IJIE Int. J. Indust. Entomol. 27(2) 237-242 (2013)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2013.27.2.237

# 1-Deoxynojirimycin Content and Blood Glucose-Lowering Effect of Silkworm (*Bombyx mori)* Extract Powder

Kang-Sun Ryu*, Heui-Sam Lee, Kee-Young Kim, Mi-Ja Kim, Gyoo-Byung Sung, Sang-Deok Ji, and Pil-Don Kang

*Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Suwon 441-853, Korea*

## Abstract

We investigated the 1-deoxynojirimycin (1-DNJ) content of extracts from silkworm larvae at each developmental stage within three silkworm varieties. We also compared the content of the following polyhydroxylated alkaloids in the silkworm extracts: 1-DNJ, fagomine, and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB). In addition, we evaluated the glucose-lowering effects of silkworm extract powder in ***db/db*** mice. The 1-DNJ content was the highest in Yeonnokjam 5$^{th}$ instar 3$^{rd}$ d larvae and Hansaengjam 5$^{th}$ instar 3$^{rd}$ d larvae, which contained 18.4 mg/100 g dry weight and 18.3 mg/100 g dry weight, respectively. The 5$^{th}$ instar 3$^{rd}$ d larvae exhibited a higher content of 1-DNJ than that of 5$^{th}$ instar 5th d larvae among all varieties. The glucose-lowering effects of silkworm extracts and Yeonnokjam powder were tested on ***db/db*** mice, and the blood glucose levels were found to decrease significantly in the YR70 group. Silkworm extracts (180 mg/kg, 90 mg/kg, 45 mg/kg, and 22.5 mg/kg) and acarbose (50 mg/kg) were administered orally for 4 wk. Changes in water intake were not statistically significant between control and silkworm extract-treated groups. Compared to the control group, blood glucose levels in the silkworm extract powder-treated group decreased in the 22.5 mg/kg/d group after being administered for 4 wk. This decrease was statistically significant. Furthermore, biochemical changes in the AST(Aspartate aminotransferase), ALT(Alanine aminotransferase), TCHO(Total Cholesterol), TG(Triglyceride), LDL(Low density lipoprotein), and HDL(High density lipoprotein) levels in blood were not observed. However, statistically significant decreases in blood GLU in the 22.5 mg/kg/d group compared to that of the control group occurred. In addition, the epididymal fat weight of the silkworm extract powder-treated group decreased significantly in both the 22.5 mg/kg/d group and 180 mg/kg/d group compared to that of the control group, but there were no statistically significant changes in perirenal fat weight. These results demonstrate that silkworm extracts inhibit changes in blood glucose levels in model diabetic mice.


Int. J. Indust. Entomol. 27(2), 237-242 (2013)

*Received* : 29 Oct 2013
*Accepted* : 15 Nov 2013

**Keywords**:
Silkworm,
Silkworm extract powder,
Blood glucose lowering

***Corresponding author.**
Kang-Sun Ryu
Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Suwon 441-853, Republic of Korea
Tel: +82-31-290-8518 / FAX: +82-31-290-8503
E-mail: ryuks@korea.kr

