Original Article

Mulberry leaves and water chestnut tea reduces postprandial blood glucose in borderline diabetic Japanese: A randomized, double-blind, placebo-controlled crossover study

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Background and Objectives: Postprandial hyperglycemia is a risk factor not only for diabetes mellitus, but also arteriosclerosis. Therefore, controlling the rapid postprandial increase in blood glucose levels is necessary. This study aimed to develop a mulberry leaf and water chestnut husk tea and investigate its effect on postprandial blood glucose levels. **Methods and Study Design:** We measured the polyphenols and 1-deoxynojirimycin contents as well as antioxidant activity of mulberry leaf and water chestnut husk tea in an in vitro experiment. The effect of the tea on postprandial blood glucose levels in 30 participants with borderline diabetes was investigated in a randomized, double-blind, placebo-controlled crossover comparison study. **Results:** The 1-deoxynojirimycin and total polyphenol contents in the tea (test food, 3g) were 10.2 ± 0.8 and 61.3 ± 1.4 mg, respectively. The test food showed higher antioxidant activity than the placebo food. Compared with those in the placebo group, blood glucose levels in the test group significantly decreased 30 and 60 min after eating rice. Additionally, insulin was significantly lower at all time points (30, 60, 90, and 120 min after rice consumption). **Conclusions:** The mulberry leaves and water chestnut mix tea may be an effective beverage to reduce insulin secretion and prevent rapid increases in blood glucose levels in patients with borderline diabetes.

Key Words: mulberry leaves, water chestnut, 1-deoxynojirimycin, polyphenols, postprandial blood glucose level

INTRODUCTION

In 2021, the number of cases of diabetes globally was estimated to be 537 million or one in ten adults.[1] It is expected to increase to 643 and 783 million by 2030 and 2045, respectively,[1] making diabetes a global challenge to health, well-being, and the economy. Type 2 diabetes, caused by a poor lifestyle, such as overeating, unbalanced diets, lack of exercise, and excessive stress, is a serious concern. Prevention of diabetes is crucial as it can reduce the quality of life and even lead to fatal complications.

In recent years, food science research to prevent the onset and progression of diabetes has focused on functional foods to control blood glucose levels. Postprandial hyperglycemia promotes glycation reactions of vascular endothelial proteins, a risk factor for cardiovascular disease (CVD).[2] Further, oxidative stress from large postprandial blood glucose fluctuations can cause intravascular damage and increase the risk of CVD,[3] and proper control of postprandial blood glucose levels is crucial in reducing the risk of developing type 2 diabetes.[4,5] Therefore, research and development of meals and foods that suppress the postprandial rise in blood glucose levels are actively ongoing.[6-8]

Mulberry leaves have long been cultivated in Japan as food for silkworms and as a popular herbal medicine. Butt et al.[9] reviewed the functional properties of mulberry and found that it exhibits antioxidant, antibacterial, anti-atherosclerosis, and anti-cancer effects, as well as suppression of blood glucose level elevation and immunostimulatory activity. The suppression of elevated blood glucose levels is attributed to 1-deoxynojirimycin (DNJ) in mulberry leaves, with α-glucosidase inhibitory activity as the primary mechanism.[10-13] Inhibition of postprandial blood glucose elevation has also been shown in animal[14-16] and human clinical studies.[17-19]

Water chestnut is a unique water plant that spreads its leaves above water and produces spiny fruits. The husk of the water chestnut contains eugeniin, 1,2,3,6-tetra-*O*-galloyl-D-glucopyranose (TGG), and trapain, which are hydrolyzed polyphenols.[20] We revealed that these polyphenols have high antioxidant activity and exhibit an inhibitory effect on postprandial blood glucose elevation in mice and humans.[20-21]

This study aimed to develop a tea mixed with mulberry leaves and water chestnut husk (MW tea), and to investigate its effect on postprandial blood glucose levels. This has the potential to contribute to the control of both blood glucose and oxidative stress, which are at the core of the pathology of diabetes. In this study, we investigated the effect of MW tea on postprandial blood glucose levels in Japanese individuals with borderline diabetes in a randomized, double-blind, placebo-controlled, crossover comparative study. We also measured the content of polyphenols and DNJ and the antioxidant activity of MW tea.

METHODS
Sample preparation
Mulberry leaves were collected from Maguwa (*Morus alba*) grown in Saga, Japan. The leaves were immediately dried using hot-air at 100°C for 15 min, and ground into powder. The water chestnut husk was obtained from water chestnut (*Trapa japonica*) grown in the wild in a creek in Saga, Japan; it was dried, the fruit or starchy part was removed, ground into powder, and roasted at 120°C for 20 min.

To determine the optimal blending ratio for MW tea, the contents and functionalities of were evaluated for the following blending ratios of mulberry leaves and water chestnut husk: 0:100, 20:80, 40:60, 60:40, 80:20, 95:5, and 100:0 (%, w/w).

