# Inhibition of postprandial blood glucose elevation in Japanese individuals with borderline diabetes by mulberry leaves and water chestnut mixed tea: A randomized, double-blind, placebo-controlled, crossover comparative study

doi: 10.6133/apjcn.202410/PP.0003
Published online: October 2024

**Running title:** Mulberry-water chestnut tea inhibits blood glucose

Midori Yasuda PhD[1,2], Kenichiro Yasutake PhD[3], Iori Yoshinaga MS[4], Kanako Nakashima MS[1], Madoka Saiki PhD[1], Ai Takeyama-Mitsuta PhD[1], Tatsuya Doi MS[2]

[1]Department of Health and Nutrition Sciences, Nishikyushu University, Saga, Japan
[2]Department of Life Support Science, Graduate School of Nishikyushu University, Saga, Japan
[3]Department of Nutritional Sciences, Nakamura Gakuen University, Fukuoka, Japan
[4]Department of Nutritional Sciences, Nakamura Gakuen University Junior College, Fukuoka, Japan

**Authors' email addresses and contributions:**
MY: midori@nisikyushu-u.ac.jp
Contribution: conceptualization, methodology, validation, investigation, resources and writing original draft.

KY: yasutake@nakamura-u.ac.jp
Contribution: methodology, data curation, data analysis and writing original draft.

IY: 0619iori@gmail.com
Contribution: data curation and data analysis.

KN: nakashimakan@nisikyu-u.ac.jp
Contribution: research implementation and data collection.

MS: hinom@nisikyu-u.ac.jp
Contribution: research implementation and data collection.

AT: takeyamaa@nisikyu-u.ac.jp
Contribution: data analysis and interpretation.

TD: 22d001@mail2.nisikyu-u.ac.jp
Contribution: data analysis and interpretation.

**Corresponding Author:** Dr Midori Yasuda, Department of Health and Nutrition Sciences, Nishikyushu University, Kanzaki, Saga 842-8585, Japan. Tel: +81952379274. Fax: +81952379274. Email: midori@nisikyushu-u.ac.jp

**ABSTRACT**

**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 and 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 food group, blood glucose levels in the test food 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 mixed tea may be an effective food 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 health, well-being, and economic challenge. 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 after we measured the content of polyphenols and DNJ and the antioxidant activity of MW tea in an in vitro experiment.

## MATERIALS AND METHODS

### *Sample preparation*

Mulberry leaves were collected from Maguwa (*Morus alba*) grown in Saga, Japan, immediately hot-air dried 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 content part (starchy) 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 each component 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). Unless otherwise noted, other reagents were special grade from Fujifilm Wako Pure Chemicals Corporation.

***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-nitrobenzofurazan23 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 the 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 to be detected from 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 study18. This was because subject background, such as gender, age, and obesity level, differed, the exclusion criteria were different, the possibility of dropouts needed to be considered, and the outcomes of this study included not only the IAUC of Δ blood glucose levels, but also the IAUC of Δ insulin.

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 follows: (1) not meeting the above 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) regular intake of 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 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) judged by the doctor of this trial to be unsuitable as a participants, and (12) request to withdraw from this trial.

We held a research information session for those interested in the study to fully explain the purpose and methods of the study 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 3g 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 Δ blood glucose level up to 120 min after the loaded food intake. The secondary outcomes were the Δinsulin (the change in insulin when the insulin before ingesting the loaded food was set to 0) and the 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*

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 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, and a large effect (inhibition rate of 70% or more) was observed when 80% or more 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 (inhibition rate of 40% or more) when 80% or more mulberry leaves were used (Figure 2B). These results show that a high ratio of mulberry leaves (80% or more) is desirable in terms of α-glucosidase inhibitory activity. It was also found that it is better to include as much water chestnut shell as possible in terms of polyphenol content and antioxidant activity.