## Introduction

Diabetes mellitus is a serious chronic metabolic disorder that has a significant impact on the health, quality of life, and life expectancy of patients, as well as the healthcare system. Nojirimycine was first discovered from a streptomycete, and its naturally occurring hydrogenated product, 1-deoxynojirimycin (1-DNJ) was first isolated from the mulberry tree (Yoshikaki and Hivonu, 1976). Today, more than 20 polyhydroxy alkaloids have been identified from mulberry and silkworm (Asano *et al*., 1994a; 1994b; 2001). 1-DNJ is a piperidine alkaloid that is a highly effective alpha-glycosidase inhibitor (Yoshikuni, 1988; Yoshikuni *et al*., 1988; Hughes and Rudge, 1994) and an effective chemical for the treatment of hyperglycemia. Presently, 1-DNJ and its analogs have been isolated from a wide range of plants and microbes (Asano *et al*., 1998; 2000; Kim *et al*., 1999), but the 1-DNJ content in the mulberry tree is the highest among plants (Kimura *et al*., 2007; Yatsunami *et al*., 2008). Mulberry has been utilized as a Chinese medicine against diabetes mellitus for years. Ryu *et al*. (1997) first reported that the silkworm larval powder of the 5th instar (prepared by lyophilization) had beneficial effects on diabetic patients (Ryu *et al*., 1997; 1999), and the lowering of blood sugar levels was further elucidated by subsequent research (Han *et al*., 2007). Silkworm powder has blood glucose-lowering effects (Ryu *et al*., 2002), and mulberry leaves the diet of silkworms effectively inhibit alpha-glucosidase in the small intestine of humans (Oku *et al*., 2006). In Korea, Japan, and China, products made from mulberry and silkworm larvae are becoming popular for auxiliary therapy for diabetes mellitus. In this report, we compared the 1-DNJ content among silkworm varieties and investigated the antidiabetic effects of silkworm extracts on the *db/db* mouse.

## Materials and Methods

### Preparation of silkworm extracts

Silkworm larvae were reared by feeding mulberry leaves in the spring season of 2012 at the National Academy Agricultural Science. The silkworm varieties used for the experiment were Yeonnokjam, Yangwonjam, and Hansaengjam. The 5$^{th}$ instar 3$^{rd}$ d larvae were quickly frozen with liquid nitrogen and lyophilized, and the resulting dried silkworm powder was extracted with ethanol.

### High pressure liquid chromatography chromatogram of extracts

1-DNJ content was measured according to the method reported by Kim *et al*. (2003). 1-DNJ in the silkworm larva or mulberry leaf powder was extracted with 0.05 mol/L HCl, treated with 9-fluorenylmethyl (FMOC) to produce a 1-DNJ-FMOC complex compound, and finally detected by high pressure liquid chromatography (HPLC).

### Measurement of fasting blood glucose levels

Fasting blood glucose levels were measured after the animals fasted for 3 h for 4 wk. The *db/db* mice were obtained from Japan SLC, Inc (Japan), and animals at 6 wk of age were used. Blood glucose levels were determined in mouse blood samples from the tail vein using a glucose analyzer (Accu-Chek Active; Roche Diagnostics GmbH, Germany). Blood glucose levels were determined in blood samples from the tail vein at 0 min (prior to maltose administration), 30 min, 60 min, 90 min, and 120 min after maltose administration for glucose tolerance tests.

### Administration of silkworm extracts to *db/db* mice

Male C57BL/KSJ-(*db/db)* mice (6 wk old) were purchased from Japan SLC, Inc (Japan). They were housed in a conventional state with the appropriate temperature (23°C ± 3°C) and humidity (55% ± 15%) under a 12-h light/12-h dark cycle with free access to food and water. All 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 the following 6 groups (n = 10 in each group): diabetic control group, low silkworm extract group (*db/db* low dose, 22.5 mg/kg), high silkworm extract group (*db/db* high dose, 180 mg/kg), and acarbose groups.

### Measurement of body weight and blood biochemical analysis

The mice fasted for 3 h, and then blood samples were taken after their autopsy. Biochemical analysis of blood TG, TCHO, LDL, GLU, AST, and ALT was performed using a blood

**Table 1.** 1-Deoxynojirimycin content in silkworm varieties and its ratio in lyophilized material and extracts

| Treatment | Lyophilization ratio (%) | Extract ratio from dried powder (%) | 1-DNJ content (mg/dried 100 g) |
|---|---|---|---|
| YR53 | 13.8 | 16.2 | 18.4 |
| YR55 | 18.3 | 10.1 | 13.2 |
| YW53 | 13.4 | 20.3 | 16.0 |
| YW55 | 17.7 | 15.8 | 14.0 |
| HS53 | 15.7 | 16.9 | 18.3 |
| HS55 | 17.2 | 14.5 | 16.9 |
| Average (53/55) | 16.0 (14.3/17.7) | 15.6 (17.8/13.5) | 16.1 (17.6/14.7) |