Reagents
Solvents used for high-performance liquid chromatography (HPLC) were HPLC-grade procured from Fujifilm Wako Pure Chemicals Corporation (Osaka, Japan). Other reagents were special grade from Fujifilm Wako Pure Chemicals Corporation unless specified.

Analysis of polyphenols
The total polyphenol content was determined based on the Folin–Ciocalteu method of Sun et al.[22] Briefly, 100 µL of the sample solution and 100 µL of Folin–Ciocalteu reagent diluted two-fold with water were placed in a microtube. After stirring well, the sample was allowed to stand at room temperature (25°C) for 3 min. Then, 100 µL of 10% (w/v) sodium carbonate solution was added, mixed well, and allowed to stand at room temperature (25°C) for 60 min under light shielding. The mixture was centrifuged at 3000 rpm for 15 min using a centrifuge (H-15FR, Kokusan Co. Ltd., Saitama, Japan). Next, 200 µL of the supernatant was placed in a microplate, and absorbance was measured at 750 nm using a microplate reader (Synergy HT; Bio-Tek Instruments Inc., Winooski, VT, USA). Total polyphenol content was calculated as the equivalent of gallic acid per 100 g.

The polyphenols of water chestnut (eugeniin, TGG and trapain) were analyzed by HPLC. This was done according to a previous study.[20]

Analysis of DNJ
The DNJ content in mulberry leaves was determined by a fluorescent derivatization method using 4-fluoro-7-nitrobenzofurazan[23] as follows: approximately 0.5 g of sample was placed in a 50 mL centrifuge tube, weighed accurately, and 30 mL of water was added. The sample was extracted for 30 min with occasional stirring using a block heater (MG-2200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) set at 100°C. The centrifuge tube was then cooled, filtered using filter paper (No. 1, Advantec Co. Ltd., Tokyo, Japan), and transferred to a 50-mL volumetric flask at a constant volume. An aliquot (0.5 mL) of this sample solution was taken into a screw-top test tube, followed by addition of 2.4 mL of 30 mM boric acid buffer solution and 100 µL of 5 mg/mL 4-fluoro-7-nitrobenzofurazan (Dojin Chemical Laboratory, Kumamoto, Japan) in ethanol. This mixture was heated at 60°C for 40 min using a block heater, then cooled, and 2 mL of 1 M hydrochloric acid was added and mixed well. The solution was filtered through a membrane filter (0.2 µm) and subjected to HPLC. The standard solution of DNJ was also derivatized via the same method, and HPLC analysis was performed under the following conditions: HPLC equipment, Prominence LC-20AT (Shimadzu Corporation, Kyoto, Japan); column, CAPCELL PAK C18 UG120 (4.6 mm φ × 150 mm, 5 µm, Osaka Soda Co., Ltd., Osaka, Japan); mobile phase, 0.05% (v/v) phosphoric acid/methanol from 100:0 (%, v/v) to 95:5 (%, v/v) (45 min linear gradient); column temperature, 40°C; flow rate, 1 mL/min; detection, fluorescence detector (Ex 470 nm, Em 530 nm).

Evaluation of antioxidant activity
The antioxidant activity was examined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity method.[24] Sample (50 µL) or Trolox solution of known concentration was placed in a 98-well microplate; 150 µL of DPPH mixture (400 µM DPPH: MES-NaOH buffer solution (pH 6): ethanol = 2:1:2) was added and mixed well. After incubation for 20 min at room temperature (25°C), the absorbance at 520 nm was measured. A calibration curve was prepared using the Trolox standard solutions, and DPPH concentration in units per gram of sample (DPPH radical-scavenging capacity equivalent to 1 µmol of Trolox) was calculated.

Evaluation of α-glucosidase inhibitory activity
The inhibitory activity of α-glucosidase (maltase and sucrase) was evaluated according to a previous study.[20]

Human clinical trials
Participants
The sample size of this study was determined based on a pilot experiment with a small number of young people and a previous Japanese study with a similar study design. First, in the pilot experiment, five people (3 men, 2 women, mean age 21.0±0.0 years) were tested with the same study design as in this study, with the change in

incremental area under the curve (IAUC) of postprandial blood glucose levels as the outcome. Based on this, the difference in IAUC of blood glucose levels between MW tea and placebo tea was 1737 mg·min/dL, and the mean standard deviation of both groups was 1531 mg·min/dL. The minimum sample size was 7 cases, calculated at a significance level of 5% and a power of 80%.

