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RESEARCH ARTICLE

The Synergistic Enhancement of Anti-Metabolic Diseases Function of *Morus alba* with the Combination of Cha (*Camellia sinensis*)

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Abstract: ***Background*:** Functional foods play an important role in the prevention and amelioration of metabolic syndromes leading to type 2 diabetes. Plant resources that have anti-metabolic syndromes activity, such as *Morus alba L.* and Cha (*Camellia sinensis L.*), have been used in functional foods against diabetes. Since Morus and Cha have different mechanisms of action against metabolic syndromes, such as prevention of sugar uptake and lipidosis, respectively, the combination of both resources will be a reliable approach for developing more efficient functional food against type 2 diabetes because certain synergism is expected in their functions.

***Methods*:** Male Wister Rats were fed the high fat-high sucrose (HFHS) diet for 12 weeks, with and without supplementation of Morus and Cha alone and their combination, and the effect of their supplementation on the markers of the metabolic syndrome such as obesity, lipidosis, and fatty liver formation, were examined.

***Results*:** Several metabolic syndrome markers, including body weight gain, lipid deposit, and fatty liver formation, were more significantly prevented by the diet supplemented with Morus and Cha combination compared to Morus or Cha given separately.

***Conclusion*:** Appropriate formulation of food resources with different functional mechanisms is a promising strategy for developing effective dietary treatment of type 2 diabetes that is a typical Mibyou.

Keywords: Anti-metabolic syndromes, synergistic effect, *Morus alba*, *Camellia sinensis*, Mibyou-care functional food, obesity.

1. INTRODUCTION

In preventive medicine, the treatment of the asymptomatic stage of diseases that was defined in the traditional oriental medicines as the Mibyou is critical [1, 2]. The Mibyou concept was currently renovated to the modern Mibyou, which was defined by the Japan Mibyou Association as the condition where individuals can spend a normal life even if clinical markers show some abnormality, separately from serious diseases that need medication [3, 4]. Therefore, the term "Mibyou-care" is implicated as the primary strategy for sustaining health and wellness in the longevity society [4, 5], and for that, functional foods need to play crucial roles rather than clinical medicines [6].

According to the Mibyou concept, type-2 diabetes is a typical Mibyou. Diabetic mellitus is a pathological condition characterized by high blood sugar caused by insulin tolerance and accompanying complications such as retinal, nephrotic, and neuropathy abnormalities and cataracts [7, 8]. As was discussed in several review articles [9-12], the diabetic condition gradually leads to fatal diseases such as cardiovascular disorders [10, 13] and dementia [12, 14], which are the risk factors for decreasing quality of life (QoL) and increasing mortality in the longevity society. Sun H *et al*. [15] discussed that according to the IDF Diabetes Atlas, the diabetes prevalence in 20-79-year-olds in 2021 was estimated to be nearly 537 million people globally, indicating over 10.5% of the world's adult population now have this condition, and the prevalence will further increase to about 783 million in 2045. Therefore, the prevention and treatment of diabetes are emerging issues that need to be resolved in the present society. Metabolic syndromes, typically obesity resulting from abnormal lifestyle habits, including daily diet and exercise, are related to the pathogenesis of diabetes, especially type-2 diabetes [16, 17]. Obviously, diet is the basic strategy for the control of metabolic diseases, but also the beneficial role of functional foods is recognized in the current society [18]. Therefore, plants or natural products that have anti-diabetes potential are attracting much attention [19-23]. and their application for the prevention and remedy of diabetes has been extensively progressed [24].

Morus alba L. is one such plant resource that has a traditionally well-known anti-diabetic function [25-27]. We previously reported that functional components of *Morus alba L* are significantly improved by in-room hydroponics, and both leaf and root preparations of the hydroponic cultivar named SARAKUWA effectively suppressed body weight gain and

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fatty liver formation in the rodents fed with the high fat - high sucrose (HFHS) diet [28].

Although such fortified Morus by itself shows reasonable activity against metabolic syndromes, the formulation consisting of the plant resources with diverse functional mechanisms in the anti-diabetic functions will be more effective and improve their functions as the Mibyou-care functional food, probably through their synergistic action. In this sense, Cha (*Camellia sinensis*) is a candidate for the component to be combined with Morus in such formulation because Cha modulates the calorie expenditure [29, 30], whereas Morus inhibits the calorie intake process [31].

In the present study, the anti-obesity and fatty liver preventive functions were precisely examined by the combination of Morus and Cha using the HFHS diet feeding obesity rat model to see if any synergistic enhancement of their anti-metabolic disorder functions occurs.

