently with various concentrations of OEE and OWE (0.00, 0.02, 0.10, 0.20, 0.30, 0.50 and 1.00 mg/ml) for 24 hr. Following treatment, a filtered MTT solution (100  $\mu$ l) was added to each well and incubated for an additional 4 hr at 37°C. Formazan was carefully aspirated and DMSO (100  $\mu$ l) was added to each well. The absorbance of the DMSO solution was assayed at 540 nm on a microplate reader (Model 680, Bio-Rad Laboratories Inc., Hercules, CA, USA).

### Inhibition of $\alpha$ -glucosidase by *O. Mishchenko* extract *in vitro*

The inhibition of  $\alpha$ -glucosidase activity was analyzed by a chromogenic process using yeast enzymes [43]. Simply, yeast  $\alpha$ -glucosidase (100 U; Sigma-Aldrich Chemical Co.) was dissolved in a 100 mM phosphate buffer (pH 7.0) con-

taining 0.2 g/l sodium azide. A 5 mM solution of *p*-nitrophenyl- $\alpha$ -D-glucopyranoside ( $\geq 99\%$ ; Sigma-Aldrich Chemical Co.) was dissolved in the same PBS (pH 7.0) to obtain the substrate solution. Then, the  $\alpha$ -glucosidase (50  $\mu$ l) and OEE or OWE (10  $\mu$ l), dissolved in dimethyl sulfoxide (DMSO; Bio Basic Inc., Markham, Ontario, Canada), were blended in a microtiter plate, and the absorbance was recorded at 405 nm using a microplate reader at 0 min. The mixture was then incubated for 5 min and the substrate solution (50  $\mu$ l) was added and incubated at room temperature for another 5 min, before the increase in absorbance was measured. The inhibitory activities of OEE and OWE at various concentrations were indicated as absorbance changes, relative to those in the vehicle control (%). The IC<sub>50</sub> values (i.e., the concentrations of OEE and OWE resulting in 50% inhibition of the maximum activity) were calculated.

### Inhibition of $\alpha$ -amylase by *O. Mishchenko* extract *in vitro*

Inhibition of  $\alpha$ -amylase was assayed using the same method specified above, using  $\alpha$ -amylase from porcine pancreas (100 U; Sigma-Aldrich Chemical Co.) and *p*-nitrophenyl- $\alpha$ -D-maltopentoglycoside ( $\geq 99\%$ ; Sigma-Aldrich Chemical Co.).

### *In vivo* experiments

Four-week-old male ICR mice were purchased from Orient Inc. (Seoul, Korea) and acclimatized for 2 weeks before being randomly assigned into the experimental groups. The animals were housed in individual cages with free access to water, in a room with a 12:12 hr light/dark cycle, temperature of 24 $\pm$ 1°C, and humidity of 55 $\pm$ 5%. The mice

Table 1. IC<sub>50</sub> values of *O. Mishchenko* extracts for  $\alpha$ -glucosidase and  $\alpha$ -amylase

Sample	IC <sub>50</sub> (mg/ml) <sup>1)</sup>	
	$\alpha$ -glucosidase	$\alpha$ -amylase
OEE	0.229 $\pm$ 0.016 <sup>c</sup>	0.106 $\pm$ 0.014 <sup>c</sup>
OWE	0.283 $\pm$ 0.015 <sup>a</sup>	0.259 $\pm$ 0.006 <sup>a</sup>
Acarbose	0.272 $\pm$ 0.008 <sup>b</sup>	0.226 $\pm$ 0.009 <sup>b</sup>

<sup>1)</sup>IC<sub>50</sub> value is the concentration of a sample required for 50% inhibition. Each value is expressed as the mean $\pm$ SD of triplicate experiments. Values with different superscript letters within a column are significantly different ( $p < 0.05$ ) based on Duncan's multiple range tests. OEE: *O. Mishchenko* 80% ethanol extract; OWE: *O. Mishchenko* water extract; Acarbose: positive control.

were fed a pelleted feed (5L79; Orient, Inc., Seoul, Korea). After an adaptation period, diabetes was induced as described below. All procedures involving in the handling and care of mice complied with the current international laws and policies (National Institutes of Health Guide for the Care and Use of Laboratory Animals), and were approved by the Animal Ethics Committee of the University (PNU-2019-2468).

#### Induction of experimental diabetes in a mouse model

Diabetes was induced by an intraperitoneal injection of streptozotocin (STZ; 60 mg/kg, Sigma-Aldrich Chemical Co.) dissolved in citrate buffer (0.1 M, pH 4.5). The fasting blood glucose level was checked at 7 days post injection to confirm the induction of diabetes, using blood from the tail vein with a glucose meter (Roche Diagnostics GmbH, Mannheim, Germany) and glucose strips. Mice with fasting blood glucose level above 250 mg/dl were regarded as being diabetic.