The number of subjects in the previous study of Japanese subjects similar to this study was 12, and the study design was a double-blind, four-period crossover study in which subjects were given 0, 3, 6, and 9 mg of DNJ followed by a loaded food (200 g of cooked rice). As a result, although the IAUC was not calculated, blood glucose levels at 120 min after a meal decreased dose-dependently, but no change was observed in insulin levels.[18]

Based on these results, the target sample size of subjects was set at 28 cases, approximately twice that of the previous study.[18] This was due to differences in subject characteristics such as gender, age, and obesity level, variations in exclusion criteria, the potential for dropouts, and the study's inclusion of both the IAUC of Δblood glucose and Δinsulin levels as outcomes.

Participants in the study were borderline diabetic adults (men and women) between the ages of 20 and 65 who lived or worked in Kanzaki, Saga, Japan. Criteria for participation were fasting blood glucose levels of ≥100 mg/dL and <126 mg/dL, or hemoglobin A1c levels of ≥5.6% and <6.5%.

The exclusion criteria for study participants were as follow: (1) not meeting the inclusion criteria, (2) blood glucose level and hemoglobin A1c were equivalent to those of diabetic patients (fasting blood glucose level 126 mg/dL or higher, hemoglobin A1c 6.5% or higher), (3) receiving drug therapy, (4) regularly taking medicines, health foods, or functional foods that may affect this trial, (5) a history of serious diseases, such as heart, liver, kidney, digestive organ diseases, (6) excessive alcohol consumption, (7) irregular working schedules, such as shift workers or late-night workers, (8) allergies to medicines or foods, (9) pregnant or planning to become pregnant, (10) breastfeeding, (11) deemed unsuitable as participants by the trial doctor, and (12) request to withdraw from this trial.

We held a research information session for those interested to fully explain the purpose and methods and the handling of research data. This clinical trial was conducted after written informed consent was obtained from the participants. Participants could drop out at any time during the trial.

Test food
Mulberry leaves were dried and ground into powder by Sato Tea Manufacturing Co., Ltd. (Kumamoto, Japan). Water chestnut husk was roasted and ground into powder by Kenkochaen, Co., Ltd. (Fukuoka, Japan). These were blended in the ratio of mulberry leaves: water chestnut husk = 95:5 (%, w/w) and individually packaged 3 g powder in a stick-like bag by Kyushu Pharmaceutical Industry Co., Ltd. (Saga, Japan). The MW tea (Kanzaki M&M Co., Saga, Japan) produced in this way was used as a test food.

The preparation method of the placebo food was based on previous studies,[25] and was designed so that the test and placebo foods were indistinguishable in appearance and smell, and with sufficient attention to hygiene. Namely, it was a 2.5 g mixture of corn flour (Pioneer Planning Corporation, Tokyo, Japan), barley tea powder (Yanagiya Co., Aichi, Japan), and food coloring (Tamaphilin, Tama Biochemical Co., Ltd., Tokyo, Japan). The test or placebo food was mixed well with 200 mL of hot water and served to the participants.

The loaded food was 200 g of vacuum-packed rice (Sato Foods Industries Co., Ltd., Aichi, Japan), heated in a microwave oven as indicated.

Study design
Prior to the main study, a screening test was conducted to confirm that participants met the participation criteria. Blood was collected to check fasting blood glucose level and hemoglobin A1c, and height, weight, waist circumference, and blood pressure were measured. Those who met the participation criteria through a screening test were registered as participations.

The clinical trial was a randomized, placebo-controlled, double-blind, cross-over comparative study. Participants were randomly assigned to Group A and Group B by a person not directly involved in the study, considering age, gender, and fasting blood glucose level. In the first test, Group A received the test food and Group B received the placebo food. After a one-week washout period, Group A received the placebo food and Group B received the test food as the second test. In each test, after blood was drawn, participants were asked to drink 200 mL of the test food or placebo food within 3 min, followed by 200 g of rice as the loaded food within 10 min. Thereafter, blood was collected 30, 60, 90, and 120 min later. The participants were allowed to rest until all blood sampling was completed. Furthermore, participants were prohibited from eating or drinking anything other than water from 9:00 pm on the day before the screening test and the main trial until the end of the test. Additionally, during the study period, participants were asked not to make any major changes to their lifestyle habits, such as diet and exercise, before participating in the study.

Outcomes
The primary outcomes were the Δblood glucose level (the amount of change in blood glucose level when the blood glucose level before ingesting the loaded food was set to 0) and the IAUC of the Δblood glucose level up to 120 min after the loaded food intake. The secondary outcomes were Δinsulin (the change in insulin when the insulin before ingesting the loaded food was set to 0) and IAUC of Δinsulin up to 120 min after the loaded food intake. IAUC was calculated using the trapezoidal method.