We are considering commercializing MW tea in the future, but water chestnut is a rare plant that grows naturally in rivers and ponds, and its production volume is very small. Therefore, it was necessary to reduce the ratio of water chestnut husk as much as possible. Therefore, we determined that the optimal ratio of mulberry leaves and water chestnut husk for MW tea is 95:5 (%, w/w). In the future, we plan to cultivate water chestnuts to increase production and increase the ratio of water chestnut husk in MW tea.

The nutritional facts of the test, placebo, and loaded foods is summarized in Table 1. And 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 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. The DNJ content in 3 g of the test food consumed by the participants in the human clinical study contained 10.2 ± 0.8 mg. The DNJ and water chestnut polyphenols, which are active ingredients in mulberry leaves and water chestnut husk, can be said to be safe because the amounts consumed were lower than those previously reported,17, 20, 21 which were also deemed safe.

***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 food 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 two groups and the test food group showed significantly lower values ($p<0.01$) than the placebo food group 30 and 60 min later, respectively. 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 of the placebo food and test food groups increased after ingestion of the loaded food, but that of the test food group was significantly ($p<0.01$) lower than that of the placebo food 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 food group than in the placebo food group (Figure 4B).

## 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. Especially, the antioxidant activity was increased by adding water chestnut husk to mulberry leaves, because water chestnut husk 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 have been published on the blood glucose suppression effect of mulberry leaves.10-19 In a study by Kimura et al.,17 after ingesting 12 mg of DNJ and sugar, a significant difference was observed in blood glucose and insulin levels only 60 min after the placebo. In a study by Asai et al.,18 after ingesting 9 mg of DNJ, a significant decrease in blood glucose and insulin levels was observed only 30 min after the loaded food. In our case, after drinking MW tea (DNJ: 10.2 mg) and then ingesting cooked rice, a significant decrease in blood glucose levels was observed 30 and 60 min after the placebo tea (Figure 3A), and a significant decrease in insulin levels was observed 30, 60, 90, and 120 min after the placebo (Figure 3B). These results indicate that mulberry leaves with water chestnut husk added 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 placebo21. However, there was no significant difference in the IAUC of Δblood glucose levels at 120 min. By contrast, in this study, there was a significant difference of blood glucose levels at 30 and 60 min (Figure 3A), and even the IAUC of Δblood glucose levels up to 120 min (Figure 4A). This suggests that adding mulberry leaves to water chestnut

husk further suppressed 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 underlying the suppression of postprandial blood glucose elevation and insulin secretion by MW tea is thought to involve α-glucosidase inhibitory activity by DNJ11-13,23 present in mulberry leaves and polyphenols20 present in water chestnut husk. However, since 95% (w/w) of the test foods used in this study were mulberry leaves, the effect due to DNJ seems to be the main one. 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 Here, we investigated the effects of a single intake of MW tea, but we plan to investigate long-term intake in the future. This is expected to reduce insulin resistance further and prevent the onset of type 2 diabetes.

This study has several limitations. First, the degree of the hypoglycemic effect of MW tea compared with that of mulberry leaves has not been confirmed. Future studies should directly compare the hypoglycemic effects of MW tea and tea extracted from mulberry leaves. Second, this study did not investigate whether the total polyphenols contained in MW tea reduce oxidative stress in humans. Future studies should be designed to reduce oxidative stress markers in humans as an outcome.

***Conclusion***

In this study, a randomized, double-blind, placebo-controlled, crossover comparative trial was conducted in 30 borderline diabetic patients to determine the effect of MW tea, a mixture of mulberry leaves and water chestnut husk, on postprandial blood glucose levels. The test food (MW tea) group had significantly lower blood glucose and insulin levels after the loaded food than the placebo food group. Water chestnuts also increased the antioxidant activity of the test food. In conclusion, MW tea has both hypoglycemic and antioxidant properties, and is 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.

## ACKNOWLEDGEMENTS

We sincerely thank all participants who took part in this clinical trial.

## CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

This study was funded by Kanzaki-shi, Saga, Japan, under the title “Product Development Research Grant” for 2018-2022.

## REFERENCES

1. 1. IDF diabetes atlas, 10th edn. https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf
2. O’Keefe JH, Bell DS. Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. Am J Cardiol. 2007;100:899–904. doi:10.1016/j.amjcard.2007.03.107.
3. Saisho Y. Glycemic variability and oxidative stress: A link between diabetes and CVD? Int J Mol Sci. 2014;15:18381–406. doi:10.3390/ijms151018381.
4. Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, Ziegelasch HJ, Lindner J. Risk factors for myocardial infarction and death in newly detected NIDDM: The diabetes intervention study, 11-year follow-up. Diabetologia. 199639:1577–83. doi:10.1007/s001250050617.
5. Solomon TPJ, Eves FF, Laye MJ. Targeting postprandial hyperglycemia with physical activity may reduce CVD risk. But what should we do, and when is the right time to move? Front Cardiovasc Med. 2018;5:99. doi:10.3389/fcvm.2018.00099.
6. Russell WR, Baka A, Björck I, Delzenne N, Gao D, Griffiths HR, et al. Impact of diet composition on blood glucose regulation. Crit Rev Food Sci Nutr. 2016;56:541–90. doi:10.1080/10408398.2013.792772.
7. Chinsembu KC. Diabetes mellitus and nature’s pharmacy of putative antidiabetic plants. J Herb Med. 2019;15:100230. doi:10.1016/j.hermed.2018.09.001.
8. Jin Y, Arroo RRJ. Application of dietary supplements in the prevention of type 2 diabetes-related cardiovascular complications. Phytochem Rev. 2021;20:181–209. doi:10.1007/s11101-020-09710-8.
9. Butt MS, Nazir A, Sultan MT, Schroën K. Morus alba L. nature’s functional tonic. Trends Food Sci Technol. 2008;19:505–12. doi:10.1016/j.tifs.2008.06.002.
10. Yoshikuni Y. Inhibition of intestinal intestinal α -glucosidase activity and postprandial hyperglycemia by moranoline and its N-alkyl derivatives. Agric Biol Chem. 1988;52:121–8. doi:10.1080/00021369.1988.10868611.
11. Asano N, Oseki K, Tomioka E, Kizu H, Matsui K. N-Containing sugars from Morus alba and their glycosidase inhibitory activities. Carbohydr Res. 1994;259:243–55. doi:10.1016/0008-6215(9484060-1.

