

Additive effects of Kothala himbutu (*Salacia reticulata*) extract and a lactic acid bacterium (*Enterococcus faecalis* YM0831) for suppression of sucrose-induced hyperglycemia in an *in vivo* silkworm evaluation system

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Summary

Using a silkworm evaluation system, we previously evaluated various substances that suppress postprandial hyperglycemia. *Enterococcus faecalis* YM0831, a lactic acid bacterium that inhibits glucose uptake by the human intestinal Caco-2 cell line, exhibited hyperglycemia-suppressing effects in the silkworm system. In the present study, we found that Kothala himbutu (*Salacia reticulata*) extract, a traditional medicine containing α -glucosidase inhibitors, suppressed sucrose-induced hyperglycemia in the silkworm system. Moreover, combined oral administration of lactic acid bacteria YM0831 with Kothala himbutu extract had stronger suppressive effects on sucrose-induced hyperglycemia than single administration of either component. These findings suggest that the silkworm system provides a simple way to evaluate the effects of supplements on the suppression of blood glucose level induced by sucrose ingestion.

Keywords: Kothala himbutu, lactic acid bacteria, *Salacia reticulata*, silkworm, hyperglycemia

1. Introduction

Medical treatments against lifestyle diseases are often problematic and must be continued for a long time. Therefore, establishing strategies to prevent the development of lifestyle-related diseases is important for human health care. Diabetes mellitus is a lifestyle disease characterized by a high blood glucose level. Long-term hyperglycemia leads to serious complications, including retinopathy, nephropathy, and neuropathy. Excess intake of sucrose is a proposed risk factor for the onset of diabetes (1,2). Sucrose, a common sweetener in various foods and beverages, is degraded to glucose and fructose by α -glucosidase in the intestine and absorbed into the blood. Therefore, suppression of the sucrose degradation or absorption process is

expected to prevent the onset of diabetes. Combinations of compounds that have different modes of action are expected to more effectively prevent diabetes.

Hypoglycemic agents are generally evaluated by *in vivo* experiments in animal models. Evaluation methods using mammals, however, are not only expensive, but are also associated with ethical issues with respect to animal welfare. We previously reported that the silkworm, an insect larva, can be used to evaluate the effects of different hypoglycemic agents (3-9). Ingestion of sucrose rapidly increases the blood glucose level in silkworms, and acarbose and voglibose, α -glucosidase inhibitors used clinically for human patients, also suppress the increase in silkworms (3). We demonstrated that a lactic acid bacterium, *Enterococcus faecalis* YM0831, suppresses sucrose-induced hyperglycemia in both silkworms and humans (10). Yogurt produced by the lactic acid bacterium YM0831 also has suppressive effects in humans (10). Therefore, the YM0831 strain is expected to be useful to suppress sucrose-induced hyperglycemia. This bacterium inhibits glucose-uptake

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into the human intestinal Caco-2 cell line, suggesting that one mechanism by which this lactic acid bacterium suppresses hyperglycemia is inhibition of glucose uptake by the intestine.

Kothala himbutu (*Salacia reticulata*, SR) extract is a traditional medicine used for diabetes patients in Asia. Hot water extract of SR is reported to inhibit α -glucosidase and to suppress sucrose-induced hyperglycemia in humans (11). α -Glucosidase inhibitors, such as salacinol and kothalanol, were isolated from SR extract (12-14). Therefore, SR extract acts to suppress sucrose-induced hyperglycemia through its effects to inhibit α -glucosidase.

Acarbose and voglibose are prescribed as anti-diabetic agents. Daily use of these medicines for preventing diabetes is not recommended for healthy humans. Therefore, it is highly desirable to screen for foods and supplements that could suppress sucrose-induced hyperglycemia. In the present study, we used the silkworm model to evaluate whether the lactic acid bacterium YM0831, a glucose uptake inhibitor, and SR extract, an α -glucosidase inhibitor, would have additive effects to suppress hyperglycemia.

