Acute effects of *Amomum villosum* Lour. fruit extract on postprandial glycemia and insulin secretion: A single-blind, placebo-controlled, crossover study in healthy subjects

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**Abstract**

**Background:** *Amomum villosum* Lour., (Zingiberaceae) an herbaceous plant in the ginger family, has been used to treat various diseases. In a single-blind, randomized, crossover study, we assessed the postprandial blood insulin and blood glucose responses in healthy subjects (n = 40) after the *Amomum villosum* water extract (AVE) (5 g/person) or a placebo (5 g/person) consumption.

**Methods:** During each treatment course, the healthy subject consumed a regular late afternoon meal, followed by fasting for 12 h, and arrived at the clinical study center the next morning. Blood insulin and blood glucose levels were assessed at 0, 30, 60, 90, and 120 min after AVE consumption. Between each treatment, the subjects accomplished one week of a washout period.

**Results:** The AVE intake demonstrated a significant (67.26%) decline in postprandial blood glucose AUC0–120 min (incremental area under the curve from 0 to 120 min) versus the placebo (*P* = 0.011). Furthermore, AVE reduced postprandial blood insulin AUC0–120 min by 59.95% compared to the placebo group (*P* < 0.003), supporting the blood glucose results.

**Conclusion:** This study revealed that AVE consumption significantly reduced postprandial insulin and glucose levels in healthy individuals, due in part to inhibition of *α*-glucosidase, and glucose transport.

1. Introduction

Prediabetes, also known as intermediate hyperglycemia, leads to diabetic conditions and is described as a glycemic fluctuation that is lower than diabetes conditions, but greater than normal. Each year, nearly 5–10% of the population with prediabetes will reach a diabetic state, with the same percentage approaching back to normal. The worldwide frequency of prediabetes has been growing significantly and specialists have estimated that by 2030 more than 470 million individuals will be affected by prediabetes (Aroda and Ratner, 2008). Notably, prediabetes is related to the concurrent occurrence of β-cell dysfunction and insulin resistance, which begins prior to glucose fluctuations. Additionally, people affected by prediabetes may frequently develop hypertension, insulin resistance-associated obesity, and hyperlipidemia (Aroda and Ratner, 2008). Hence, decreasing the cardiovascular disease risk factors, especially lipid and blood pressure abnormalities and intensive lifestyle intervention, are necessary to avoid the progress of prediabetes to diabetes (Twigg et al., 2007).

In patients, the prominent approach used in diabetes management is the regulation of postprandial blood glucose levels. Dietary sugars such as starch, sucrose, and maltose are degraded in the small intestine into monosaccharides (glucose, fructose, galactose) by *α*-glucosidases before absorption. Acarbose is an *α*-glucosidase inhibitor widely used in the treatment of type 2 diabetes or impaired glucose tolerance (Van de Laar, 2008, Van de Laar,
In the small intestine, α-glucosidase inhibitors hinder the digestion or absorption of simple carbohydrates, indicating a lower effect on postprandial blood glucose level (Van de Laar, 2008). Notably, α-glucosidase inhibitors demonstrated considerable efficacy in hyperglycemia when compared with other anti-hyperglycemic drugs. 

Ever since the prehistoric period, medicinal plants have been used effectively in all cultures worldwide for monitoring and preventing diabetes. *Amomum villosum* Lour., belonging to the genus Zingiberaceae, has more than 1300 years of medicinal history in healthcare (Duang et al., 2009). Earlier pharmacological research has reported that Amomi Fructus has significant anti-diarrheal, anti-inflammatory, anti-ulceration, and antimicrobial effects (Huang et al., 2014). While *A. villosum* has broadly been used in the management of numerous diseases, its role in the treatment of postprandial glycemia and insulin secretion remains unclear. Here, we revealed the effects of *A. villosum* water extract (AVE) on postprandial insulin secretion and glycemia in healthy subjects as a single-blind, placebo-controlled, and crossover study.

### 2. Materials and methods

#### 2.1. Preparation of crude AV extract

*A. villosum* Lour. (AV) was authenticated by G.S. Lee, a Herbolary specialist, and a voucher specimen was placed at the Department of Herbolary, Wonkwang University Korean Medical School. AV extracts (AVE, 100 g) was extracted using an electric boiling pot for 2–3 h with 1000 mL of deionized water, followed by filtration using a filter paper, and the resulting decoction was spray-dried for 24 h. The final yield of the AVE was 9.25 g. This step was repeated until a sufficient quantity of the water extract was obtained for the clinical trial. The test group material (5 g) contained 24.2% AVE.