12. Breitmeier D, Günther S, Heymann H. Acarbose and 1-deoxynojirimycin inhibit maltose and maltooligosaccharide hydrolysis of human small intestinal glucoamylase-maltase in two different substrate-induced modes. Arch Biochem Biophys. 1997;346:7–14. doi:10.1006/abbi.1997.0274.
13. Oku T, Yamada M, Nakamura M, Sadamori N, Nakamura S, Nakamura S. Inhibitory effects of extractives from leaves of Morus alba on human and rat small intestinal disaccharidase activity. Br J Nutr. 2006;95:933–8. doi:10.1079/BJN20061746.
14. Kimura M, Chen FJ, Nakashima N, Kimura I, Asano N, Koya S. Antihyperglycemic effects of N-containing sugars derived from Mulberry leaves in streptozocin-induced diabetic mice. J Trad Med. 1995;12:214–9.
15. Park JM, Bong HY, Jeong HI, Kim YK, Kim JY, Kwon O. Postprandial hypoglycemic effect of mulberry leaf in Goto-Kakizaki rats and counterpart control Wistar rats. Nutr Res Pract. 2009;3:272–8. doi:10.4162/nrp.2009.3.4.272.
16. Kwon HJ, Chung JY, Kim JY, Kwon O. Comparison of 1-deoxynojirimycin and aqueous mulberry leaf extract with emphasis on postprandial hypoglycemic effects: In vivo and in vitro studies. J Agric Food Chem. 2011;59:3014–9. doi:10.1021/jf103463f.
17. Kimura T, Nakagawa K, Kubota H, Kojima Y, Goto Y, Yamagishi K, Oita S, Oikawa S, Miyazawa T. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J Agric Food Chem. 2007;55:5869–74. doi:10.1021/jf062680g.
18. Asai A, Nakagawa K, Higuchi O, Kimura T, Kojima Y, Kariya J, Miyazawa T, Oikawa S. Effect of mulberry leaf extract with enriched 1-deoxynojirimycin content on postprandial glycemic control in participants with impaired glucose metabolism. J Diabetes Investig. 2011;2:318–23. doi:10.1111/j.2040-1124.2011.00101.x.
19. Thondre PS, Lightowler H, Ahlstrom L, Gallagher A. Mulberry leaf extract improves glycaemic response and insulaemic response to sucrose in healthy participants: Results of a randomized, double blind, placebo-controlled study. Nutr Metab (Lond). 2021;18:41. doi:10.1186/s12986-021-00571-2.
20. Yasuda M, Yasutake K, Hino M, Ohwatari H, Ohmagari N, Takedomi K, Tanaka T, Nonaka G. Inhibitory effects of polyphenols from water chestnut (Trapa japonica) husk on glycolytic enzymes and postprandial blood glucose elevation in mice. Food Chem. 2014;165:42–9. doi:10.1016/j.foodchem.2014.05.083.
21. Yasuda M, Yasutake K, Ohmagari N, Saiki M, Kojima Y. Properties of polyphenols in hot water extract of water chestnut husk and suppressive effect on postprandial blood glucose elevation in humans. Food Preserv Sci. 2018;44:179–87. in Japanese. doi:10.5891/jafps.44.179.
22. Sun T, Tang J, Powers JR. Effect of pectolytic enzyme preparations on the phenolic composition and antioxidant activity of asparagus juice. J Agric Food Chem. 2005;53:42–8. doi:10.1021/jf0491299.
23. Anno T, Tamura K, Oono H, Tomi H. Maltase, sucrase and α-amylase inhibitory activity of Morus leaves extract. Food Preserv Sci. 2004;30:223–9. in Japanese. doi:10.5891/jafps.30.223.

24. Yasuda M, Ikeoka M, Kondo SI. Skin-related enzyme inhibitory activity by hydrolyzable polyphenols in water chestnut (Trapa natans) husk. Biosci Biotechnol Biochem. 2021;85:666–74. doi:10.1093/bbb/zbaa076.
25. Wasai M, Urata N, Fujii Y, Kawaoka A, Tachibana H, Fujihara S, Sakano K, Hayashi M, Ebihara S. Effect of Sunrouge on the postprandial blood glucose level-a randomized, double-blind, crossover-study. Jpn Pharmacol Ther. 2017;45:105–11. in Japanese
26. Ceriello A. The emerging role of post-prandial hyperglycaemic spikes in the pathogenesis of diabetic complications. Diabet Med. 1998;15:188–93. doi:10.1002/(SICI)1096-9136(199803)15:3<188::AID-DIA545>3.0.CO;2-V.
27. Maruhashi T, Higashi Y. Pathophysiological association between diabetes mellitus and endothelial dysfunction. Antioxidants (Basel). 2021;10:1306. doi:10.3390/antiox10081306.
28. Sheng Y, Zheng S, Zhang C, Zhao C, He X, Xu W, Huang K. Mulberry leaf tea alleviates diabetic nephropathy by inhibiting PKC signaling and modulating intestinal flora. J Funct Foods. 2018;46:118–27. doi:10.1016/j.jff.2018.04.040.
29. Parida IS, Takasu S, Ito J, Ikeda R, Yamagishi K, Kimura T, Miyazawa T, Eitsuka T, Nakagawa K. Physiological effects and organ distribution of bacillus amyloliquefaciens AS385 culture broth powder containing 1-deoxynojirimycin in C57BL/6J mice. J Nutr Sci Vitaminol (Tokyo). 2019;65:157–63. doi:10.3177/jnsv.65.157.
30. Tanabe K, Nakamura S, Omagari K, Oku T. Repeated ingestion of the leaf extract from Morus alba reduces insulin resistance in KK-Ay mice. Nutr Res. 2011;31:848–54. doi:10.1016/j.nutres.2011.09.023.