#### Measurement of blood glucose levels

The mean blood glucose level in each group (normal mice and diabetic mice) was similar, and each group was divided into four sub-groups of seven mice. A total of 8 groups were used and the following were orally administered after overnight fasting: 1) control: soluble starch (2 g/kg of body weight [BW]), 2) OEE: soluble starch with OEE (300 mg/kg of BW), 3) OWE: soluble starch with OWE (300 mg/kg of BW), 4) acarbose: soluble starch with acarbose (100 mg/kg of BW). Blood samples were collected from the tail vein at 0, 15, 30, 60, and 120 min, and blood glucose was checked using the blood glucose meter. Areas under the concentration-time curves (AUCs) were identified using the trapezoidal rule.

#### Statistical analysis

Data are represented as the mean  $\pm$  standard deviation (SD). Statistical analysis was conducted using SAS software ver. 9.1 (SAS Institute, Inc., Cary, NC, USA). The *t*-test was utilized to compare the control and sample groups. Dissimilarity between groups was assessed by one-way analysis of variance, followed by Duncan's post-hoc multiple range tests. A *p*-value < 0.05 was regarded as being significant.

## Results

#### Cytotoxic effect of *O. Mishchenko*

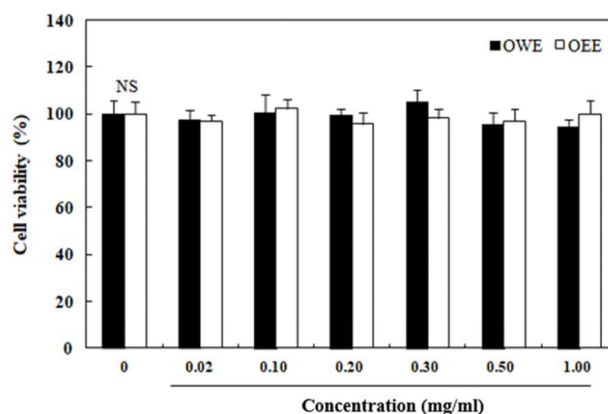


Fig. 1. Effect of *O. Mishchenko* extracts on cytotoxicity in 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0.02, 0.10, 0.20, 0.30, 0.50, and 1.00 mg/ml) of OEE and OWE for 20 hr, and cytotoxic effects were measured by the MTT assay. Each value is expressed as the mean  $\pm$  SD of triplicate experiments. NS: not significant. OEE: *O. Mishchenko* 80% ethanol extract; OWE: *O. Mishchenko* water extract; Acarbose: positive control.

The viability of 3T3-L1 cells treated with various concentrations of OEE and OWE, as described above, was evaluated using the MTT assay. OEE and OWE did not affect cell viability till a concentration of 1.00 mg/ml, the highest used in this study (Fig. 1).

#### Inhibition of $\alpha$ -glucosidase by *O. Mishchenko* extracts *in vitro*

Alpha-glucosidase converts the carbohydrates degraded by  $\alpha$ -amylase into glucose [8]. Inhibition of this enzyme is thought to prevent the rapid rise of blood glucose after meals by delaying glycolysis and absorption. Therefore, the inhibitory activity of OEE and OWE on  $\alpha$ -glucosidase (EC 3.2.1.20) was measured. The inhibitory activity of OEE was found to be dose-dependent in nature, i.e., 20.53, 33.47, 37.84, 46.87, and 57.42% at 0.02, 0.05, 0.10, 0.20, and 0.30 mg/ml, respectively (Fig. 2), same was the case with OWE, and the levels of inhibition were 19.04, 28.63, 36.99, 42.11, and 51.52% at concentrations of 0.02, 0.05, 0.10, 0.20, and 0.30 mg/ml, respectively. In particular, OEE showed significantly higher  $\alpha$ -glucosidase inhibitory activity than OWE. IC<sub>50</sub> values for OEE with respect to  $\alpha$ -glucosidase activity were 0.229 mg/ml, and the same was 0.283 mg/ml for OWE. Acarbose, a commercial hypoglycemic pharmaceutical product, inhibited enzyme activity by 52.45% at a concentration of 0.30 mg/ml. Thus, at the same concentration (0.30 mg/ml), OEE showed markedly higher inhibitory activities than acarbose.

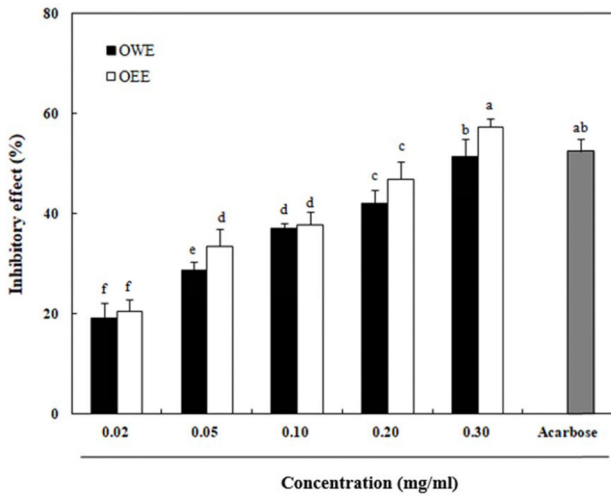


Fig. 2. Effect of *O. Mishchenko* extracts on  $\alpha$ -glucosidase inhibitory activities. Each value is expressed as the mean  $\pm$  SD of triplicate experiments. Values with different superscript letters are significantly different ( $p < 0.05$ ) based on Duncan's multiple range tests. The concentration of acarbose, used as a positive control, was 0.30 mg/ml. OEE: *O. Mishchenko* 80% ethanol extract; OWE: *O. Mishchenko* water extract; Acarbose: positive control.

