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# Heat Stability and Glucose-Lowering Effect of 1-Deoxynojirimycin from Silkworm (*Bombyx mori*) extract Powder

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## Abstract

Silkworm powder, which contains 1-deoxynojirimycin (DNJ), is a promising complementary and alternative medicine (CAM) in Korea. Silkworm powder was produced from Yeonnokjam pupae at d 3 of the 5th instar at the National Academy of Agricultural Science. The powder was derivatized with 9-fluorenylmethyl chloroformate (FMOC-Cl), and the DNJ-FMOC content was measured by HPLC. We investigated the content of 1-DNJ in the silkworm powder and its glucose-lowering effect when it was treated at different temperatures. The content of 1-DNJ was the lowest at 150°C, while it was constant at other temperatures. The silkworm extract powder was orally administered to diabetic mice (20 mg/kg/d) for 4 wk. Water intake did not significantly change when compared with the control group (T0). The blood glucose levels significantly decreased when mice where administered silkworm powder treated at 60°C (T60) compared to the control group, but no difference was observed between the groups T100 and T150. Moreover, the blood levels of TG significantly decreased compared with the control group. Based on these results, we surmise that the properties of the silkworm extract powder were stable upon heating at 100°C but not at 150°C.


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## Introduction

Silkworm and silkworm droppings have long been used in China and Korea as a folk remedy for the treatment of diabetes.1-Deoxynojirimycin (1-DNJ) is the hydrogenation product of nojirimycine, which was firstly discovered in Streptomyces. Natural DNJ was first isolated from the mulberry tree (Yoshikaki and Hivonu, 1976), and to date, more than 20 polyhydroxy alkaloids have been identified in mulberry and silkworm (Asano *et al*., 1994a; 1994b; 2001). As a piperidine alkaloid, DNJ is known to possess highly effective α-glycosidase inhibition activity (Yoshikuni, 1988; Yoshikuni *et al*., 1988; Hughes and Rudge, 1994) and is an effective anti-hyperglycemia agent. Currently, DNJ and its analogs have been extracted from a wide range of plants and microbes (Asano *et al*., 1998; 2000; Kim *et al*., 1999), but its content in the mulberry tree is the highest compared with other plants (Kimura *et al*., 2007; Yatsunami *et al*., 2008). Mulberry has been used in Chinese medicine against diabetes mellitus for a long time. Ryu *et al*. (1997) first reported that the silkworm larval powder of the 5th instar (prepared by lyophilization) had a positive effect on diabetic patients (Ryu *et al*., 1997; 1999), and the action of lowering the blood sugar level was further proved by subsequent research (Han *et al*., 2007). Silkworm powder possesses blood glucose-lowering effects (Ryu *et al*., 2002), and mulberry leaves, which form the diet of silkworms,

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effectively inhibit α-glucosidase in the human small intestine (Oku *et al*., 2006). DNJ from silkworm powder is stable upon heating to 121°C for 15 min (Yatsunami *et al*., 2011). In recent times, in Korea, Japan, and China, mulberry and silkworm larva products are becoming a popular auxiliary therapy for diabetes mellitus. In this report, we measured the content of 1-DNJ in the powder according to different heating temperatures and investigated the glucose-lowering effect of heated silkworm powder on *db/db* mice.

## Materials and Methods

### Preparation of silkworm powder

Silkworm larvae (*Bombyx mori*) were reared by feeding mulberry leaves during the spring season in 2012 at the National Academy of Agricultural Science. The silkworm varieties used for the experiment was Yeonnokjam. The larvae of 3$^{rd}$ d of the 5th instar were quickly frozen with in liquid nitrogen and lyophilized.

Heat treated at 60°C, 100°C and 150°C for 30 min in the oven.

### Content of 1-DNJ in silkworm extract powders

DNJ content was measured according to the method reported by Kim *et al*. (2003). DNJ in the silkworm extract powder was extracted with 0.05 mol/L HCl, treated with 9-fluorenylmethyl (FMOC) to produce the DNJ-FMOC complex, and finally analyzed by high-pressure liquid chromatography (HPLC).

### Administration of silkworm extract powders to *db/db* mice

Male C57BL/KSJ-(db/db) mice (6 wk old) were purchased from Japan SLC Inc. (Japan). Mice were housed in a conventional cage at the appropriate temperature (23°C ± 3°C) and humidity (55% ± 15%) under a 12-h light/dark cycle, and had free access to food and water. All the groups were fed a standard diet (certified irradiated global 18% protein rodent diet). After a 1-wk adaptation period, the 7-wk-old mice were divided into five groups (n = 10 in each group): G1( control group : no silkworm powder), G2(T0 ; silkworm extract powder no-heating), G3(T60 ; 60°C / 30 min), G4(T100 ; 100°C/30 min), and G5(T150 ; 150°C/30 min), the silkworm powder was remade with diet.