**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

| Group | Age (year) | Height (cm) | Weight (kg) | BMI (kg/m$^2$) |
|---|---|---|---|---|
| Total | 20.8±1.0 | 178.6±4.8 | 71.2±7.3 | 22.3±1.8 |
| PAEE | | | | |
| Gp1 | 20.5±0.8 | 176.0±3.5 | 68.9±5.58 | 22.2±1.1 |
| Gp2 | 20.9±1.2 | 177.6±4.4 | 68.3±5.2 | 21.6±1.4 |
| Gp3 | 21.3±1.1 | 180.7±6.6 | 72.5±7.9 | 22.2±1.4 |
| Gp4 | 20.5±1.0 | 180.3±3.0 | 75.1±8.8 | 23.1±2.8 |
| *F* | 1.309 | 2.435 | 2.149 | 1.193 |
| *p* | 0.285 | 0.080 | 0.110 | 0.325 |
| MET | | | | |
| Gm1 | 20.5±0.9 | 177.2±4.1 | 68.4±5.7 | 21.7±1.3 |
| Gm2 | 20.8±0.9 | 180.5±7.6 | 74.6±8.1 | 22.9±1.9 |
| Gm3 | 21.0±1.3 | 177.0±2.7 | 68.1±3.1 | 21.7±0.8 |
| Gm4 | 20.9±1.1 | 180.1±3.3 | 74.1±9.3 | 22.8±2.7 |
| *F* | 0.366 | 1.552 | 2.749 | 1.365 |
| *p* | 0.778 | 0.217 | 0.056 | 0.268 |

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.

**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 and DBP indicate systolic blood pressure and diastolic blood pressure, respectively.
Statistical analysis was performed using Fisher's exact probability test and Student's t-test.