**Inhibition of  $\alpha$ -amylase by *O. Mishchenko* extracts *in vitro***

The inhibitory activities of OEE and OWE against  $\alpha$ -amylase are shown in Fig. 3. OEE inhibited  $\alpha$ -amylase by 20.69, 35.37, 49.03, 63.42, and 65.98% at concentrations of 0.02, 0.05,

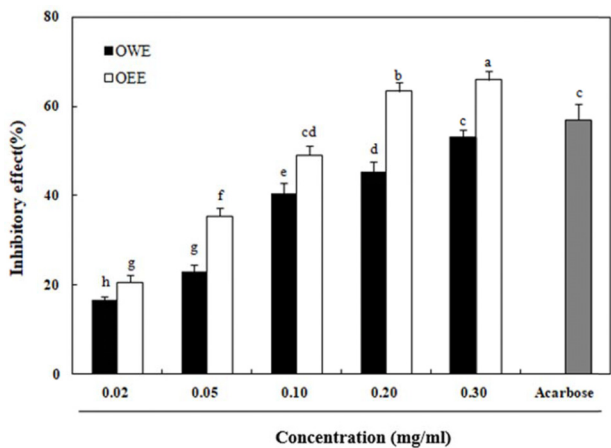


Fig. 3. Effect of *O. Mishchenko* extracts on  $\alpha$ -amylase inhibitory activities. Each value is expressed as the mean  $\pm$  SD of triplicate experiments. Values with different superscript letters are significantly different ( $p < 0.05$ ) based on Duncan's multiple range tests. The concentration of acarbose, used as a positive control, was 0.30 mg/ml. OEE: *O. Mishchenko* 80% ethanol extract; OWE: *O. Mishchenko* water extract; Acarbose: positive control.

0.10, 0.20, and 0.30 mg/ml, respectively. The inhibitory effect of OWE against  $\alpha$ -amylase was also concentration-dependent, and the levels of inhibition were 16.55, 22.99, 40.54, 45.37 and 53.11% at concentrations of 0.02, 0.05, 0.10, 0.20, and 0.30 mg/ml, respectively. OEE inhibited  $\alpha$ -amylase more effectively than OWE. The inhibitory effect of OEE against  $\alpha$ -amylase was markedly higher than that of acarbose at the same concentration (0.30 mg/ml). The  $IC_{50}$  value of OEE against  $\alpha$ -amylase was 0.106 mg/ml, and that of OWE and acarbose was 0.259 and 0.226 mg/ml, respectively, indicating that OEE had significantly higher inhibitory activity than acarbose.

**Effect of *O. Mishchenko* extract on blood glucose levels *in vivo***

OWE (300 mg/kg body weight, BW), OEE (300 mg/kg BW), and acarbose (100 mg/kg BW) were orally administered to mice, with soluble starch (2 g/kg BW), to confirm the inhibitory effect of the *O. Mishchenko* extracts on the levels of postprandial blood glucose. After administration of the extracts, blood was collected from the tail vein of normal mice and STZ-induced diabetic mice at 0, 15, 30, 60, and 120 min, and the change in postprandial blood glucose levels was measured. In normal mice, blood glucose levels increased to 242.33 mg/dl at 30 min administration of starch (Fig. 4A). Normal mice administered starch with OEE (155.00, 179.40, 188.80, and 127.40 mg/ml at 15, 30, 60, and 120 min, respectively) or OWE (172.00, 192.50, 190.83, and 139.67 mg/ml at 15, 30, 60, and 120 min, respectively) showed significantly decreased levels of blood glucose. In diabetic mice, the blood glucose level in the control group, administered with only soluble starch, increased to 406.50, 431.50, and 473.50 mg/ml after 15, 30, and 60 min, respectively dropping to 460.00 mg/ml after 120 min (Fig. 4B). The results with OEE and soluble starch were 327.00, 361.00, 358.67, and 291.00 mg/ml after 15, 30, 60, and 120 min, respectively. When OWE was administered with starch, postprandial hyperglycemia also decreased (374.50, 381.00, 397.00, and 365.00 mg/ml after 15, 30, 60, and 120 min, respectively), but this was not as pronounced as that observed with OEE. Peak postprandial blood glucose was also significantly lower in the normal (non-diabetic) group, following starch and OEE administration, than in those administered starch alone. The AUC of the diabetic group treated with OEE ( $663.58 \pm 78.62$  mg/ml) was significantly lower than that of the diabetic control group ( $880.68 \pm 53.77$  mg/ml)