* YR53, Yeonnokjam 5th instar 3rd d; * YR55, Yeonnokjam 5th instar 5th d; YW53, Yangwonjam 5th instar 3rd d; * YW55, Yangwonjam 5th instar 5th d; HS53, Hansaengjam 5th instar 3rd d; and * HS55, Hansaengjam 5th instar 5th d.

biochemical analyzer (AU680, Beckman Coulter, Japan). Fat was taken from the circumference of the perirenal and epididymis during the autopsy, and the weight was measured.

## Results and Discussion

### 1-DNJ content in silkworm varieties

We compared the 1-DNJ content among silkworm extracts of 5th instar 3rd d larvae and 5th instar 5th d larvae of the Yeonnokjam, Yangwonjam, and Hansaengjam. The Yeonnokjam and Hansaengjam larvae contained higher 1-DNJ than those of Yangwonjam. Furthermore, the 1-DNJ content of 5th instar 5th d larva powder was lower than that of the 5th instar 3rd d larvae. The best new candidate among these extracts was YR53. Yeonnokjam is one of the special silkworm varieties in Korea (Table 1).

### HPLC analysis of highly polyhydroxylated alkaloids

We established a profile pattern of highly polyhydroxylated alkaloids, such as 1-deoxynojirimycin (1-DNJ), fagomine, and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), of Yeonnokjam, a silkworm variety. We standardized the manufacturing process with this profile pattern, and now its development, including the establishment of standards as a natural medicine, is in progress (Fig. 1).

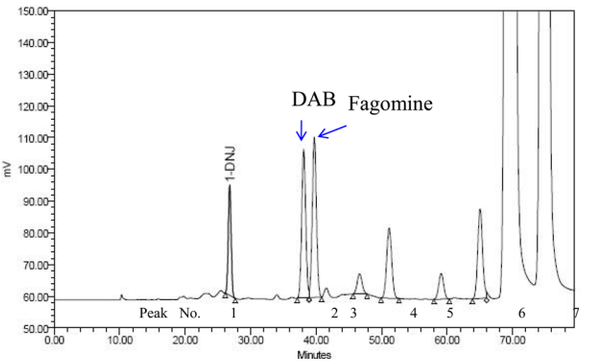


**Fig. 1.** HPLC chromatogram of 1-deoxynojirimycin (1-DNJ), fagomine, and DAB of silkworm (Yeonnokjam) extract.

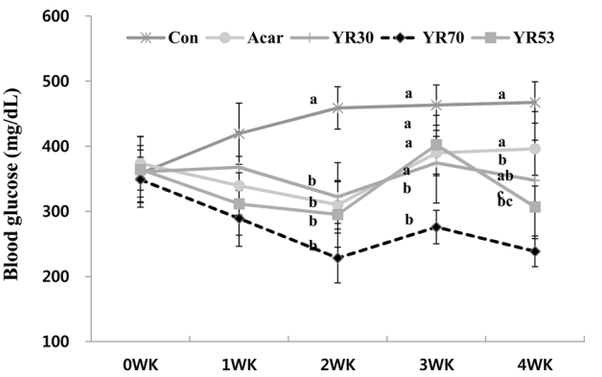


**Fig. 2.** Effects of silkworm (Yeonnokjam) extracts on blood glucose levels in *db/db* mice
* Con (control), Acar (Acarbose), YR30 (YR30% ETOH), YR70 (YR70%), YR53 (YR 5th instar 3rd d larva powder)

### Effects of silkworm extracts and powder on blood glucose levels

In order to determine the optimal silkworm powder extraction, we selected conditions based on how they lowered glucose levels with varying ethanol concentrations. All groups, with the exception of control and YR30 groups, exhibited glucose-lowering effects. Among these, the YR70 group showed the highest glucose-lowering effects for 4 wk. In conclusion, the optimal extraction condition was 70% ethanol (Fig. 2).