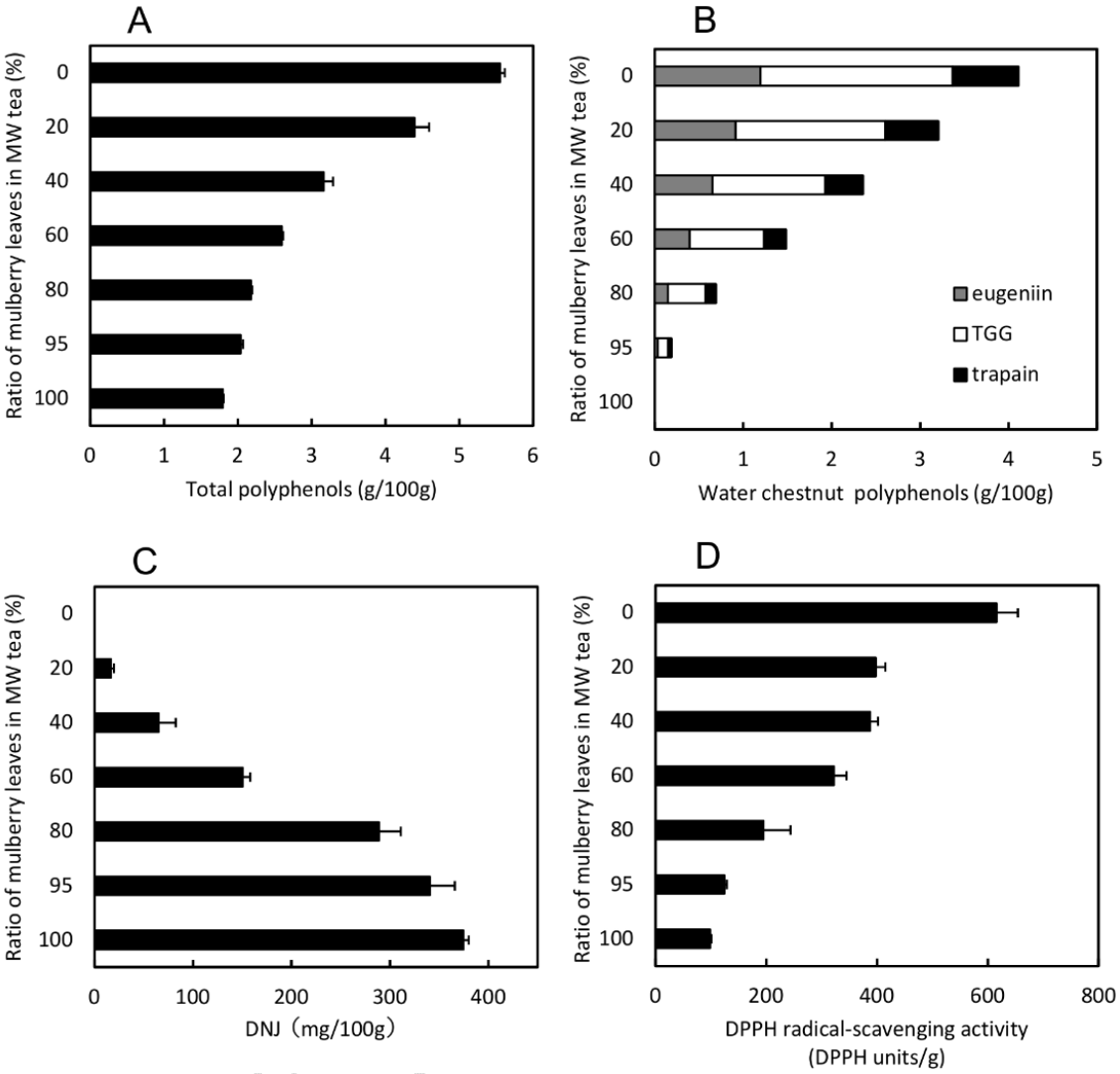



**Figure 1.** Effect of mulberry leaves and water chestnut husk ratio in MW tea on chemical composition and antioxidant activity. Error bars indicate standard deviation. (A) total polyphenols; (B) water chestnut polyphenols; (C) 1-deoxynojirimycin (DNJ); (D) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging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

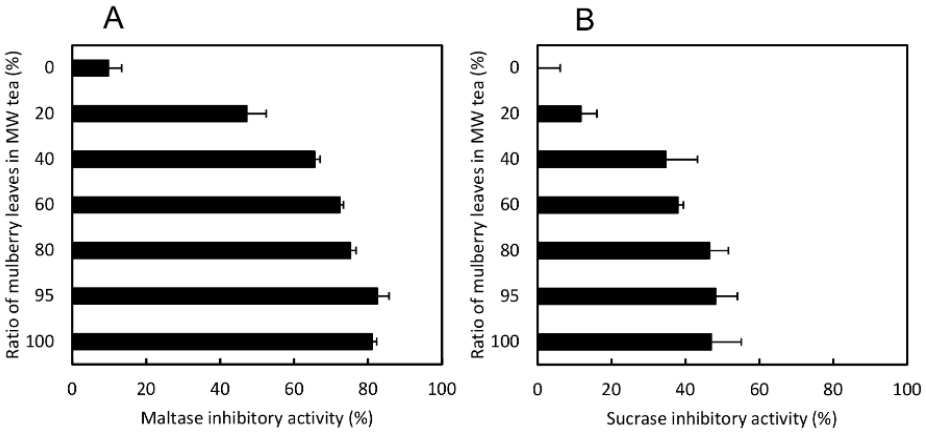



**Figure 2.** Effect of mulberry leaves and water chestnut husk ratio in MW tea on α-glucosidase inhibitory activity. Error bars indicate standard deviation. (A) maltase; (B) sucrase. MW: mulberry leaves and water chestnut husk.