2. MATERIALS AND METHODS

2.1. Test Samples and Solid Chows Preparation

Dried powder of leaf and stem parts mix (ML) prepared from the *Morus alba L* cultivated in the in-room hydroponics (trademark: SARAKUWA) was generously provided by Morera Co. Ltd (Suwa, Nagano, Japan. Cha (*Camellia sinensis*) leaf powder (CL) was purchased from Shizupack Co. Ltd. (Shizuoka, Japan). The Cha sample powder used in the present study is the Kawane-cha, one of the Japanese green tea brands, which is made from the selected leaves of a tree cultivated in a specific region of Shizuoka (Kawane district) in Japan.

Solid chow supplemented with Morus and Cha was prepared by Oriental Yeast Co. Ltd (Tokyo).

The solid chows prepared for five experimental groups are as follows: 1) Normal control (AIM-93M), 2) HFHS control, 3) ML group (HFHS supplemented with ML), 4) CL group (HFHS supplemented with CL), 5) MC group (HFHS supplemented with both ML and CL).

The concentrations of supplemented ML and CL powders were 2% (w/w) and 3% (3% w/w), respectively, and thus MC mix concentration was 5% (w/w). This ML/CL ratio was determined according to that of commercially available Morus powder product blended with Cha, which is named SO-KAN-RO (MORERA Co. Ltd.).

The nutritional composition of each chow diet was adjusted to match the total calorie with HFHS based on the nutritional component analysis data of ML and CL, respectively, in the same way as reported in our previous reports (Supplement Tables **I** and **II**) [28, 32].

2.2. Long-term Feeding Experiment in Rats

2.2.1. Animals

Four-week-old male Wister/ST rats were purchased from SLC Japan (Shizuoka, Japan). The rats were divided into two and put in one cage each and kept under the conditions of constant temperature at 22±1°C, illumination with a 12-h light /dark cycle, and free access to water and diet during both acclimation and long-term feeding trial. After acclimation for one week with basal diet (Labo MR stock, Nihon. Nosan Kogyo, Shizuoka, Japan), rats were randomly grouped into four designed diet groups (n=8 in each group) described above.

2.2.2. Ethical Approval

The experimental protocol and animal treatment procedure were approved by the Niigata University of Pharmacy and Applied Life Sciences and complied with the Guideline of Animal Care and Treatment (Approved No.2026-12).

2.2.3. Experimental Protocol

The experimental protocol for the animal experiments and biochemical assay were essentially the same as described in our previous reports [28, 32]. Briefly, body weight was recorded weekly, and the dietary intake volume was recorded every day during the feeding trial for 12 weeks. At the end of the feeding trial, rats were starved overnight, anesthetized with pentobarbital, and the blood was corrected in a heparinized syringe from the subclavian veins for biochemical analysis, as described precisely in our previous report [32]. The blood plasma separated by centrifugation at 3000 rpm and 4°C for 5 min using a micro-centrifuge apparatus (Sigma 1-14) was stored in a freezer at -80°C until use. At the same time, the tissues, including visceral fats (mesenteric, epididymal, and perirenal fats) and liver, were removed and weighed after rinsing in chilled saline. A piece of liver tissue was sampled and fixed in 10% formalin-neutral buffer solution for histological analyses, and the rest of the liver was stored at -80°C until biochemical analysis.

2.2.4. Biochemical Analysis

The plasma levels of glucose, cholesterol, and triglyceride were determined using commercially available kits, Glucose C II-test Wako, Cholesterol E-test Wako, and Triglyceride E-test Wako (Wako Pure Chemical Industries Ltd. Osaka, Japan), respectively, and the procedures followed the protocols attached. Furthermore, to determine plasma insulin level, the LBIS Rat Insulin ELISA Kit (FJIFILM Wako Shibayagi Corporation, Gunma, Japan) was used.

Liver lipids were extracted according to the method reported by Folch *et al*. [33]. Briefly, the liver was homogenized in AcOEt. The AcOEt layer was recovered by centrifugation, evaporated off, and the residual lipids were dissolved in 200 µL of 10% Triton-X 100/isopropanol to determine triglycerides and total cholesterol as described above.

2.2.5. Statistic Evaluation

All the data given as mean ± SEM were analyzed by Statcel 4 software (OMS Publishing Inc., Saitama, Japan). The statistical significance of the data was evaluated using the unpaired Student's *t*-test to mean of two groups, and one-way ANOVA followed by the Tukey-Kramer HSD test applied for comparisons between multiple experimental groups. The differences were considered statistically significant at p-value < 0.05.