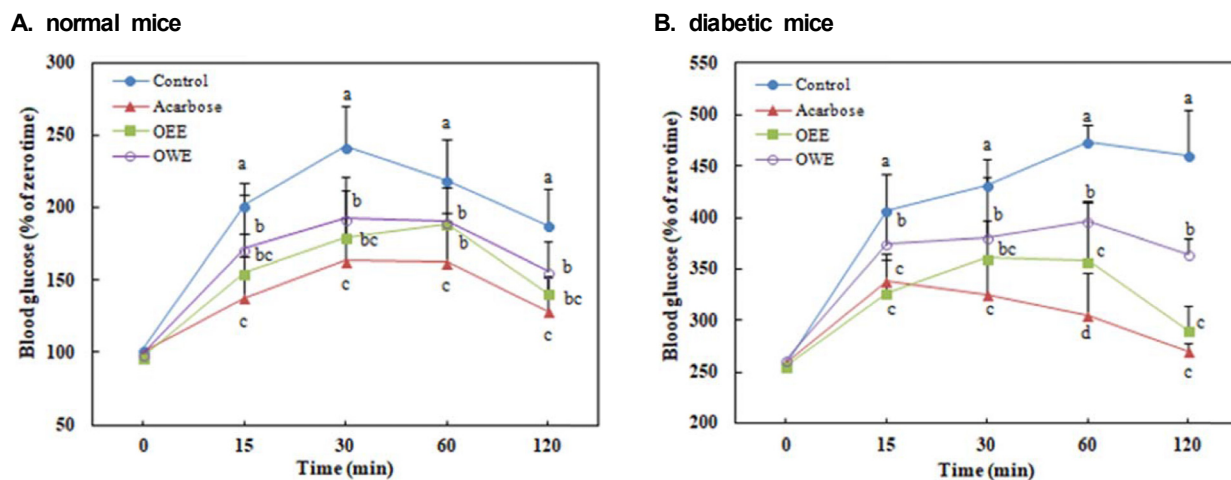


Fig. 4. Blood glucose levels after administration of *O. Mishchenko* extracts to streptozotocin-induced diabetic mice and normal mice. Control (distilled water), OEE (300 mg/kg), OWE (300 mg/kg), or acarbose (100 mg/kg) were orally co-administered with starch (2 g/kg). Each value is expressed as the mean  $\pm$  SD of seven mice per group. Values with different superscript letters are significantly different ( $p < 0.05$ ) based on Duncan's multiple range tests. OEE: *O. Mishchenko* 80% ethanol extract; OWE: *O. Mishchenko* water extract; Acarbose: positive control.

(Table 2). The acarbose (oral hypoglycemic agent used as a positive control) and OEE groups showed similar patterns for blood glucose levels and AUCs.

## Discussion

Diabetes is a metabolic disease characterized by hyperglycemia. Chronic hyperglycemia causes complications such as macrovascular and microvascular issues, diabetic neuropathy, and kidney disease [6, 20, 28]. The treatment of diabetes is generally a combination of medication, diet and exercise. The goal of treatment is to maintain ideal blood

glucose, to prevent or delay diabetic complications [18, 35]. It is critical to maintain the postprandial and fasting blood glucose levels as close as possible to the normal levels [6]. It has been reported that postprandial hyperglycemia reduces insulin sensitivity, impairs pancreatic function, and reduces insulin secretion, thereby exacerbating the diabetic condition and causing macrovascular and microvascular complications [20]. In general, starch ingested via the diet is digested into small sugars by  $\alpha$ -amylase, and then into glucose by  $\alpha$ -glucosidase on the brush border of the mucosa in the small intestine, which leads to an increase in blood glucose after absorption. In this respect, inhibition of carbohydrate hydrolases can play an important role in controlling blood glucose and provide effective anti-diabetic control by targeting postprandial hyperglycemia [45].

Alpha-glucosidase is an enzyme that breaks down carbohydrates in the diet and converts them into glucose, and  $\alpha$ -glucosidase inhibitors slow down the increase in postprandial blood glucose by delaying carbohydrate digestion and absorption [15, 16]. Long-term use of  $\alpha$ -glucosidase inhibitors may cause side effects such as bloating, vomiting and diarrhea in some patients, which may limit their use. Therefore, research is under way to search for hypoglycemic agents from among natural products that have few side effects [23].

As natural products, edible insects have been used to treat various diseases. *Oxya chinensis sinuosa* Mishchenko has long been used, orally, as a medicine, particularly in Korea.

Table 2. Areas under the concentration - time curves (AUCs) of postprandial glucose responses in normal and streptozotocin-induced diabetic mice

Group <sup>1)</sup>	AUC (mg · hr/dl)	
	Normal mice	Diabetic mice
Control	400.04 $\pm$ 39.25 <sup>a</sup>	880.68 $\pm$ 53.77 <sup>a</sup>
OEE	323.42 $\pm$ 43.87 <sup>c</sup>	663.58 $\pm$ 78.62 <sup>c</sup>
OWE	340.56 $\pm$ 51.76 <sup>b</sup>	749.43 $\pm$ 51.52 <sup>b</sup>
Acarbose	287.81 $\pm$ 56.39 <sup>d</sup>	602.25 $\pm$ 53.11 <sup>c</sup>

<sup>1)</sup>OEE (300 mg/kg), OWE (300 mg/kg), acarbose (100 mg/kg), and distilled water (control) were orally co-administered with starch (2 g/kg). Each value is expressed as the mean $\pm$ SD of seven mice. Values with different superscript letters within a column are significantly different ( $p < 0.05$ ) based on Duncan's multiple range tests. OEE: *O. Mishchenko* 80% ethanol extract; OWE: *O. Mishchenko* water extract; Acarbose: positive control.