Statistical analysis
Data are expressed as mean ± standard deviation. Changes in blood glucose and insulin over time were tested for significant differences by two-way analysis of variance (with correspondence). If there was a significant difference in the interaction, a corresponding t-test was used to test for significant differences as a post-hoc test. To

compare the area under the curve of the rise in blood glucose and insulin between the two groups, a test for difference between the population means (with correspondence) was performed. If the distribution was normal, a corresponding t-test was performed. If it did not follow a normal distribution, a Wilcoxon's signed rank test was performed. Statistical analysis of the human clinical trials was performed using BellCurve for Excel (Ver. 4.06, Social Survey Research Information Co., Ltd., Tokyo, Japan). The significance level was set at $p<0.05$ for all testing methods.

Ethical considerations

The human clinical trial was conducted in compliance with the Declaration of Helsinki and after review and approval by the Ethics Committee of Nishikyushu University (approval number: 21BIQ03). The study plan was registered with the University Hospital Medical Information Network Center (UMIN, registration No. UMIN000045377) prior to the start of the study.

RESULTS

Chemical composition and functional properties of MW tea

The total polyphenols and water chestnut polyphenols in MW tea were found to increase with the ratio of water chestnut husk (Figure 1A and 1B). The DNJ content increased with an increasing ratio of mulberry leaves (Figure 1C). The DPPH radical-scavenging activity (antioxidant activity) increased with the ratio of water chestnut husk (Figure 1D). When the sample was extracted at a concentration of 7.5 mg/mL, the maltase inhibitory activity increased with an increase in the ratio of mulberry leaves. A large effect ($\geq$ 70% inhibition rate) was observed when $\geq$ 80% mulberry leaves were used (Figure 2A). When the sample was extracted at a concentration of 0.94 mg/mL, the sucrase inhibitory activity was large ($\geq$ 40%) ($\geq$ 80% mulberry leaves used) (Figure 2B). These results show that a high ratio of mulberry leaves ($\geq$ 80%) is desirable in terms of α-glucosidase inhibitory activity. It was found that maximizing the inclusion of water chestnut husk enhances polyphenol content and antioxidant activity.

We are exploring the commercialization of MW tea, but water chestnut is a rare plant with limited natural production. To address this, we reduced the proportion of water chestnut husk. The optimal mulberry leaf-to-water chestnut husk ratio for MW tea was determined to be 95:5 (%, w/w). In the future, we plan to cultivate water chestnuts to boost production and increase the husk ratio in MW tea.

The nutritional facts of the test, placebo, and loaded foods is summarized in Table 1. Table 2 summarizes the content of total polyphenol, water chestnut polyphenol, DNJ and DPPH radical-scavenging activity in mulberry leaves, water chestnut husk, test food, and placebo food. In the human clinical study, participants consumed 3 g of the test food and 2.5 g of the placebo food, and the total
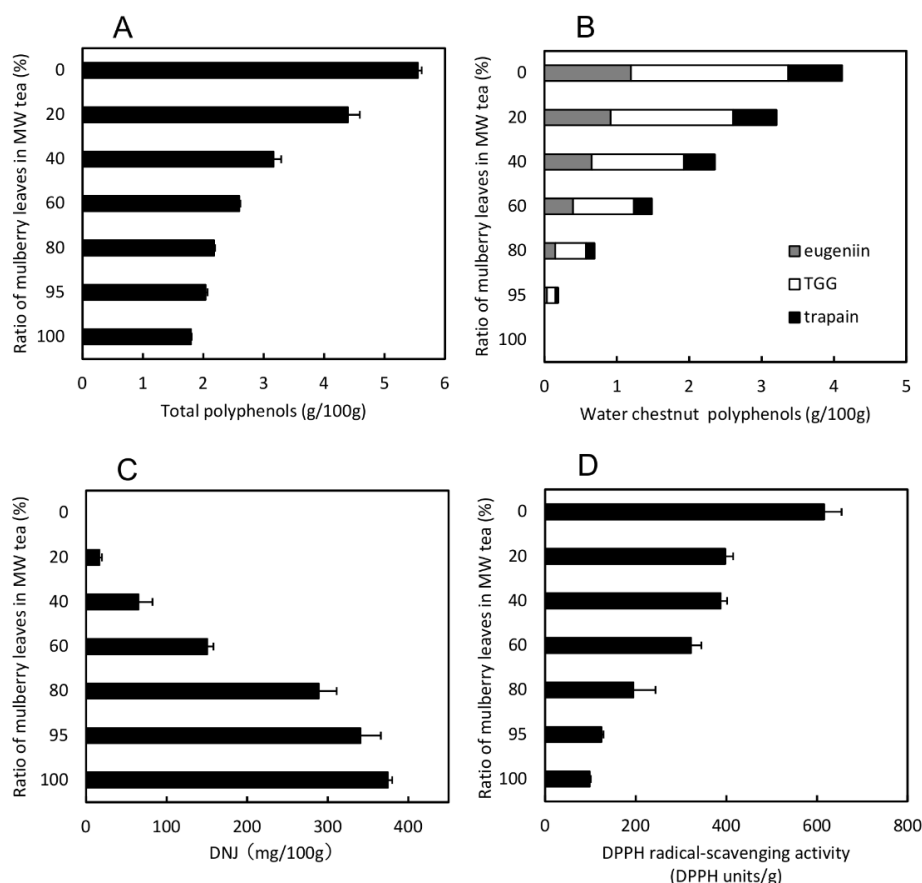