### Measurement of body fat weight and blood biochemical analysis

The mice were fasted for 3 h, and then blood samples were taken after autopsy. The biochemical analysis of blood included the measurement of the levels of TG(Triglyceride), TCHO(Total cholesterol), LDL(Low density lipoprotein), GLU(Glutamic acid), AST(Aspartate aminotransferase), and ALT(Alanine aminotransferase), which was performed with a blood biochemical analyzer (AU680, Beckman Coulter, Japan). The fat weight was estimated by measuring the circumference of the perirenal area and of the epididymis at autopsy.

## Results and Discussion

### 1-DNJ contents in silkworm powder

The content of 1-DNJ in silkworm powder of Yeonnokjam pupae at d 3 of the 5$^{th}$ instar heated at different temperatures was determined. The content of 1-DNJ in the powders heated at 60°C and 100°C for 30 min was similar to that of the powder without heat treatment. However, in the powder treated at 150°C for 30 min, the 1-DNJ content was approximately half of that in the other samples (6.83 mg/dL). The amount of 1-DNJ remained the same when the powder was heated to 121°C for 15 min (Yatsunami *et al.*, 2011). Therefore, 1-DNJ in the silkworm powder was relatively stable at up to 100°C for 30 min and 121°C for 15 min (Table 1). The mice were fed these diets for 3–4 wk.

### Changes in body weight

Our analysis focused on the comparison between the changes in body weight of the treatment groups and the control group. In

**Table 1.** 1-Deoxynojirimycin content in silkworm powder as a function of the heating temperature

| Treatment | 1_ Deoxynojirimycin contents(mg/dL) |
|---|---|
| G2(T0 ; Control) | 12.54 |
| G3(T60 ; 60°C/30 min) | 14.65 |
| G4(T100 ; 100°C/30 min) | 13.82 |
| G5(T150 ; 150°C/30 min) | 6.83 |

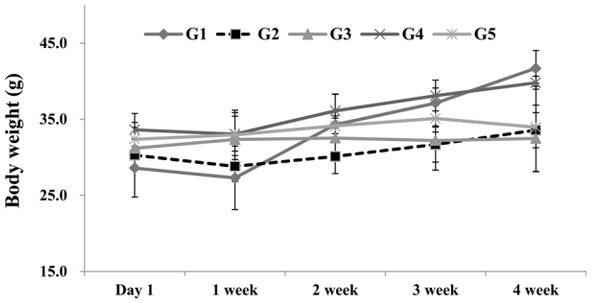


**Fig. 1.** Change in body weight of *db/db* mice.
* G1 (control), G2 (non-heating), G3 (60°C/30 min), G4 (100°C/30 min), G5 (150°C/30 min)

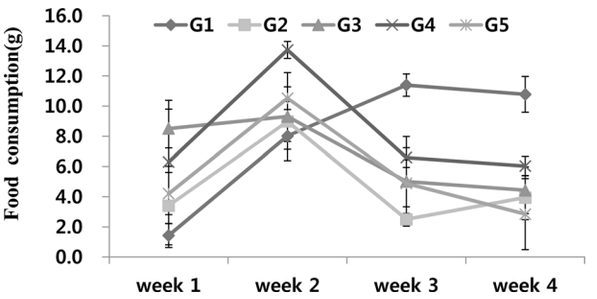


**Fig. 2.** Change in body weight of *db/db* mice administered silkworm powder.
* G1 (control), G2 (non-heating), G3 (60°C/30 min), G4 (100°C/30 min), G5 (150°C/30 min)

the T60 group, a statistically significant weight gain inhibition was observed compared to the T0 group (Fig. 1).

## Changes in feed intake

The weekly diet used in the experiments consisted of a fixed amount. The feed intake was observed once per week for 4 wk after silkworm powder administration. Mice in the T0, T60, T100, and T150 groups showed statistically significant reduction in feed intake compared to the control group (no silkworm powder administration) at 3–4 wk after administration (Fig. 2).

## Changes in water intake

We prepared a fixed amount of water the day before water intake, for determining water intake quantity. The water that remained unused in the period of 24 h was subtracted from the total amount of water that was offered daily, and the difference was considered as the water intake quantity (g/mouse/d).