2. Materials and Methods

2.1. Reagents

Freeze-dried powder of Kothala himbutu (*Salacia reticulata*) hot water extract was obtained from Snowden Co., Ltd. (Tokyo, Japan). The salacinol content in the freeze-dried powder was determined to be 0.36% using a liquid chromatography-mass spectrometry method at Japan Food Research Laboratories (Tokyo, Japan) (15).

2.2. Culture of lactic acid bacteria

Enterococcus faecalis YM0831 was anaerobically cultured in MRS medium at 30°C for 2 days. Bacterial cells were harvested by centrifuge and the wet pellet was used as a sample.

2.3. Silkworm rearing conditions and sucrose tolerance test

Silkworms were reared according to a previously reported method (4,16). The silkworms were tested for sucrose tolerance as previously reported (3). Test samples were mixed in artificial diet containing 10% glucose or 10% sucrose. After feeding for 1 h, the glucose level in the silkworm hemolymph was measured using a glucometer (Accu-Chek, Roche).

2.4. Statistical analysis

All experiments were performed at least twice. The significance of differences was calculated using a two-

tailed Student's *t*-test at the significance level of $\alpha = 0.05$.

3. Results

3.1. Suppression of sucrose-induced hyperglycemia by Kothala himbutu (*Salacia reticulata*, SR) extract

We first tested whether SR extract suppresses sucrose-induced hyperglycemia in silkworms. The blood glucose levels were much lower in silkworms that ingested the SR extract compared with the control group, which did not ingest SR extract (Figure 1A). The SR extract, however, did not affect glucose-induced hyperglycemia in silkworms (Figure 1B).

3.2. Additive effect of *E. faecalis* YM0831 and SR extract on sucrose-induced hyperglycemia in silkworms

We previously reported that a sucrose-induced increase in the blood glucose level was suppressed by the ingestion of *E. faecalis* YM0831 in both silkworms and humans (10). The suppressive effect of this lactic acid bacterium is due to its activity to inhibit glucose transport in the intestine (10). Therefore, the mechanisms by which SR extract and *E. faecalis* YM0831 inhibit sucrose-induced hyperglycemia are different. We used the silkworm evaluation system to test our hypothesis that these substances would have an additive effect. Compared with SR extract (0.31% in diet) alone, administration of both lactic acid bacteria YM0831 and SR extract induced a greater decrease in the blood glucose level in silkworms that ingested sucrose (Figure 2A). Further, the blood glucose level was much lower in silkworms

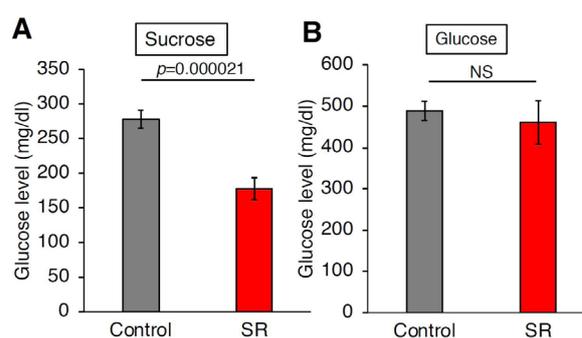


Figure 1. Effects of SR extract on dietary sucrose-induced increases in glucose levels in silkworm hemolymph. A. Silkworms were fed a diet containing 10% (w/w) sucrose with 1% (w/w) SR extract (SR, $n = 20$) or without SR extract (Control, $n = 28$) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean \pm SEM. Statistically significant differences between the control and testing groups were evaluated using Student's *t*-test. **B.** Silkworms were fed a diet containing 10% (w/w) glucose with 1% (w/w) SR extract (SR, $n = 12$) or without SR extract (Control, $n = 14$) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean \pm SEM. Statistically significant differences between control and testing groups were evaluated using Student's *t*-test. NS: not significant.