In recent years, it has been recognized as a non-polluting, nutritional foodstuff, and its supply has increased annually. *O. Mishchenko* contains abundant amounts of energy, protein, unsaturated fatty acids, trace nutrients—such as zinc, phosphorus, and calcium—and pigments, such as chlorophyll, carotenoids, and polyphenols [30]. It has been used in traditional anti-diabetic medicines [13]. Nevertheless, there is no experimental data demonstrating a relationship between the suppression of glucose absorption in the intestine, postprandial blood glucose levels and *O. Mishchenko*. Thus, this study aimed to investigate whether supplementation by *O. Mishchenko* inhibits  $\alpha$ -glucosidase. We investigated the effect of OEE and OWE on the activity of  $\alpha$ -glucosidase. The results showed that both OEE and OWE inhibited  $\alpha$ -glucosidase, in particular OEE showed higher inhibitory activity than acarbose, a commercial inhibitor of  $\alpha$ -glucosidase.

*O. Mishchenko* has a higher content of unsaturated fatty acids, chlorophyll, carotenoids, and polyphenols than other edible insects. In the case of freeze-dried samples, polyphenols, such as flavonoids, terpenoids, and phenolic acids are present at 14.06-17.61 mg/100 g [30, 31]. Polyphenols are known to exhibit physiological activities, such as antioxidant, anti-obesity, anti-inflammatory, and anti-diabetic effects, and play an important role in the inhibition of glycosidase enzymes [1, 22]. Phenolic acids are generally classified into two major groups, i.e., benzoic acids containing 7 carbon atoms and cinnamic acids containing 9 carbon atoms. Cinnamic acid and its derivatives are a major group of molecules that are ubiquitously distributed in fruits, vegetables, and edible insects [3, 5, 11]; they have been investigated for their potential as inhibitors of carbohydrate hydrolases. Studies have reported that the hydroxyl groups of cinnamic acid can play an important role in the inhibition of enzymes such as pancreatic  $\alpha$ -amylase. Inhibition of  $\alpha$ -amylase is largely due to the presence of hydroxyl groups at the para and meta-positions of the cinnamic acid molecule [3, 4, 36]. Buszewska-Forajra [11] detected cinnamic acid derivatives as components of grasshoppers, using gas chromatography-tandem mass spectrometry (GC-MS/MS). Cinnamic acid and its derivatives are one of the most abundant groups of compounds found in grasshoppers, and may contribute, at least in part, to the inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase *in vitro*. In addition, when extracting ethanol, it is expected that the carbohydrate digestive enzyme inhibitory activity of the ethanol extract rich in polyphenol content would be higher than that of the water extract because polyphenols

are more polar than water [5, 26]. Some studies have shown that the anti-inflammatory, apoptosis-protective effects, and antioxidant activities of the ethanol extract of *O. Mistshenk* were more potent than those of the water extract, which was consistent with our results [38, 44].

In patients with type 2 diabetes, blood glucose increases rapidly after eating, and if higher levels of blood glucose persist, various diabetic complications occur [42]. There are two major factors that control diabetes, one is blood glucose regulation through insulin secretion, and the other is delayed digestion and absorption of carbohydrates resulting in suppression of the rapid increase in blood glucose after eating [32]. In this study, the focus was on the latter, and it is an important factor in controlling diabetes, which regulates fasting blood glucose and postprandial blood glucose levels. In particular, it is more important to control blood glucose levels after eating than to control fasting blood glucose [7]. In this study, to verify the results of the *in vitro* test, we used an animal model in which hyperglycemia was induced with STZ to confirm the ability of the extracts to control postprandial blood glucose levels.

The hypoglycemic effect of OEE was greater than that of OWE, after starch loading in the animal model. OEE significantly reduced postprandial hyperglycemia when administered to diabetic mice. These results suggest that the OEE supplementation slows postprandial hyperglycemia by delaying the absorption of starch. Acarbose is an oral hyperglycemic agent that lowers blood glucose levels after meals, and significantly reduces the AUC value [23]. OEE also significantly reduced the AUC value in our study, in addition to reducing the maximum blood glucose level. Our results indicate that OEE alleviates postprandial hyperglycemia by delaying the absorption of dietary carbohydrates, due to the inhibitory activity of OEE on carbohydrate digestive enzymes.

Postprandial hyperglycemia is an independent contributor to diabetes complications as well as being a feature of diabetes [19]. Various epidemiological studies have suggested that postprandial hyperglycemia may be more closely correlated with cardiovascular morbidity and mortality than fasting hyperglycemia [10]. Pharmaceutical agents, especially acarbose, can reduce postprandial hyperglycemia by alleviating blood glucose levels after eating. However, these are usually associated with side effects, such as weight gain, abdominal discomfort, and diarrhea [14, 21]. Our results indicate that *O. Mishchenko* can improve postprandial hyper-

glycemia and help prevent the occurrence of diabetic complications.