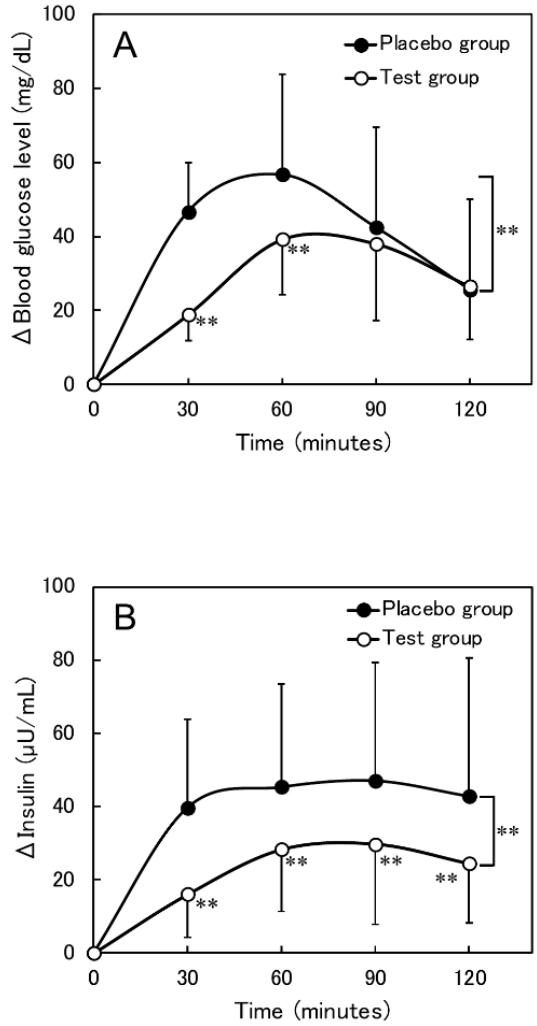






**Figure 3.** Effect of MW tea on postprandial blood glucose and insulin. (A) blood glucose; (B) insulin. The Δblood glucose represents the change in blood glucose level when the blood glucose level before ingestion of the load food is set to 0, and Δinsulin represents the change in insulin when the 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)