Figure 1. Effect of mulberry leaves and water chestnut husk ratio in MW tea on chemical composition and antioxidant activity. Error bars indicate standard deviation. (A) total polyphenols; (B) water chestnut polyphenols; (C) 1-deoxynojirimycin (DNJ); (D) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. MW: mulberry leaves and water chestnut husk
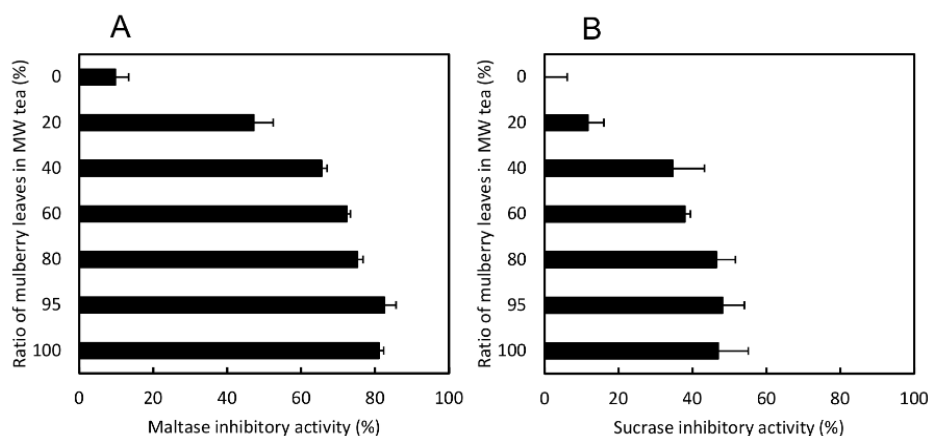
Figure 2. Effect of mulberry leaves and water chestnut husk ratio in MW tea on α-glucosidase inhibitory activity. Error bars indicate standard deviation. (A) maltase; (B) sucrase. MW: mulberry leaves and water chestnut husk.

Table 1. Nutrition facts of test, placebo, and loaded foods

Nutrition facts	Test food (3.0 g)	Placebo food (2.5 g)	Loaded food (200 g)
Energy (kcal)	8.5	9.3	294
Protein (g)	0.6	0.1	4.2
Fat (g)	0.1	0.0	0.0
Carbohydrates (g)	1.8	2.2	67.8
Salt equivalent (g)	0.0	0.0	0.0

Table 2. Total polyphenols, water chestnut polyphenols, DNJ, and DPPH radical-scavenging activity of the samples

Sample	Total poly-phenols (g/100 g)	Water chestnut polyphenols (g/100g)			DNJ (mg/100 g)	DPPH radical-scavenging activity (DPPH units/g)
		Eugeniin	TGG	Trapain		
Mulberry leaves	1.80 ± 0.05	–	–	–	375 ± 51	99 ± 2
Water chestnut husk	5.56 ± 0.17	1.19 ± 0.04	2.17 ± 0.05	0.74 ± 0.03	–	616 ± 38
Test food[†]	2.04 ± 0.05	0.03 ± 0.00	0.12 ± 0.00	0.04 ± 0.00	341 ± 25	125 ± 4
Placebo food[‡]	0.11 ± 0.01	–	–	–	–	6 ± 1

TGG, 1,2,3,6-tetra-*O*-galloyl-D-glucopyranose; DNJ, 1-deoxynojirimycin; DPPH, 2,2-diphenyl-1-picrylhydrazyl

[†]The sample was a mixture of mulberry leaves: water chestnut husk at 95:5.

[‡]Placebo food was made similar to test food by mixing corn flour, barley tea powder, and coloring. All data are presented as mean ± standard deviation.

polyphenol content was 61.3 ± 1.4 and 2.8 ± 0.2 mg, respectively. The content of water chestnut polyphenols in the MW tea (3 g) consumed herein was 0.97 ± 0.04, 3.54 ± 0.13 and 1.21 ± 0.01 mg for eugeniin, TGG and trapain, respectively. Test food used in the human clinical study contained 10.2 ± 0.8 mg DNJ. DNJ and water chestnut polyphenols, the active compounds in mulberry leaves and water chestnut husk, are considered safe, as the amounts consumed were lower than previously reported safe levels.[17, 20, 21]

Effect of MW tea on postprandial blood glucose and insulin in humans

A screening test was performed on 46 individuals who agreed to participate in the study, of whom 32 met the inclusion criteria. Of these, one withdrew, and one dropped out for personal reasons, leaving a total of 30 participants for the final analysis. These participants were randomly divided into two groups (Groups A and B); the characteristics are shown in Table 3.