Recently, there have been an increased number of studies investigating the anti-diabetic effects of edible insects. For example, an extract of *Gryllus bimaculatus* effectively inhibited the occurrence of diabetes by protecting pancreatic islet function, and significantly reduced blood glucose in a diabetic animal model [41]. In another example, the increase in plasma membrane GLUT4 expression upon administration of the mealworm extract has been known to promote the uptake of blood glucose into cells and relieved hyperglycemia in diabetic C57BL/Ksj-db/db mice [29]. In another study, *Bombycis corpus* normalized the blood glucose and serum insulin levels in diabetic rats [24]. Silkworms also exhibit therapeutic potential for reducing the plasma glucose levels in db/db mice, and show inhibitory activity against  $\alpha$ -glucosidase [39].

In summary, in our study we were able to demonstrate that *O. Mishchenko* extract strongly inhibits the activities of  $\alpha$ -glucosidase and  $\alpha$ -amylase. Comparison of the two extracts shows that OEE has a stronger inhibitory effect than OWE *in vitro*. Administration of OEE or OWE with starch delayed the digestion of carbohydrates and absorption of glucose, resulting in alleviation of postprandial hyperglycemia *in vivo*. OEE co-administration alleviated postprandial blood glucose levels more than OWE alone in diabetic mice. Thus, OEE seems to be a better candidate than OWE as a potential functional food to decrease postprandial hyperglycemia.

### Acknowledgement

This research was supported by the Global Ph.D. Fellowship Program through the National Research Foundation of Korea (NRF-2019H1A2A1074826).

### The Conflict of Interest Statement

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

### References

1. Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, M. T., Bashir, M. S., Ishtiaq, A., Hussain, S. and Suleria, H. A. 2016. Natural polyphenols: an overview. *Int. J. Food Prop.* **20**, 1689-1699.
2. Abid, S., Lekchiri, A., Mekhfi, H., Ziyat, A., Legssyer, A., Aziz, M. and Brouham, M. 2014. Inhibition of  $\alpha$ -glucosidase and glucose intestinal absorption by *Thymelaea hirsuta* fractions. *J. Diabetes* **6**, 351-359.
3. Adisakwattana, S., Sookkongwaree, K., Roengsumran, S., Petsom, A., Ngamrojnavanich, N., Chavasiri, W., Deesamer, S. and Yibchok-anun, S. 2004. Structure-activity relationships of trans-cinnamic acid derivatives on  $\alpha$ -glucosidase inhibition. *Bioorg. Med. Chem. Lett.* **14**, 2893-2896.
4. Adisakwattana, S., Chantarasinlapin, P., Thammarat, H. and Yibchok-Anun, S. 2009. A series of cinnamic acid derivatives and their inhibitory activity on intestinal  $\alpha$ -glucosidase. *J. Enzyme Inhib. Med. Chem.* **24**, 1194-1200.
5. Adisakwattana, S. 2017. Cinnamic acid and its derivatives: mechanisms for prevention and management of diabetes and its complications. *Nutrients* **9**, 163.
6. Asano, N., Tomioka, E., Kizu, H. and Matsui, K. 1994. Sugars with nitrogen in the ring isolated from the leaves of *Morus bombycis*. *Carbohydr. Res.* **253**, 235-245.
7. Avignon, A., Radauceanu, A. and Monnier, L. 1997. Non-fasting plasma glucose is a better marker of diabetic control than fasting plasma glucose in type 2 diabetes. *Diabetes Care* **20**, 1822-1826.
8. Baron, A. D. Postprandial hyperglycaemia and  $\alpha$ -glucosidase inhibitors. 1998. *Diabetes Res. Clin. Pract.* **40**, 51-55.
9. Basu, S., Yudkin, J. S., Kehlenbrink, S., Davies, J. I., Wild, S. H., Lipska, K. J., Sussman, J. B. and Beran, D. 2018. Estimation of global insulin use for type 2 diabetes, 2018-30: a microsimulation analysis. *Lancet Diabetes Endocrinol.* **7**, 25-33.
10. Bonora, E. and Muggeo, M. 2001. Postprandial blood glucose as a risk factor for cardiovascular disease in type II diabetes; the epidemiological evidence. *Diabetologia* **44**, 2107-2114.
11. Buszewska-Forajta, M., Struck-Lewicka, W., Bujak, R., Siluk, D. and Kaliszan, R. 2014. Determination of water-soluble components of abdominal secretion of grasshopper (*Chorthippus* spp.) by GC/MS/MS in search for potential wound healing agents. *Chromatographia* **77**, 1091-1102.
12. Chinenye, S. and Young, E. E. 2012. Isolated postprandial hyperglycemia in type 2 diabetic patients in a Nigerian Tertiary Health Center. *Indian J. Endocrinol. Metab.* **16**, 604-608.
13. Chung, M. Y., Kwon, E. Y., Hwang, J. S., Goo, T. W. and Yun, E. Y. 2013. Pretreatment conditions on the powder of *Tenbrio molitor* for using as a novel food ingredient. *J. Seric. Entomol. Sci.* **51**, 9-14.
14. Clissold, S. P. and Edwards, C. 1988. Acarbose: a preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. *Drugs* **3**, 214-243.
15. Forman, L. J., Estilow, S., Lewis, M. and Vasilenko, P. 1986. Streptozotocin diabetes alters immunoreactive beta-endorphin levels and pain perception after 8 wk in female rats. *Diabetes* **35**, 1309-1313.
16. Fujita, H., Yamagami, T. and Ohshima, K. 2001. Efficacy and safety of Touchi extract,  $\alpha$ -glucosidase inhibitor derived