3. RESULTS

3.1. Effects of Morus, Cha, and their Combination on the Body Weight Gain Caused by HFHS Diet

Rats were fed with HFHS control diet and HFHS diets supplemented with ML, CL, and their combination (MC) for 12 weeks, and the change in body weight of each group was observed. The time course of body weight changes of each experimental group during the feeding period and the body weight gain at the end of the feeding period are given in Figs. (**1A** and **B**), respectively. It was found that the HFHS diet increased body weight gain significantly during the feeding trial, but the body weight gain was significantly low in the MC group fed the HFHS diet containing both ML and CL together. In contrast, either solo ML or solo CL supplementation did not show a significant effect on the body weight gain.

During the feeding trial, both the net intake of chow and net calories taken by rats in each experimental group showed no significant differences among all experimental groups, except the normal diet group showed a higher amount of diet intake and slightly lower calorie intake compared to other experimental groups (Supplementary Fig. **1**).

3.2. Effect of Morus, Cha and their Combination on Fat Deposit after HFHS Diet Feeding Trial

After the HFHS feeding trial, the tissue weight and fat deposit were determined, and the results are shown in Table **1**. There was a significant increase in liver mass in the HFHS control group. ML solo supplementation group did not show any effect on the liver mass increase, however, the supplementation of both ML and CL together (MC group) significantly inhibited the HFHS diet-dependent increase of liver mass. The effect of ML and CL combination was also apparently huge compared to CL solo. Except for the liver, heart mass also significantly increased, and the increase was suppressed only in the MC diet group.

It was apparent that HFHS diet feeding increases visceral fat deposit significantly as determined in mesenteric, perirenal, and epididymal fats, respectively. ML supplementation did not show any suppressive effect on the fat deposit in these three regions, and also in the visceral fat as total. CL supplementation, on the other hand, significantly suppressed the fat accumulation in these fat tissues. However, it is notable that the combination of ML and CL [MC group] remarkably enhanced the suppressive effect compared to CL solo, and that the fat deposit in the MC group was the smallest of all the fat tissues that were examined (Table **1**).

3.3. Fat Excretion in the Feces

The feces were collected throughout the feeding period, and the recovered volumes were weighed after drying. In the HFHS diet control group, the daily feces excreted were significantly small compared to the normal control rats. The excretions were slightly enhanced in the groups supplemented with ML solo and CL solo, although the effect was not significant (Fig. **2**). The MC combination group enhanced the fecal fat excretion compared to both solo ML and solo CL groups, although it was not significant (Fig. **3A**).

When the fat contents per gram of the dried feces were compared, the MC group showed the highest value, and then, for normal diet control, the ML and CL groups followed. HFHS showed the lowest value. Using these values and the feces volumes in Fig. (**3A**), the daily total excretion of fat was calculated as in Fig. (**3B**). The fat excretion rate was markedly decreased in the HFHS diet group compared to the normal control diet group, but MC supplementation significantly enhanced the fecal fat excretion. ML solo and CL solo also stimulated the fat excretion, although the extents were significantly smaller compared to the MC combination group. It was also noted that ML solo tend to stimulate the fecal fat excretion a bit stronger than CL.

3.4. Inhibitory Function against Fatty Liver

The HFHS diet group apparently developed the fatty liver condition when assessed both by mg fat/liver tissue and mg fat/g liver tissue (Fig. **4**). ML solo supplementation did not suppress the liver fat deposit at all. However, CL solo supplementation decreased the fat deposit in the liver, as expected from its stimulative function on lipid metabolism. However, the effect observed in the MC group was clearer than in the CL group, indicating that the MC combination is more effective for preventing fatty liver formation than CL alone.

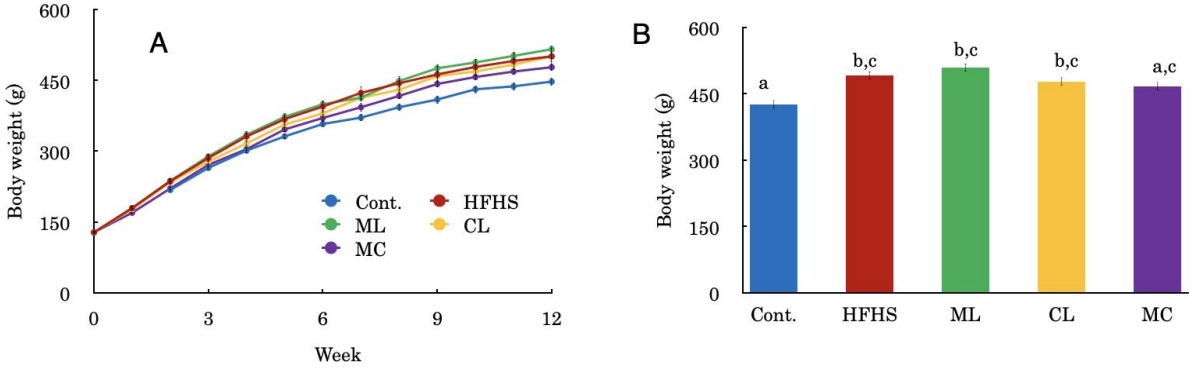

Fig. (1). Body Weight Change during Feeding Period.
A) body weight changing profiles during feeding period, **B)** body weight attained after feeding trials.
There is significant difference between the groups noted with different alphabet. ($P < 0.05$). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 1. Organ and tissue weight (n = 8, Means +/- SE).