#### 2.2. Study subjects, and design

This clinical trial was a randomized, crossover trial carried out at Wonkwang University, Korea, from August to October 2018. The study was performed in agreement with the Declaration of Helsinki, and all processes performed in human subjects were approved by the Iksan Korean Medicine Hospital Institutional Review Board (IRB), permitting the trial (WKUJMHH-IRB-2018-1). Prior to screening, written informed consent was received from all participants. A total of 44 subjects were screened after clarifying the investigational procedures. Finally, 40 healthy individuals were enrolled (Fig. 1).

At each visit, prior to the oral consumption of drugs, the health of the individual was tested by routine blood chemistry (fasting blood glucose, HbA1c). Additionally, other demographic information (sex, age, height, weight, BMI, SBP, DBP, pulse, alcohol intake, smoking) was also recorded before the clinical trial (Table 3). The individuals take part in two 24-h investigational stages that were initiated the evening before the clinical trial. The selected subjects were required to have consumed a standard dinner before each visit. After fasting for 12 h, the subjects arrived at the study center the following morning. Before drug consumption, fasting blood samples were taken from each individual subject.

This study was designed as a single-blinded, randomized two-visit crossover study, with a 7-day washout period. At every stage, after an overnight fasting (12-hour), the participants consumed the following drugs orally: (1) 5 g placebo + 75 g sucrose in 250 mL of water (placebo group); (2) 5 g AVE + 75 g sucrose in 250 mL of water (test group); within 5 min from the starting time point. Blood samples from each subject were collected before (0 min) and after 30, 60, 90, and 120 min of oral consumption. During the investigational time period, the participants were requested to avoid phenolic-rich nutrition (e.g., fruit juices, coffee, tea, berries, chocolate, etc.), alcoholic beverages, antioxidant-rich diets, and extreme exercise 7 days before each clinical trial. Furthermore, the participants were requested to report their food intake and physical activity details at every single visit (Kang et al., 2016; Nymabe-Silavwe and Williamson, 2016).

At the end of the clinical trial, participants were requested to fill up a post-test questionnaire. Each participant spent a total of approximately 2.5 h at the study center. Next, the participants were offered a snack and then permitted to leave. A blood glucose assay kit (Caressens II, ICENS Co., Ltd., Wonjoo, Korea) was used for blood glucose quantification. Subjects completed a week of washout period in the middle of each clinical trial. The blood glucose levels were evaluated using PKSolver, an easily accessible menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications. The AUCt was evaluated by the linear trapezoidal rule.

#### 2.3. The estimation of serum insulin level

Individual serum separator tubes were assigned for each participant and venous blood samples (7–8 mL) were collected at 0, 30, 60, 90, and 120 min from the test (AVE) and placebo healthy subjects. The blood samples were allowed to clot at room temperature for 15 min and then centrifuged at 1345g for 7 min. All serum samples were examined for insulin quantity using the ELISA assay (Precise Lab Solution).

#### 2.4. Statistical analysis

All experimental data are presented as mean ± SD. Statistical evaluations were performed using the statistical package SPSS 9.3 (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) and significance was verified using a linear mixed effect model for the comparison of means.

### 3. Results and discussion

#### 3.1. Subjects

We assessed the activity of AVE on postprandial blood insulin and glucose levels in healthy subjects with normal blood glucose levels. Forty-four individuals were enrolled in this study in accordance with the flowchart (Fig. 1). After the vital inclusion and exclusion specifications of the clinical trial, four individuals were excluded from the clinical trial. The remaining 40 subjects were randomly allocated into two different groups (placebo and control), with 20 individuals in each group. All 40 participants attended the second phase of the study. Two subjects in the test group were lost to follow-up during phase 1 of the study due to participation after meals. In total, 38 subjects, men and women, successfully completed the investigation. The baseline features of the 40 individuals are presented in Table 1. In addition, all participants were requested to maintain their lifestyle or physical activities without any modifications during the clinical trial. No significant differences in alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, total protein, albumin, blood urea nitrogen (BUN), and creatinine levels were reported between the AVE and placebo groups (Table 2).

#### 3.2. Glycemic response

The AVE treatment indicated significantly lower glucose concentration levels at 30, 60, and 90 min compared to the placebo
Fig. 1. Flowchart for the randomized, single-blinded, two-visit crossover study.