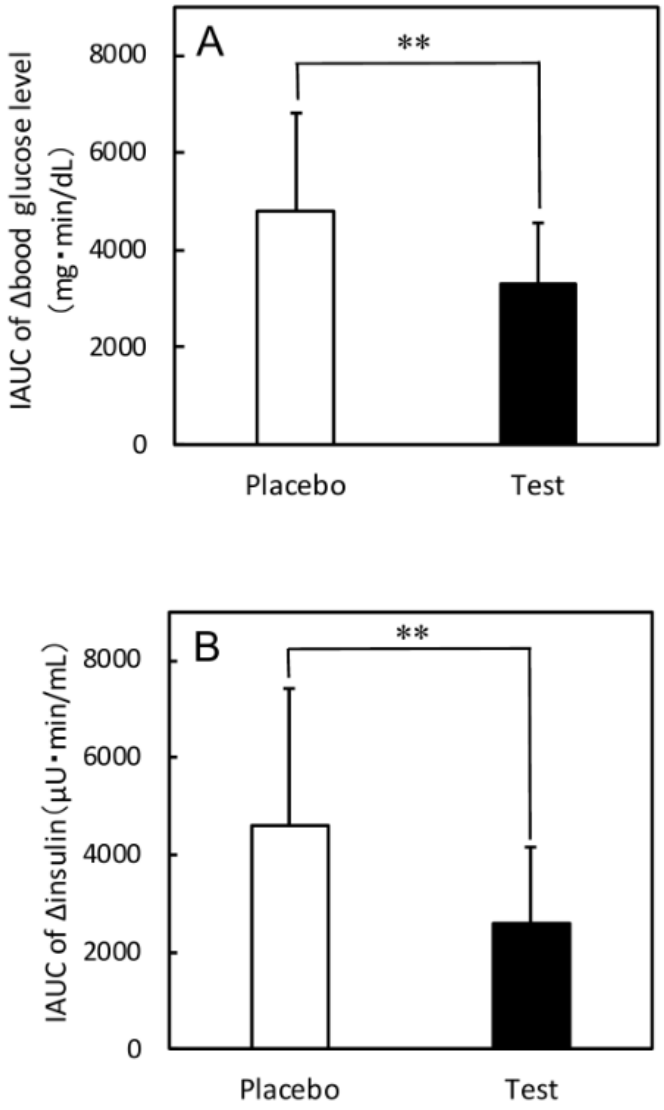
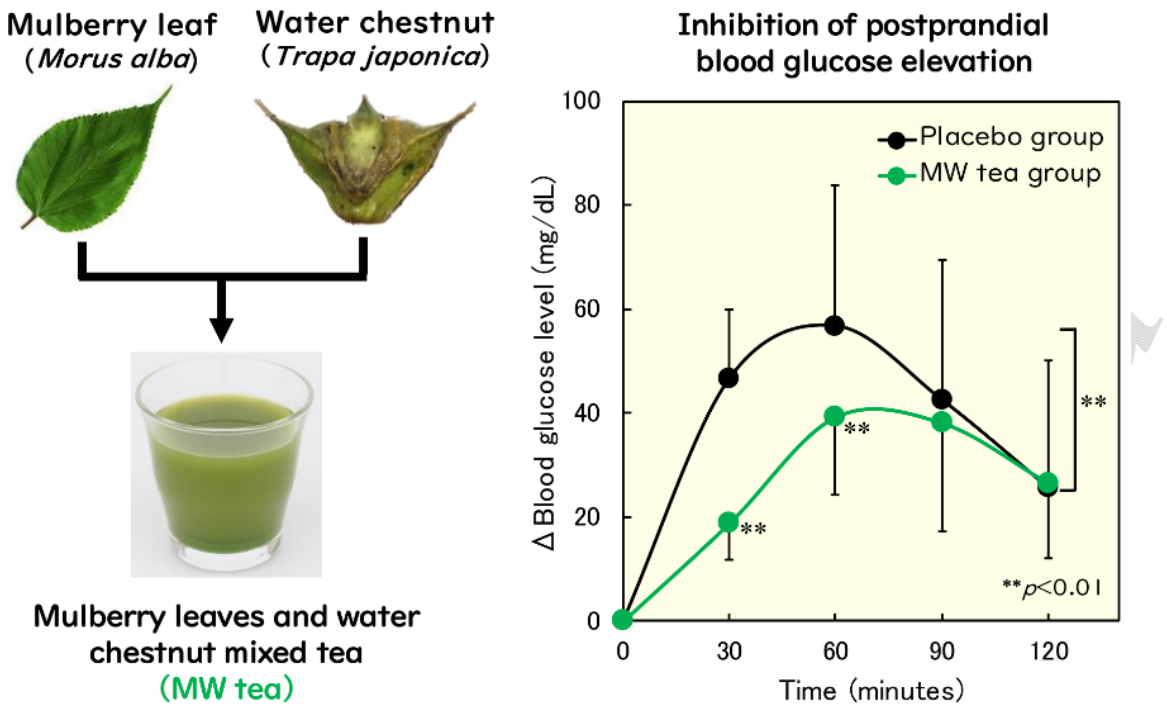



**Figure 4.** Effect of MW tea on IAUC Δblood glucose and Δinsulin. (A) blood glucose; (B) insulin. The IAUC means the incremental area under the curve of the Δblood glucose level or Δinsulin up to 120 min after the load food intake. The explanation of Δblood glucose and Δinsulin is the same as in Figure 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.**