- from fermented soybeans, in non-insulin-dependent diabetic mellitus. *J. Nutr. Biochem.* **12**, 351-356.
17. Gerich, J. E. 2003. Clinical significance, pathogenesis, and management of postprandial hyperglycemia. *Arch. Intern. Med.* **163**, 1306-1316.
  18. Goldmann, A., Milat, M. L. and Ducrot, P. H. 1990. Tropane derivatives from *Calystegia sepium*. *Phytochemistry* **29**, 2125-2128.
  19. Grundy, S. M., Benjamin, I. J., Burke, G. L., Chait, A., Eckel, R. H. and Howard, B. V. 1999. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* **100**, 1134-1146.
  20. Haller, H. 1998. The clinical importance of postprandial glucose. *Diabetes Res. Clin. Pract.* **40**, 43-49.
  21. Hanefeld, M. 1998. The role of acarbose in the treatment of non-insulin-dependent diabetes mellitus. *J. Diabetes Complications* **12**, 228-237.
  22. Hanhineva, K., Törrönen, R. and Bondia-Pons, I. 2010. Impact of dietary polyphenols on carbohydrate metabolism. *Int. J. Mol. Sci.* **11**, 1365-1402.
  23. Inoue, I., Takahashi, K., Noji, S., Awata, T., Negishi, K. and Kataya-ma, S. 1997. Acarbose controls postprandial hyperproinsulinemia in non-insulin-dependent diabetes mellitus. *Diabetes Res. Clin. Pract.* **36**, 143-151.
  24. Jeong, B. M., Hyun, M. K., Sin, W. Y., Kim, M. R., Shin, H. C., Yoon, C. H. and Jeong, J. C. 2004. Effects of *Bombycis corpus* on streptozotocin-induced diabetic rats. *J. Internal. Kor. Med.* **25**, 288-297.
  25. Kalita, D., Holm, D. G., LaBarbera, D. V., Petrash, J. M. and Jayanty, S. S. 2018. Inhibition of alpha-glucosidase, alpha-amylase, and aldose reductase by potato polyphenolic compounds. *PLoS One* **13**, 0191025.
  26. Kim, D. S., Choi, M. H. and Shin, H. J. 2018. Polyphenol contents and antioxidant activities of domestic bamboo leaves with different extraction solvents. *J. Adv. Engin. Technol.* **11**, 7-13.
  27. Kim, H. J., Kang, S. J., Kim, S. G., Kim, J. E., Koo, H. Y., Park, J. H. and Choi, H. C. 2015. Antioxidant activity and antimicrobial activity of the grasshopper, *Oxya chinensis sinuosa*. *J. Seric. Entomol. Sci.* **53**, 130-134.
  28. Kim, J. S., Kwon, C. S. and Son, K. H. 2000. Alpha-glucosidase inhibitory activities of some wild vegetable extracts. *J. Food Sci. Nutr.* **5**, 174-176.
  29. Kim, S. Y., Park, J. E. and Han, J. S. 2019. *Tenebrio molitor* (Mealworm) extract improves insulin sensitivity and alleviates hyperglycemia in C57BL/Ksj-db/db mice. *J. Life Sci.* **5**, 570-579.
  30. Kim, T. S., Lee, J. H., Choi, B. D. and Ryu, H. S. 1987. Nutritional value of dried paddy grasshopper, *Oxya chinensis formosana*. *J. Kor. Soc. Food Nutr.* **16**, 98-104.
  31. Kim, Y. S. and Kwon, T. D. 2018. Effects of grasshopper diet and treadmill exercise performance of blood lipid profile and antioxidant enzyme in rats. *Kor. J. Sports Sci.* **27**, 1605-1614.
  32. Krentz, A. J. and Bailey, C. J. 2005. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs* **65**, 385-411.
  33. Laakso, M. 1999. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes* **48**, 937-942.
  34. Li, Y., Wen, S., Kota, B. P., Peng, G., Li, G. Q., Yamahara, J. and Roufogalis, B. D. 2005. *Punica granatum* flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. *J. Ethnopharmacol.* **99**, 239-244.
  35. Madar, Z. 1984. Effect of brown rice and soybean dietary fiber on the control of glucose and lipid metabolism in rats. *Am. J. Clin. Nutr.* **43**, 388-396.
  36. Narita, Y. and Inouye, K. 2009. Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas  $\alpha$ -amylase isozymes I and II. *J. Agric. Food Chem.* **57**, 9218-9225.
  37. Paek, M. K., Hwang, J. M., Jung, K. S., Kim, T. W., Kim, M. C., Lee, Y. J., Cho, Y. B., Park, S. W., Lee, H. S., Ku, D. S., Jeong, J. C., Kim, K. G., Choi, D. S., Shin, E. H., Jwang, J. H., Lee, J. S., Kim, S. S. and Bea, Y. S. 2010. Checklist of Korean Insects. Nature and Ecology. Pub. Seoul, Korea. **36**.
  38. Park, J. Y., Heo, J. C., Woo, S. U., Yun, C. Y., Kang, S. W., Hwang, J. S. and Lee, S. H. 2006. Anti-inflammatory and cellular protective effects on hydrogen peroxide induced cytotoxicity of grasshopper extracts. *Kor. J. Food Preserv.* **13**, 796-802.
  39. Ryu, K. S., Lee, H. S., Kim, K. Y., Kim, M. J., Kang, P. D. and Chun, S. N. 2012. Anti-diabetic effects of the silkworm (*Bombyx mori*) extracts in the db/db mice. *Planta Med.* **78**, 458.
  40. Salimifar, M., Fatehi-Hassanabad, Z. and Fatehi, M. 2013. A review of natural products for controlling type 2 diabetes with an emphasis on their mechanisms of action. *Curr. Diabetes Rev.* **9**, 402-411.
  41. Taek, C. H., Sang, S. K., Yeona, K., Han, C. M., Taewan, K., Hwan, L. S., Lee, D. H. and Ho, K. J. 2019. Anti-diabetic activity of edible insect *Gryllus bimaculatus* extracts in insulin-deficient diabetic mice. *J. Kor. Soc. Food Sci. Nutr.* **10**, 1165-1171.
  42. Tai, E., Lim, S. C., Tan, B. Y., Chew, S. K., Heng, D. and Tan, C. E. 2000. Screening for diabetes mellitus-a two-step approach in individuals with impaired fasting glucose improves detection of those at risk of complications. *Diabet. Med.* **17**, 771-775.
  43. Watanabe, J., Kawabata, J., Kurihara, H. and Niki, R. 1997. Isolation and identification of alpha-glucosidase inhibitors from tochucha (*Eucommia ulmoides*). *Biosci. Biotechnol. Biochem.* **61**, 177-178.
  44. Yoon, Y. I., Chung, M. Y., Hwang, J. S., Goo, T. W., Ahn, M. Y., Lee, Y. B., Han, M. S. and Yun, E. Y. 2014. Anti-inflammatory effect of *Oxya chinensis sinuosa* ethanol extract in LPS-induced RAW 264.7 cells. *J. Life Sci.* **24**, 370-376.
  45. Young, I. R. and Stout, R. W. 1987. Effects of insulin and glucose on the cells of the arterial wall: Interaction of insulin with dibutyryl cyclic AMP and low density lipoprotein in arterial cells. *Diabete Metab.* **13**, 301-306.