-	Control	HFHS	ML	CL	MC (g)
Liver	10.86 +/- 0.24a	12.98 +/- 0.73a,b	13.27 +/- 0.64b	11.95 +/- 0.51a,b	11.66 +/- 0.52a,b
Spleen	0.72 +/- 0.06	0.73 +/- 0.03	0.69 +/- 0.02	0.70 +/- 0.06	0.65 +/- 0.05
Kidney	2.57 +/- 0.07	2.71 +/- 0.08	2.70 +/- 0.08	2.73 +/- 0.10	2.74 +/- 0.08
Heart	1.22 +/- 0.04	1.40 +/- 0.06	1.34 +/- 0.04	1.49 +/- 0.09	1.29 +/- 0.03
Lung	1.56 +/- 0.07	1.60 +/- 0.06	1.59 +/- 0.06	1.64 +/- 0.05	1.58 +/- 0.05
Mesenteric fat	6.47 +/- 0.68a	10.87 +/- 1.01b,c	12.68 +/- 0.80b	9.79 +/- 0.85b,c	7.76 +/- 0.60a,c
Epididymal fat	6.58 +/- 0.36a	13.15 +/- 1.59b	14.61 +/- 1.03b	10.59 +/- 0.75b,c	8.84 +/- 0.63a,c
Perirenal fat	7.67 +/- 0.71a	17.51 +/- 1.79b	17.95 +/- 1.69b	13.48 +/- 0.77b,c	10.27 +/- 0.88a,c
Visceral fat	20.73 +/- 1.64a	41.54 +/- 4.32b	45.23 +/- 3.36b	33.85 +/- 2.23b,c	26.88 +/- 1.99a,c

Note: There is statistically significant difference between the groups noted by different alphabet ($P < 0.05$).

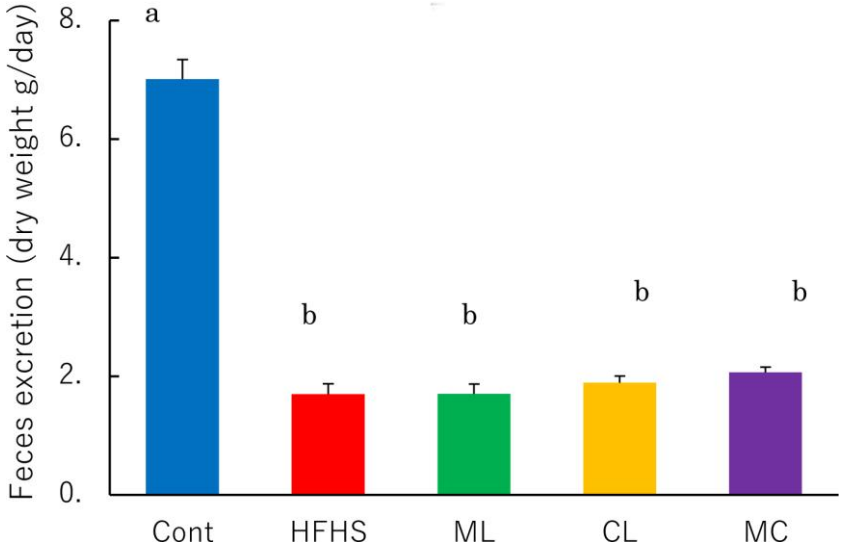

Fig. (2). Feces excretion during 24 hr. Feces recovered during 24 hr was dried and weighted. There is significant difference between the groups noted with different alphabet. ($P < 0.05$). (n=8 Means ± SE). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