The Δblood glucose levels after ingestion of the loaded food are shown in Figure 3A. Both the placebo and test groups showed an increase in Δblood glucose levels after ingestion of the loaded food, reaching a maximum at 60 min and then gradually decreasing until 120 min. A significant interaction ($p<0.01$) was observed between the groups, with the test group showing significantly lower values than the placebo group at both 30 and 60 minutes ($p<0.01$). In addition, the change in insulin after the intake of the loaded food is shown in Figure 3B. A significant interaction ($p<0.01$) was observed between the two groups. The Δinsulin values increased in both the placebo and test groups after food ingestion, but the test group had significantly lower values ($p<0.01$) than the placebo group at all sampling times.

Comparison of the IAUC of Δblood glucose levels from before to 120 min after ingestion of the loaded food showed that the test food had a significantly lower value ($p<0.01$) than the placebo food (Figure 4A). The IAUC of Δinsulin was also significantly lower ($p<0.01$) in the test group than in the placebo group (Figure 4B).

Table 3. Characteristics of the participants

Item	Group A	Group B	p value
Number of people (person)	15	15	1.000
Gender (men / women)	11/4	10/5	1.000
Age (age)	44.9±7.4	42.9±9.4	0.523
BMI (kg/m^2)	26.7±3.5	27.0±3.3	0.776
Abdominal circumference (cm)	94.1±9.0	94.3±8.6	0.951
SBP (mmHg)	122.5±18.4	122.1±12.5	0.945
DBP (mmHg)	77.7±11.8	80.2±7.4	0.487
Fasting blood glucose level (mg/dL)	94.3±6.3	93.5±5.7	0.718
HbA1c (%)	5.8±0.2	5.8±0.2	0.927

All data are expressed as mean ± standard deviation. SBP, DBP and Hb indicate systolic blood pressure, diastolic blood pressure and hemoglobin, respectively. Statistical analysis was performed using Fisher's exact probability test and Student's t-test.
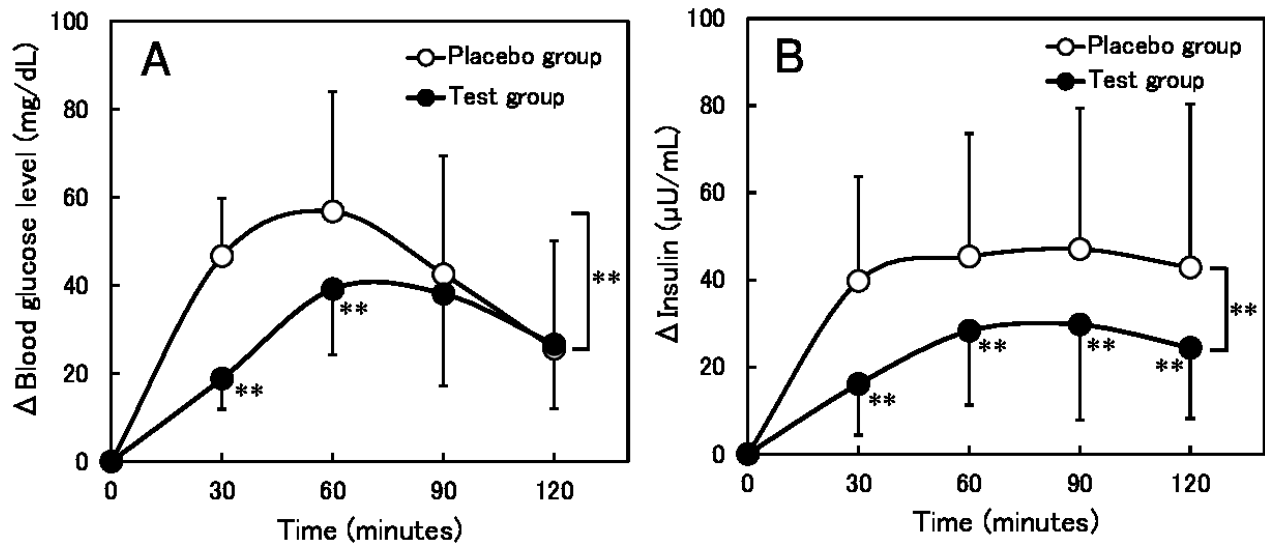