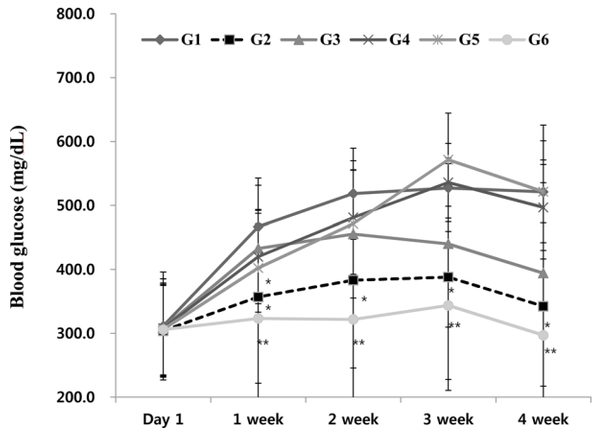


**Fig. 3.** Effects of Silkworm (Yeonnokjam) extracts on change of blood glucose in *db/db* mice.
*G1, control; G2, 22.5 mg/kg/d; G3, 45 mg; G4, 90 mg; G5, 180 mg; and G6, Acarbose.

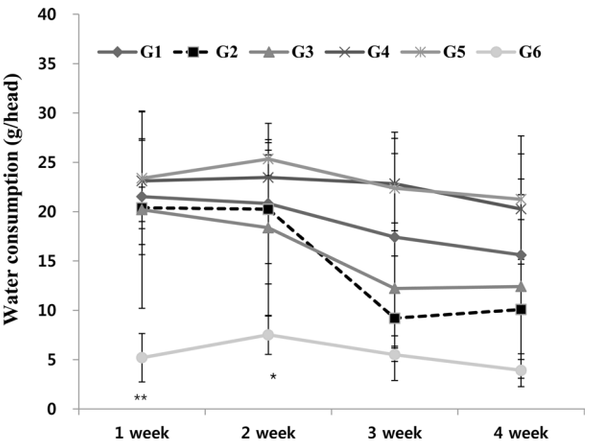


**Fig. 4.** Effects of silkworm (Yeonnokjam) extracts on *db/db* mouse water consumption *
**G1, control; G2, 22.5 mg/kg/d; G3, 45 mg; G4, 90 mg; G5, 180 mg; and G6,* Acarbose.

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

Based on blood glucose measurements, there was no statistically significant reduction in blood glucose levels in the G5 (180 mg/kg/d) group compared to that of the control group ($P < 0.05$ or $P < 0.01$). The blood glucose levels from wk 2 to 4 of the G3 (45 mg/kg/d) group did not decrease significantly. In contrast, a statistically significant reduction was observed for the G2 (22.5 mg/kg/d) group for 4 wk after administration ($P < 0.05$). The positive control (G6) group also exhibited a statistically significant reduction for 4 wk after administration ($P < 0.01$). The dosage test of silkworm extract powder needed an additional dosage lower than 22.5 mg/kg/d. Nevertheless, it is unclear why the effects of silkworm extract powder on blood glucose levels are dose independent (Fig. 3).

## Changes in water intake

The silkworm extract group did not show statistically significant changes in water intake compared to that of the control group. However, the 22.5 mg/kg/d and 45 mg/kg/d groups reduced their water intake at 3 and 4 wk after administration by approximately 47% and 33% and 41% and 20%, respectively. The water intake of the positive control group was reduced by approximately 70–75% compared to that of the control group over the course of 4 wk (Fig. 4).