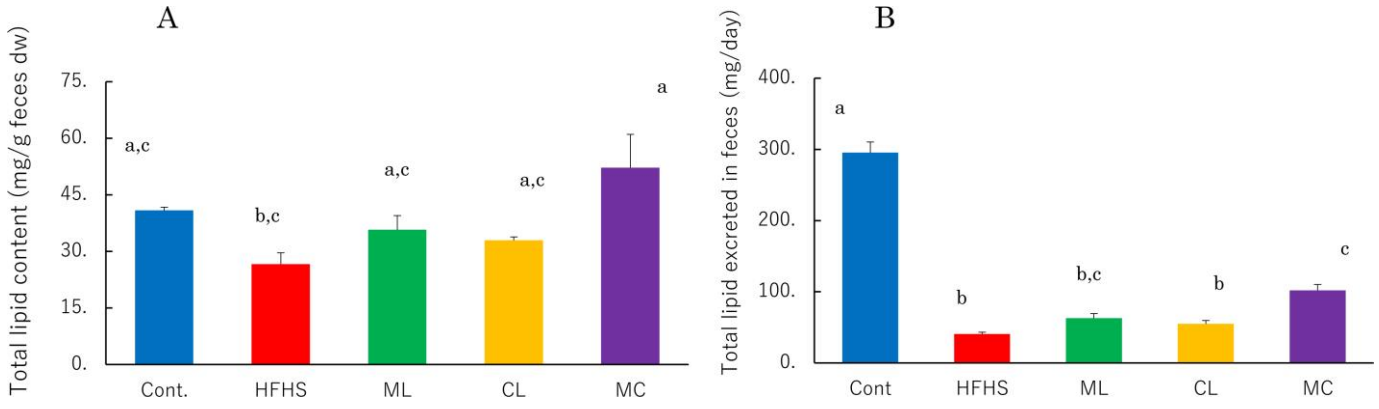

Fig. (3). Lipid excretion in Feces. (**A**) total lipid in feces. (**B**) total lipid excreted during 24 hrs. There is significant difference between the groups noted with different alphabet. ($P < 0.05$). (n=8, Means±SE). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

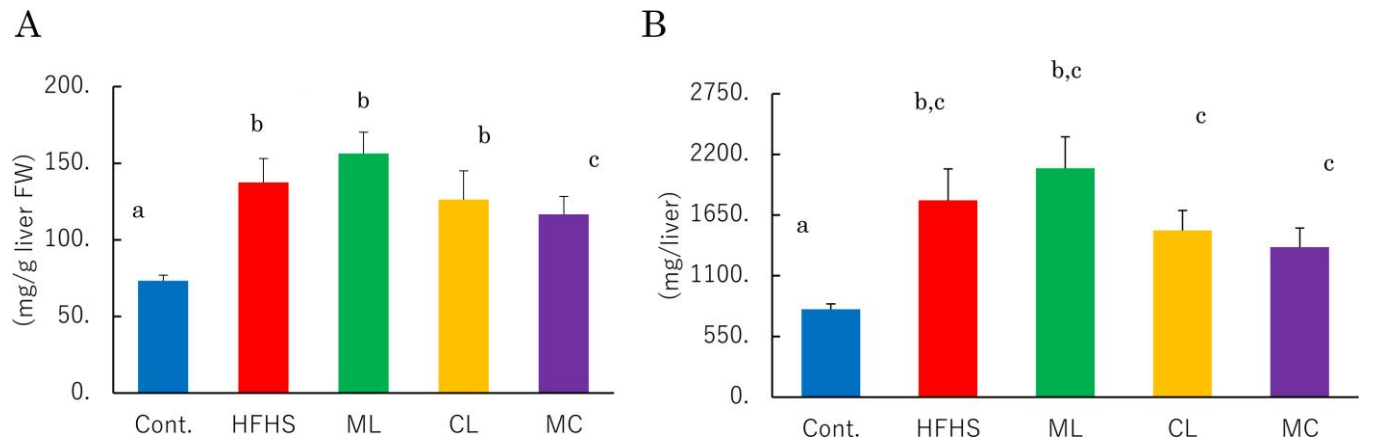

Fig. (4). Comparison of fat deposit in Liver. (**A**) lipid per 1g raw liver tissue. (**B**) total lipid in total liver. There is significant difference between the groups noted with different alphabet. ($P < 0.05$) (n=8, Means ± SE). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