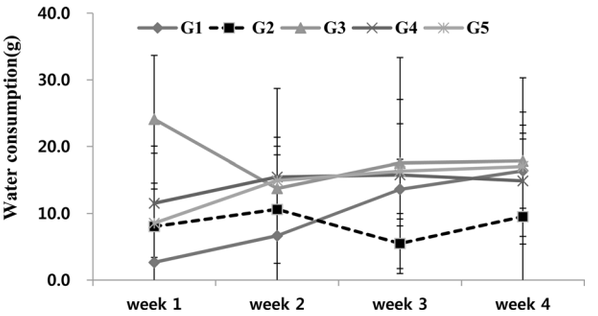


**Fig. 3.** Change in water consumption in *db/db* mice administered silkworm powder.
* G1 (control), G2 (non-heating), G3 (60°C/30 min), G4 (100°C/30 min), G5 (150°C/30 min)

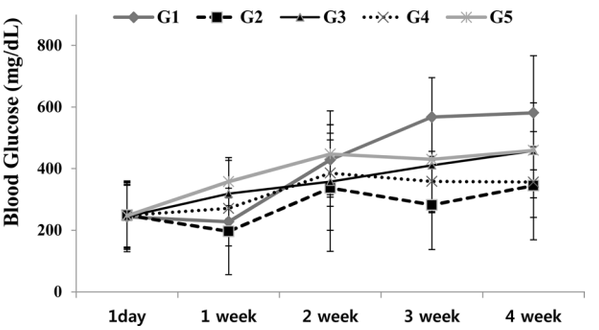


**Fig. 4.** Blood glucose-lowering effect of silkworm powder in *db/db* mice.
* G1 (control), G2 (non-heating), G3 (60°C/30 min), G4 (100°C/30 min), G5 (150°C/30 min)

The water intake quantity of each cage was the sum of each individual water intake value in each cage. The result showed no significant difference among the different groups (Fig. 3).

## Blood glucose-lowering effect of silkworm extracts in the *db/db* mice

In the T0 and T60 groups, a statistically significant reduction in the blood glucose level was observed compared to the control group ($P < 0.05$ and $P < 0.01$, respectively). However, in the T100 and T150 group, no significant reduction in the blood glucose level occurred. This result indicates that the silkworm powder as a supplement raw material has to be sterilized at temperatures below 100°C (Fig. 4). Heat-treated silkworm powder also had a decreased effect in blood glucose levels, but the non-heat-treated silkworm powder was 6–52% better than the heat-treated silkworm powder in reducing blood glucose level.

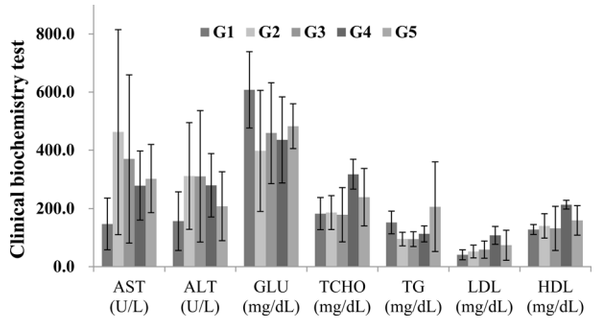


**Fig. 5.** Clinical biochemical analysis of the blood of *db/db* mice administered silkworm powder.
* G1 (control), G2 (non-heating), G3 (60°C/30 min), G4 (100°C/30 min), G5 (150°C/30 min)

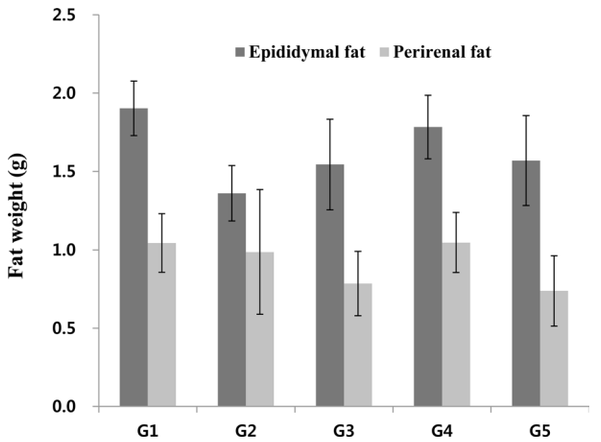


**Fig. 6.** Change in body fat in *db/db* mice administered silkworm powder.
* G1 (control), G2 (non-heating), G3 (60°C/30 min), G4 (100°C/30 min), G5 (150°C/30 min)

### Blood biochemical analysis and change in body fat

Blood biochemical analysis indicated that in the T150 group, the level of AST was lower than that in the other groups. The levels of TCHO in the T100 group significantly decreased compared to the control groups. The levels of TG in the T0 and T60 mice were significantly decreased compared to the control groups. For GLU level, no treatment showed significant changes compared to the control groups, but GLU decreased by approximately 20% compared to control group without silkworm powder treatment (Fig. 5).

The epididymal fat weight of mice in the T0, T60, and T150 groups significantly decreased compared to the control groups, while the perirenal fat weight of mice in the T60 and T150 groups significantly decreased compared to the control groups (Fig. 6).

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