---

**초록 : 당뇨 모델을 이용한 벼메뚜기(*O. Mistshenk*) 추출물의 식후 고혈당 완화 효과**

박재은 · 한지숙\*

(부산대학교 식품영양학과)

벼메뚜기는 메뚜기과에 속하는 불완전 변태 곤충으로, 국내에서 오랫동안 식용으로 이용되어 왔다. 현재까지 벼메뚜기의 항산화 및 항염증 효과 등의 기능들이 연구되었으나, 탄수화물 소화 효소나 식후 혈당 수치에 미치는 영향에 관한 연구는 부족한 실정이다. 이에 본 연구는 벼메뚜기 에탄올 추출물(OEE)과 물 추출물(OWE)이 탄수화물 소화 효소를 저해하고, streptozotocin (STZ)으로 유도된 당뇨병 마우스에서 식후 고혈당을 강하시키는 효과에 대해 조사하였다. 그 결과 OEE에 의한  $\alpha$ -glucosidase와  $\alpha$ -amylase 저해 효과가 OWE 보다 더 효과적이었다. 당뇨병 마우스에 전분(2 g/kg)을 투여한 후의 혈당 증가는 15, 30, 60, 120분에 각각 406.50, 431.50, 473.50 and 460.00 mg/dl로 나타났고, 전분(2 g/kg)과 OEE 추출물(300 mg/kg)을 투여한 후 혈당 증가는 15, 30, 60, 120분에 각각 327.00, 361.00, 358.67 and 291.00 mg/dl로 나타나, OEE 추출물 투여군이 대조군에 비해 식후 혈당 강하가 효과적으로 나타남을 알 수 있었다. 이러한 결과는 벼메뚜기 추출물이  $\alpha$ -glucosidase와  $\alpha$ -amylase를 저해함으로써 식후 고혈당을 완화시키고, 특히 벼메뚜기 에탄올 추출물(OEE)이 벼메뚜기 물 추출물(OWE) 보다 식후 고혈당을 완화시키는데 더욱 효과가 있는 것으로 나타났다. 따라서 OEE가 탄수화물 소화 효소 저해 효과로 식후 고혈당을 완화시키는 유용한 천연 기능성 식품이 될 것으로 사료된다.