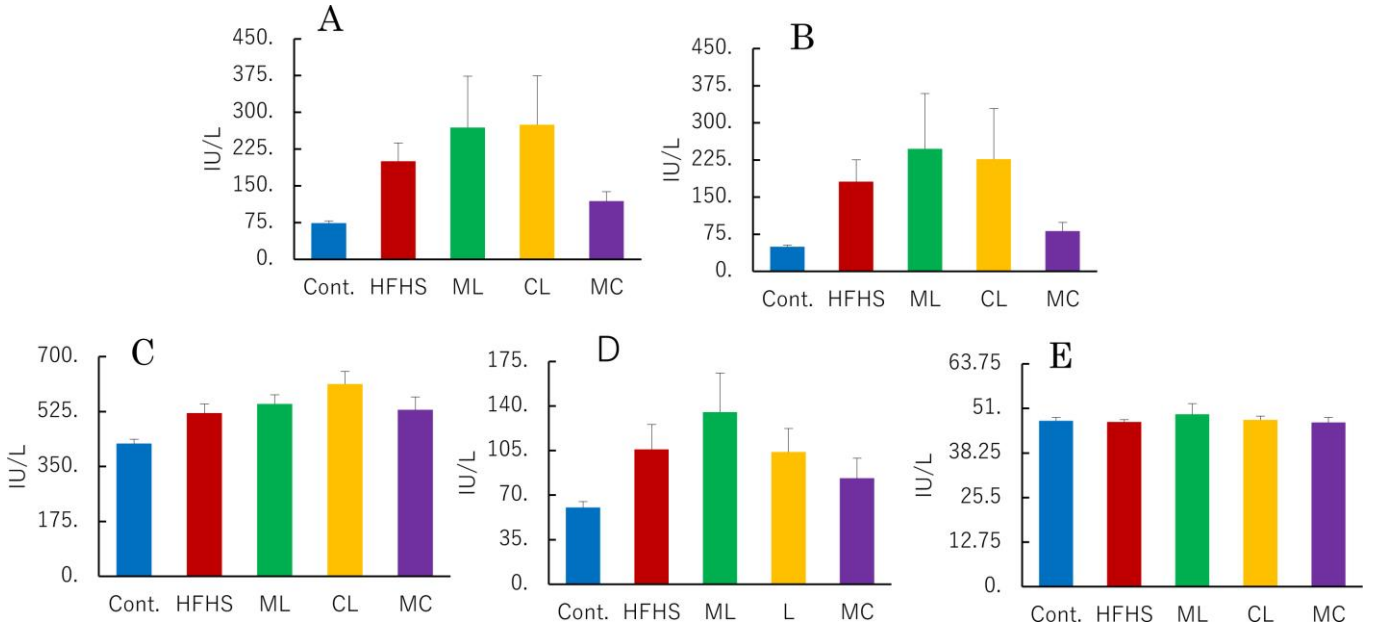

Fig. (5). Comparison of liver damage markers. (**A**) AST, (**B**) ALT, (**C**) ALP, (**D**) LDH, (**E**) LAP , (n=8, Means±SE). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

3.5. Amelioration of Liver Damage Markers

Blood markers of liver damage were biochemically determined after a long-term feeding trial, and the results are given in Fig. (**5**). All the liver damage markers, including aminotransferases (AST and ALT), Alkaline phosphatase (ALP) and Lactate Dehydrogenase (LDH), except Leucine aminopeptidase (LAP) were significantly increased in the HFHS diet group. However, it was found that each of the MC, ML, and CL supplementations showed different effects on each marker. Both ML solo and CL solo supplementations did not affect any of the liver damage markers or even increase the levels, typically, as shown in the ML-supplemented group in that AST, ALT, and LDH levels were rather high compared to the HFHS diet group. However, in the MC-supplemented group, the levels of AST, ALT, and LDH, especially the level of AST, were significantly low, indicating a synergy effect was occurring between ML and CL.

3.6. Change of Insulin Sensitivity after Long-term Feeding Trial

After the long-term feeding trial, the rats were starved for 24hr, and both blood glucose and insulin levels were determined (Fig. **6**). The blood glucose levels of the respective diet groups did not show significant differences, but the insulin levels significantly differed. The plasma insulin level was significantly high in the HFHS diet group, and, the CL-supplemented group, also showed a high level similar to the HFHS group. In contrast, both MC and ML supplementation significantly reduced the insulin level compared to the HFHS group, and the effect was clearer in the MC group. As a result, HOMA-IR was significantly reduced in the MC group, followed by ML.