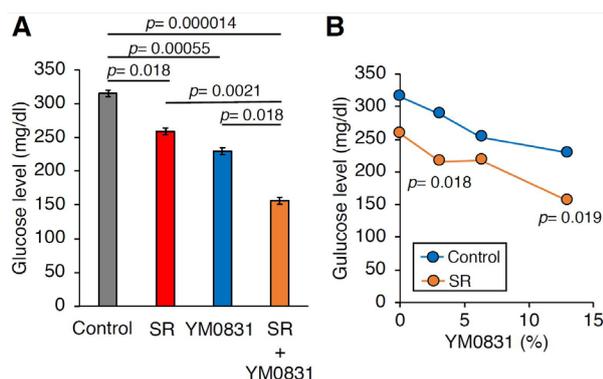


Figure 2. Combined effects of SR extract and *Enterococcus faecalis* YM0831 on dietary sucrose-induced increases in glucose levels in silkworm hemolymph. **A.** Silkworms were fed a diet containing 10% (w/w) sucrose (Control, $n = 14$), the 10% sucrose diet with 13% (w/w) *E. faecalis* YM0831 (*E. faecalis*, $n = 14$), the 10% sucrose diet with 0.3% (w/w) SR extract (SR, $n = 14$), or the 10% sucrose diet with 13% (w/w) *E. faecalis* YM0831 and 0.3% (w/w) SR extract (*E. faecalis* + SR, $n = 10$) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean \pm SEM. Statistically significant differences between control and testing groups were evaluated using Student's *t*-test. **B.** Silkworms were fed a diet containing 10% (w/w) sucrose and 0-13% (w/w) *E. faecalis* YM0831 with 0.3% (w/w) SR extract (SR; orange line) or without SR extract (Control; blue line) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean \pm SEM. Statistically significant differences between control and testing groups were evaluated using Student's *t*-test. $n = 10-14$.

that ingested viable lactic acid bacteria YM0831 with SR extract (0.31% in diet) than in silkworms ingesting the bacteria without SR extract (Figure 2B).

4. Discussion

In the present study, using silkworms as a model animal, we showed that combined administration of lactic acid bacteria *E. faecalis* YM0831 and SR extract had additive effects to suppress sucrose-induced hyperglycemia. To our knowledge, this is the first report to show additive effects of anti-hyperglycemia substances that act by different mechanisms in an invertebrate evaluation system.

SR is a plant used as a traditional medicine in the Indian subcontinent. Hot water extract of SR has α -glucosidase inhibitory activity (13) and suppresses postprandial hyperglycemia in mammalian animals, including humans (11). SR extract suppresses sucrose-induced hyperglycemia, but not glucose-induced hyperglycemia, in rats (13). These findings suggest that the suppression of hyperglycemia by SR extract is due to its effects to inhibit the degradation of sucrose in the intestine. Our findings indicated that SR extract also suppressed sucrose-induced hyperglycemia, but not glucose-induced hyperglycemia, in silkworms.

E. faecalis YM0831 was screened using the silkworm evaluation system as a functional lactic acid bacterium that suppresses sucrose-induced hyperglycemia (10). This lactic acid bacterium also suppressed sucrose-

induced hyperglycemia in humans (10). Moreover, it inhibited glucose-uptake by the human intestinal Caco-2 cell line (10). Therefore, we consider that the inhibition of sugar absorption from the intestine is the likely mechanism for this bacterium to suppress a sucrose-induced increase in the blood glucose level. In this paper, we demonstrated that combined administration of this lactic acid bacteria and the SR extract had additive effects to suppress sucrose-induced hyperglycemia. On the basis of these results, we propose that combinations of foods or supplements with α -glucosidase inhibitory activity and glucose transport inhibition activity may efficiently suppress the postprandial increase in blood glucose and may contribute to prevent the lifestyle diseases such as diabetes and obesity.

In conclusion, the findings of the present study suggest that SR extract and *Enterococcus faecalis* YM0831 have additive effects to inhibit postprandial sucrose-induced increases in blood glucose and that silkworms are a useful animal model for testing the additive effects of anti-hyperglycemia substances.

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