Figure 3. Effect of MW tea on postprandial blood glucose and insulin. (A) blood glucose; (B) insulin. The Δblood glucose represents the change in blood glucose level when the blood glucose level before ingestion of the load food is set to 0, and Δinsulin represents the change in insulin when the insulin level before ingestion of the load food is set to 0. MW: mulberry leaves and water chestnut husk. Error bars indicate standard deviation. **$p<0.01$ (test group vs. placebo group)
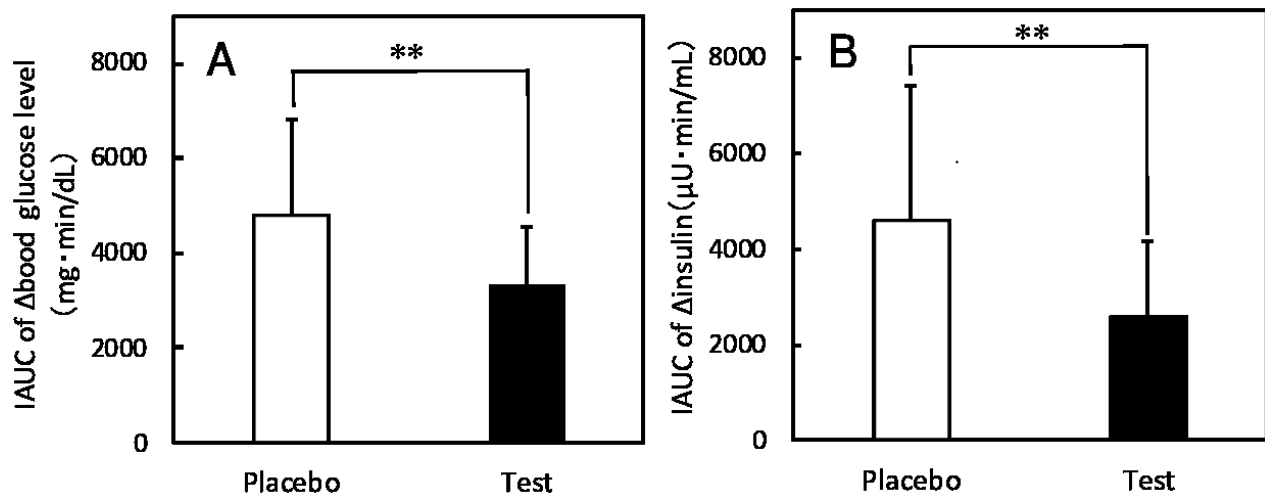

Figure 4. Effect of MW tea on IAUC Δblood glucose and Δinsulin. (A) blood glucose; (B) insulin. The IAUC means the incremental area under the curve of the Δblood glucose level or Δinsulin up to 120 min after the load food intake. The explanation of Δblood glucose and Δinsulin is the same as in Figure 3. MW: mulberry leaves and water chestnut husk; IAUC: incremental area under the curve. Error bars indicate standard deviation. **$p<0.01$ (test group vs. placebo group)
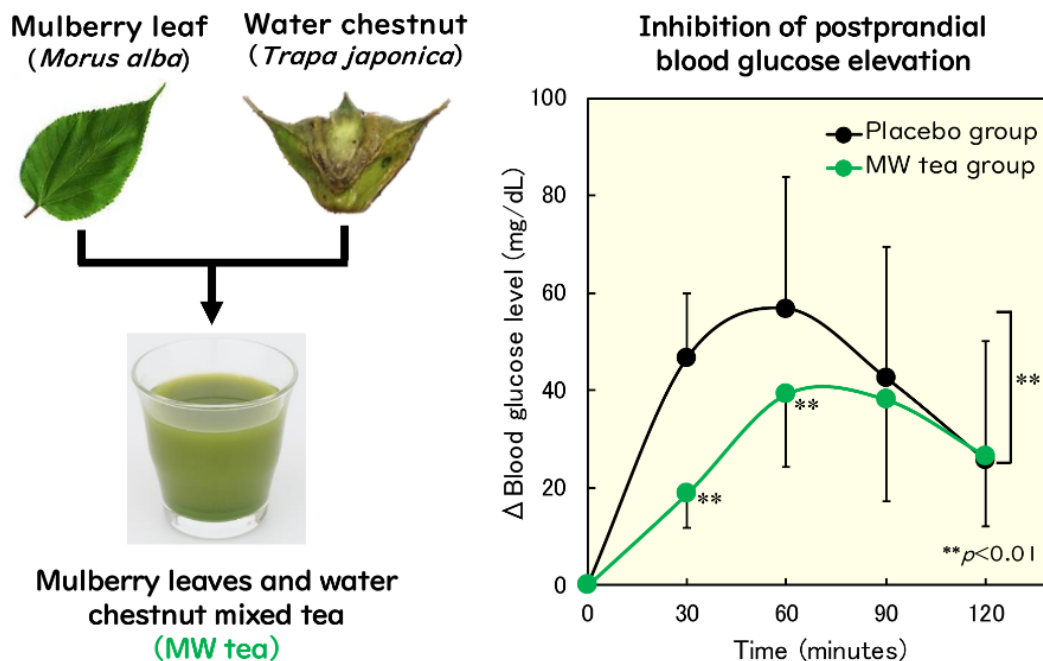
Graphical abstract.