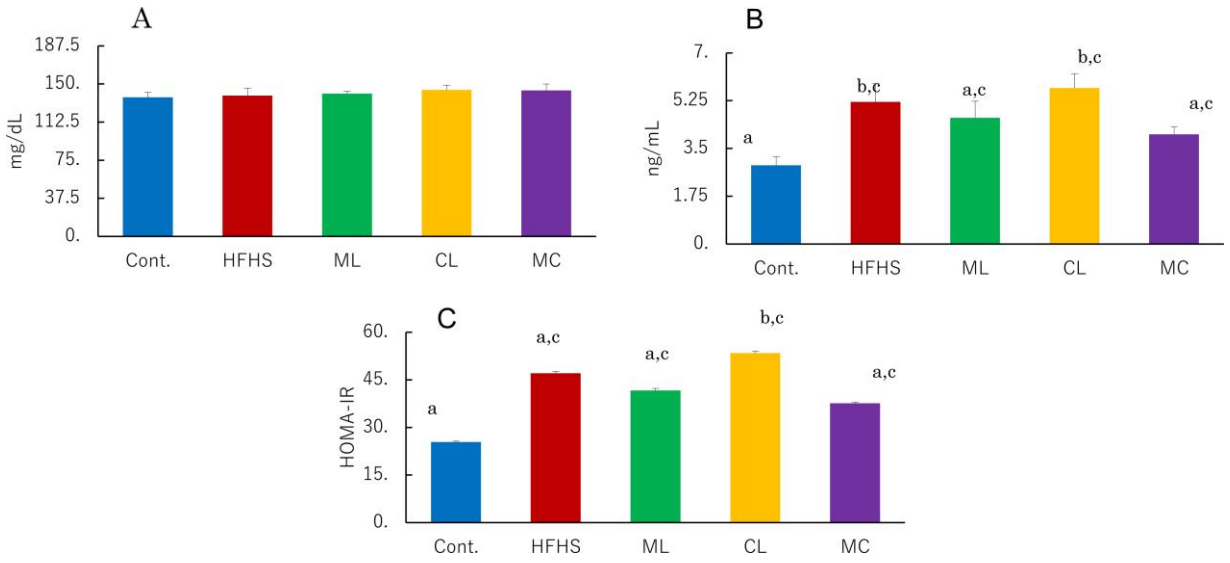

Fig. (6). Comparison of fasting blood sugar and insulin levels, and HOMA-IR. (**A**) fasting blood sugar level, (**B**) fasting insulin level, (**C**) HOMA-IR. There is significant difference between the groups noted with different alphabet. ($P < 0.05$). (n=8, Means±SE). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

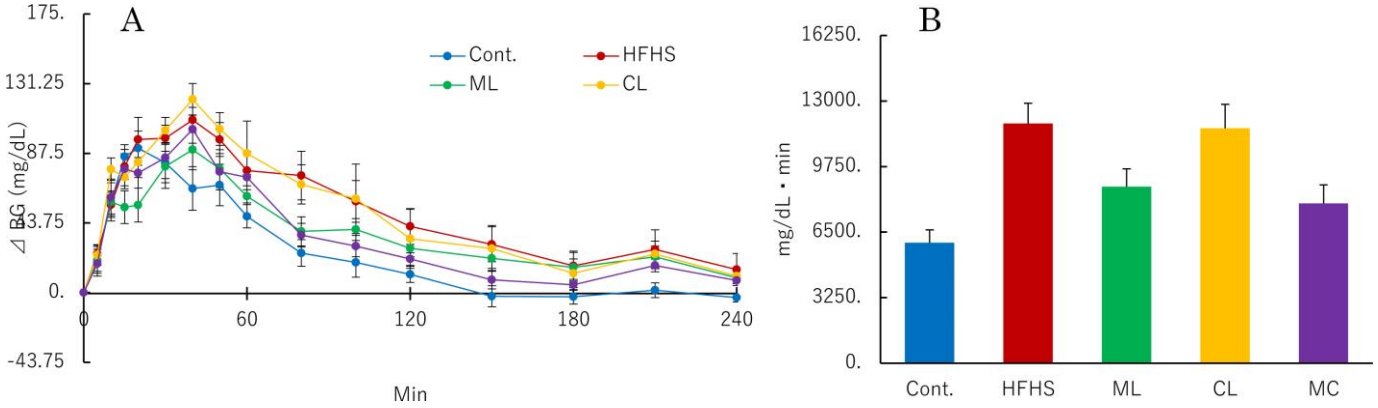

Fig. (7). Profiles of blood sugar level after OGTT, and IAUC. (**A**) changing profile of blood sugar level (ΔBG), (**B**) AUC during 0-120 min after glucose intake. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

3.7. Glucose Tolerance Test

After the long-term feeding trials, the OGTT (oral glucose tolerance test) was examined for the rats of each experimental group. The highest blood glucose spike was observed after glucose load in the HFHS diet group but the initial rise in the blood glucose level was significantly suppressed by ML or MC supplementations. Contrarily, CL supplementation did not give rise to any effect on the blood glucose behavior observed in the HFHS group when compared both in time-dependent profile and AUC (Fig. **7**).