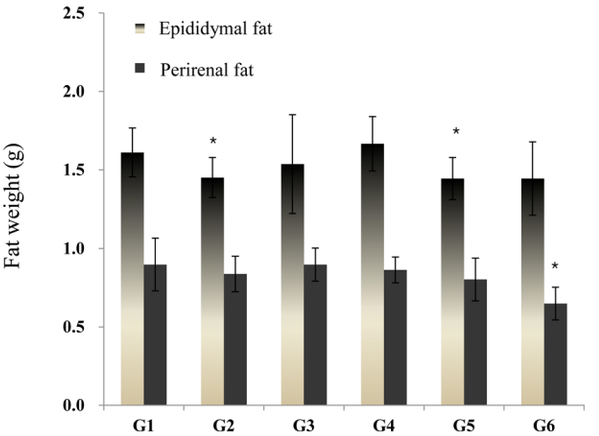


**Fig. 5.** Effects of silkworm (Yeonnokjam) extracts on the weight of epididymal and perirenal fat weights
** G1, control; G2, 22.5 mg/kg/d; G3, 45 mg); G4, 90 mg; G5, 180 mg; and G6,* Acarbose.

## Changes in body fat and blood biochemical analysis

The two groups of G2 (22.5 mg/kg/d) and G5 (180 mg/kg/d) showed statistically significant decreases in epididymal fat weight ($P < 0.05$) compared to those of the control groups. For perirenal fat weights, the silkworm extract group did not show statistically significant decreases compared to those of control groups (Fig. 5).

The positive control group did not show statistically significant

**Table 2.** Blood biochemical analysis in ***db/db*** mice.

| CLINICAL BIOCHEMISTRY TEST | | | | | | |
|---|---|---|---|---|---|---|
| Groups | G1 | G2 | G3 | G4 | G5 | G6 |
| AST (U/L) | 709.6 ± 627.6 | 425.9 ± 365.7 | 447.2 ± 307.7 | 503.2 ± 258.0 | 226.0 ± 229.8* | 184.9 ± 91.0* |
| ALT (U/L) | 653.7 ± 568.5 | 636.1 ± 544.5 | 502.7 ± 336.2 | 480.0 ± 290.7 | 206.6 ± 189.1* | 225.6 ± 125.6* |
| GLU (mg/dL) | 644.5 ± 124.1 | 416.8 ± 250.6* | 529.6 ± 190.6 | 626.1 ± 65.4 | 665.8 ± 48.5 | 374.4 ± 187.8** |
| TCHO (mg/dL) | 315.2 ± 64.3 | 243.6 ± 88.9 | 318.2 ± 39.4 | 317.7 ± 38.5 | 256.2 ± 76.8 | 229.6 ± 41.0** |
| TG (mg/dL) | 100.2 ± 26.0 | 73.6 ± 20.9 | 76.4 ± 13.4* | 256.2 ± 7.8 | 97.0 ± 20.1 | 89.1 ± 30.5 |
| LDL (mg/dL) | 89.0 ± 25.5 | 66.2 ± 32.7 | 89.6 ± 18.7 | 229.6 ± 11.3 | 70.3 ± 30.4 | 60.2 ± 15.0** |
| HDL (mg/dL) | 187.3 ± 27.3 | 160.7 ± 54.6 | 204.3 ± 10.8 | 196.8 ± 14.7 | 168.4 ± 38.7 | 157.9 ± 21.2** |

** G1, control;, G2, 22.5 mg/kg/d; G3, 45 mg; G4, 90 mg; G5, 180 mg; and G6, Acarbose.*

decreases in epididymal fat weight, whereas its perirenal fat weight increased ($P < 0.05$ or $P < 0.01$).

The blood biochemical analysis indicated that the silkworm extract (180 mg) showed similar decreases in AST and ALT levels. Similar decreases were found in the positive control group as well. G2 (22.5 mg/kg/d) showed statistically significant decreases in GLU levels compared to that of the control groups. The positive control group (G6) also exhibited statistically significant decreases in GLU levels compared to the control groups ($P < 0.05$ or $P < 0.01$). The silkworm extract groups did not show statistically significant changes in TCHO, LDL, or HDL levels compared to those of the control groups, whereas the positive control group (G6) showed a statistically significant decrease compared to those of control groups ($P < 0.05$ or $P < 0.01$). G3 (45 mg/kg/d) showed statistically significant decreases in TCHO, LDL, and HDL levels compared to those of control groups ($P < 0.05$). The positive control group (G6) did not show statistically significant changes compared to those of control groups ($P < 0.05$ or $P < 0.01$) (Table 2).