### Table 1
Baseline characteristics of 40 subjects.

| Parameters      | Total (n = 40) |
|-----------------|---------------|
| Sex (M/F)       | 5/35          |
| Age (years)     | 35.23 ± 13.10 |
| Height (cm)     | 161.20 ± 4.99 |
| Weight (kg)     | 59.85 ± 6.47  |
| BMI (kg/m²)     | 23.06 ± 6.47  |
| SBP (mmHg)      | 115.03 ± 8.68 |
| DBP (mmHg)      | 77.83 ± 6.05  |
| Pulse (BPM)     | 81.53 ± 9.63  |
| Glucose (mg/dL) | 75.58 ± 7.39  |
| HbA1c (%)       | 5.29 ± 0.38   |
| Alcohol (n)     | 14 (35.00)    |
| Alcohol (units/week) | 15.24 ± 16.62 |
| Smoking (n)     | 1 (2.50)      |

Values are presented as mean ± SD or number (%)

### Table 2
Diagnostic test results of 40 subjects.

| Parameters      | Test group (n = 40) | Placebo group (n = 40) | p-value¹ |
|-----------------|---------------------|------------------------|----------|
| ALP (IU/L)      | 194.28 ± 62.79      | 194.88 ± 60.51         | 0.876    |
| AST (IU/L)      | 25.28 ± 7.95        | 27.58 ± 12.04          | 0.198    |
| ALT (IU/L)      | 15.80 ± 9.75        | 16.80 ± 9.88           | 0.164    |
| Total bilirubin (mg/dL) | 0.69 ± 0.27        | 0.72 ± 0.39            | 0.408    |
| Total Protein (g/dL) | 7.23 ± 0.48        | 7.15 ± 0.40            | 0.164    |
| Albumin (g/dL)  | 4.32 ± 0.20         | 4.26 ± 0.25            | 0.120    |
| BUN (mg/dL)     | 11.72 ± 3.96        | 11.45 ± 3.53           | 0.551    |
| Creatinine (mg/dL) | 0.85 ± 0.16       | 0.83 ± 0.11            | 0.163    |

Values are presented as mean ± SD.

¹ Analyzed by linear mixed effect model.
Fasting, postprandial glucose response (0–120 min), and glycemic area under the curve (AUC) (0–120 min).

| Parameters                  | Test group (n = 38) | Placebo group (n = 40) | p-value
|------------------------------|--------------------|------------------------|---------
| Fasting blood glucose (mg/dL)| 84.58 ± 10.91      | 88.88 ± 7.20           | 0.040** |
| Blood glucose (mg/dL) 30 min after meal | 123.58 ± 18.77 | 138.13 ± 27.31         | 0.004** |
| Blood glucose (mg/dL) 60 min after meal | 97.34 ± 23.33  | 118.90 ± 36.31         | 0.0006*** |
| Blood glucose (mg/dL) 90 min after meal | 84.50 ± 16.16 | 94.85 ± 22.01          | 0.005** |
| Blood glucose (mg/dL) 120 min after meal | 80.16 ± 12.97 | 84.05 ± 13.71          | 0.107   |
| Glucose AUC               | 1857.40 ± 1516.47  | 2761.57 ± 1778.62      | 0.011*  |

Values are presented as mean ± SD.
*P < 0.05, **P < 0.01, ***P < 0.001.
1) Analyzed by linear mixed effect model.

Group (Table 3; P = 0.004, P = 0.0006 and P = 0.005, respectively). There was a significant (67.26%) reduction in glucose AUC0–120 min in AVE subjects compared to the placebo group (Table 3; P = 0.011).

3.3. Insulinemic response

The blood insulin levels at the distinct time points supported the glucose levels and were significantly lower following AVE consumption compared to the placebo group at 60 and 90 min of post-ingestion (Table 4; P < 0.018, and P < 0.0001, respectively). The AVE group demonstrated significantly lower (59.95%) AUC0–120 min (Table 4, P < 0.003) when compared to the placebo group. In summary, the hypoglycemic effect of AVE was most intense at 60 min (glucose) and 90 min (insulin) compared to the placebo group. Based on these results, we proposed that the elevated postprandial blood glucose level was significantly reduced by AVE oral consumption. The main chemical components in A. villosum are camphor, borneol, borneol acetate, and copaene (Chen et al., 2014).

Among these main ingredients, both camphor and borneol have reported significant anti-diabetic effects in previous studies (Chen et al., 2014; Kodikonda and Naik, 2017). Based on our findings, we believe that these compounds (camphor and borneol) in A. villosum may be responsible for these observed effects. The results of this study have empirically indicated that AVE was effective in the treatment of diabetes. In conclusion, oral AVE augments the reduction of postprandial blood glucose and insulin level compared to the placebo. The long-term effects of AVE need further evaluation.

Declaration of Competing Interest

The authors have no conflict of interest to declare.