4. DISCUSSION

The prevention and remedy of type 2 diabetes is a typical target of Mibyou-care, which is key for maintaining Quality of Life (QoL) in the longevity society [4]. Functional foods are being used in society for beneficial reasons, not only to preserve health and wellness but also to prevent diseases [34]. Therefore, Mibyou, typically type 2 diabetes, is the target of the functional foods [6, 35]. It is implicated that the Mibyou, diabetes as a typical example, is caused by the disturbance of homeostatic control of metabolic systems where many factors and metabolic steps are coordinately involved [36, 37]. For example, the blood sugar level that is the target for anti-obesity or anti-diabetic strategy is regulated by several metabolic steps or factors such as intestinal uptake, cellular uptake through the transporter, intracellular metabolisms, and excretion of hormones such as insulin, glucagon, and incretin [38]. Therefore, it is recognized that in the Mibyou-care strategy, foods are superior to Western medicines, which are made of pure single pharmacological molecules [4-6, 39]. Functional foods are foods which are the primary source of nutrients, but also are characterized by the presence of certain bioactive ingredients, namely, food factor, which contributes to the pharmacological functions of foods such as suppression of blood sugar level or lipidosis [40].

For a more beneficial use of functional food in Mibyou-care, the combined use of different functional food resources with different functional properties will be designed [41]. The reliability of herbal combination has been established in oriental medicine, such that at least two herbs are prescribed based on the character of the herbs and the physiological con-

dition of the patients [42]. This multi-targeted approach is currently attracting attention in both food factors and clinical medicines [43-45]. For example, Wei *et al*. evaluated the gut-protecting effect of chlorogenic acid (CA) and epigallocatechin-3-gallate (EGCG), alone or in combination, on D-galactose-induced aging mice and found the combination more effectively improved the cognition deficits and protected the gut barrier function, compared with the agents alone [43]. Moreover, the combination of catechins and chlorogenic acid was studied in relation to postprandial glycemic responses in healthy humans [44]. The advantage of combined treatment of diverse targets was also discussed in cancer chemotherapy in that simultaneous treatment of two distinct suppressers of p53 potentiates cancer cell death through activation of a complementary gene network [45]. The beneficial use of herbal combinations with clinical medicines is also discussed [46].

In the present study, we examined the effect of the combination of *Morus alba L*. (hydroponic cultured Morus) and *Camellia sinensis* (Cha) using a high fat-high sucrose (HFHS) diet-feeding obesity model of rats to examine whether any synergism occurs between them. While the major mechanism of anti-obesity function of *Morus alba L.* is in the inhibitory function of intestinal sugar uptake process, in that 1-DNJ is implicated to play a role through its α-glucosidase enzyme inhibition activity [47-49], Cha catechins such as epigallocatechin (EGC and EGCG) play a pivotal role in the anti-diabetic function through their stimulative function on lipid metabolism [29, 30, 50]. Moreover, it is expected that the differential polyphenols from these resources will synergistically contribute to the anti-oxidant and anti-inflammation functions to improve insulin tolerance [51, 52].

The Results obtained in the present study showed that the preventive functions of both Morus and Cha against metabolic syndromes were synergistically enhanced, as shown in visceral fat deposit, fecal fat excretion, and liver damage protection (Table **1** and Figs. **3**-**5**). It was found that a blend of Morus and Cha reduced body weight gain, insulin tolerance, and blood sugar more extensively than either Morus or Cha alone (Figs. **1**, **6** and **7**).

The mixed ratio of Morus and Cha in the diet chow used in the present study was referred to as that of a commercially available product (So-Kan-Ro) made of both hydroponic Morus leaf and stem (SARAKUWA) and Japanese green tea (Kawane Cha). Therefore, the Morus amount in the diet chow was 2% (W/W) in the present study. This concentration is less than half of the concentration (5% w/w) used in our previous anti-obesity study of hydroponic cultured Morus alone [28]. Therefore, in the present study, Morus alone did not give rise to significant effects on body weight gain, fat accumulation, and liver damage protection, but the combination with Cha apparently enhanced the effects of Morus, and even of Cha alone, indicating synergy effect took place between Morus and Cha.

CONCLUSION

The present study indicated that the combination of two plant resources having different mechanisms of action will be a promising approach for the prevention and remedy of metabolic syndromes leading to type-2 diabetes. Since the foods are a mixed formulation of both bioactive components and nutrients that may play a pivotal role in Mibyou-care, the design and formulation consisting of appropriate herbs are an important strategy for Mibyou-care functional food [53].

AUTHORS' CONTRIBUTIONS

SS designed and set up the experimental systems and gained data. **TK** drew the basic design of research and manuscript preparation. **SS** also contributed to writing the manuscript.

ABBREVIATION

IDF = International Diabetes Federation

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol and animal treatment procedure were approved by the Niigata University of Pharmacy, Japan (Approved No.2026-12).

HUMAN AND ANIMAL RIGHTS

The experimental protocol applied life sciences and complied with the Guideline of Animal Care and Treatment (All methods are reported in accordance with ARRIVE guidelines).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article and in supplementary material.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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