DISCUSSION

In this study, mulberry leaf and water chestnut husk were used as tea ingredients. The total polyphenol content and antioxidant activity of water chestnut husk were about 3 and 6 times higher than those of mulberry leaf, respectively. The antioxidant activity was increased by adding water chestnut husk to mulberry leaves as the latter contains hydrolysable polyphenols, namely eugeniin, TGG and trapain.[20] These polyphenols have been shown to exhibit high antioxidant activity.[20,24] In diabetic patients, acute postprandial hyperglycemia increases oxidative stress, which impairs endothelial function and causes cardiovascular and other complications.[26-27] Therefore, it is necessary to decrease oxidative stress as much as possible. Polyphenols with high antioxidant activity found in water chestnut husk may reduce complications associated with high blood glucose level.

The MW tea was found to suppress insulin secretion, while reducing the increase in postprandial blood glucose levels. Many studies on the blood glucose suppression effect of mulberry leaves have been published.[10-19] In a study by Kimura et al.,[17] a significant difference in blood glucose and insulin levels was observed 60 minutes after ingesting 12 mg of DNJ and sugar, compared to the placebo. In a study by Asai et al.,[18] a significant decrease in blood glucose and insulin levels was observed only 30 min after ingesting 9 mg of DNJ, compared to the loaded food. In our study, after consuming MW tea (containing 10.2 mg of DNJ) followed by cooked rice, a significant decrease in blood glucose levels was observed at 30 and 60 min compared to the placebo tea (Figure 3A). Additionally, a significant decrease in insulin levels was observed at 30, 60, 90, and 120 min after the placebo (Figure 3B). These findings suggest that mulberry leaves combined with water chestnut husk are more effective in controlling blood glucose and insulin levels than mulberry leaves alone.

When only water chestnut husk was used, blood glucose levels were significantly low at 20 and 30 min after ingestion of the hot water extract of water chestnut husk followed by rice compared to placebo.[21] However, there was no significant difference in the IAUC of Δblood glucose levels at 120 min. In contrast, this study found that there was a significant difference of blood glucose levels at 30 and 60 min (Figure 3A), with the IAUC of Δblood glucose levels remaining significantly different up to 120 min (Figure 4A). This suggests that adding mulberry leaves to water chestnut husk further could further suppress the rise in blood glucose levels. Therefore, the mixed MW tea is more effective in suppressing the rise in blood glucose levels than mulberry leaves or water chestnut husk alone.

The mechanism behind the suppression of postprandial blood glucose elevation and insulin secretion by MW tea is believed to involve the α-glucosidase inhibitory activity of DNJ[11-13,23] found in mulberry leaves, and polyphenols[20] found in water chestnut husk. However, since 95% (w/w) of the test foods used in this study consisted of mulberry leaves, the effect of DNJ appears to be the primary contributor. In this study, ingestion of 10.2 ± 0.8 mg of DNJ significantly reduced the increase in postprandial blood glucose levels and insulin secretion. DNJ can also improve insulin sensitivity by inhibiting insulin secretion and is thought to inhibit protein kinase c (PKC) signaling.[28] Additionally, DNJ is absorbed from the gastrointestinal tract and distributed and accumulated in organs, including the kidneys, to control blood glucose.[29]

Long-term consumption of mulberry leaf extract in mice was reported to maintain hypoglycemia and decrease insulin secretion.[30] In this study, we examined the effects of a single intake of MW tea, but we plan to

investigate the effects of long-term intake in the future. This is expected to further reduce insulin resistance and help prevent the onset of type 2 diabetes.

This study has several limitations. First, the extent of the hypoglycemic effect of MW tea compared to mulberry leaves alone has not been confirmed. Future studies should directly compare the hypoglycemic effects of MW tea and mulberry leaf extract. Secondly, this study did not examine whether the total polyphenols in MW tea reduce oxidative stress in humans. Future studies should aim to assess the reduction of oxidative stress markers in humans as an outcome.

Conclusion

In this study, a randomized, double-blind, placebo-controlled, crossover comparative trial was conducted with 30 participants with borderline diabetes to evaluate the effect of MW tea (a mixture of mulberry leaves and water chestnut husk) on postprandial blood glucose levels. The test group (MW tea) had significantly lower blood glucose and insulin levels after the loaded food than the placebo group. Water chestnut also increased the antioxidant activity of the test food. In conclusion, MW tea has both hypoglycemic and antioxidant properties, thus expected to be an effective food for borderline diabetics to prevent a rapid rise in blood glucose levels without a large increase in insulin levels.

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