## Acknowledgement

This study was performed with financial support from the Cooperative Research Program for Agricultural Science & Technology Development (Project No. PJ0091252013), Rural Development Administration, Republic of Korea.

## References

Asano N, Tomioka E, Kizu H, Oseki K, Matsui K (1994a) Sugars with nitrogen in the ring isolated from the leaves of Morus bombycis. Carbohydr. Res., 253(3), 235-245.

Asano N, Kato A, Miyauchi M, Kizu H, Kameda Y, Watson AA, Nash RJ, Fleet GW (1998) Nitrogen containing furanose and pyranose analogues from Hyacinthus orientalis. J. Nat. Prod., 61(5), 625-628.

Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kata A, Nash RJ, Lee HS, Ryu KS (2001) Polyhydroxylated alkaloids isolated from mulberry trees (Morus alba L.) and silkworms (*Bombyx mori* L.). J. Agric. Food Chem., 49(9), 4208-4213.

Han J, Inoue S, Isoda H (2007) Effects of silkworm powder on glucose absorption by human intestinal epithelial cell line Caco-2. J. Nat. Med., 61(4), 387-390.

Hughes AB, Rudge AJ (1994) Deoxynojirimycin: synthesis and biological activity. Nat. Prod. Rep., 11(2), 135-162.

Kim HS, Kim YH, Hong YS, Paek NS, Lee HS, Kim TH, Kim KW, Lee JJ (1999) Alpha-glucosidase inhibitors from commelina communis. Planta Med., 65(5), 437-439.

Kim JW, Kim SU, Lee HS, Kim I, Ahn MY, Ryu KS (2003) Determination of 1-deoxynojirimycin in *Morus alba* L. leaves by derivatization with 9-fluorenylmethyl chloroformate followed by reversed phase high-performance liquid chromatography. J Chromatogr. A, 1002(1-2), 93-99.

Kimura T, Nakagawa K, Kubota H, Kojima Y, Yamaqishi K, Oita S, Oikawa S, Miyazawa T (2007) Food- grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J. Agric. Food Chem., 55(14),

5869-5874.

Oku T, Yamada M, Nakamura M, Sadamori N, Nakamura S (2006) Inhibitory effects of extractives from leaves of Morus Alba on human and rat small intestinal disaccharidase activity. British J. of Nutrition 95, 933-938

Ryu KS, Lee HS, Chung SH, Kang PD (1997) An activity of lowering blood-glucose levels according to preparative conditions of silkworm powder. Korean J. Seric. Sci. 39, 79-85.

Ryu KS, Lee HS, Kim SY (1999) Pharmacodynamic study of silkworm powder in mice administered to maltose, sucrose and lactose. Korean J. Sric. Sci. 41, 9-13.

Ryu KS, Lee HS, Kim I (2002) Effects and mechanism of silkworm powder as a blood glucose lowering agent.Int. J. Indust. Entomol. 4. 93-100.

Yatsunami K, Ichida M, Onodera S (2008) The relationship between 1-deoxynojirimycin content and alphaglucosidase inhibitory activity in leaves of 276 mulberry cultivars (Morus spp.) in Kyoto. Jpn Nat. Med. (Tokyo), 62(1):63-66.

Yoshikaki A, Hivonu M (1976) The structure of moranoline, a piperidine alkaloid form Morus species. Nippon Nogei Kagaku Kaishi, 50(11):571-572.

Yoshikuni Y (1988) Inhibition of intestinal alpha-glucosidase activity and postprandial hyperglycemia by moranoline and its N-alkyl derivatives. Agric. Biol. Chem., 52(1):121-128.

Yoshikuni Y, Ezure Y, Aoyagi Y, Enomoto H (1988) Inhibition of intestinal alpha- glucosidase and postprandial hyperglycemia by N-substituted moranoline derivatives. J. Pharmacobiodyn., 11(5):356-362.