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Anti-obesity effects of two herbal extracts in C57BL/6N mice fed high-fat diet

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Abstract

The objective of this study was to investigate the anti-obesity effects of adding *Momordica charantia* (MC) and *Chrysanthemum zawadskii* var. *latilobum* (CZ) extracts to drinking water on obesity-induced mice. A total of 84 eight-week-old C57BL/6N male mice with an initial body weight (BW) of 28.11 ± 1.39 g were used in this study. All treatments were fed a high-fat diet for d 28. Mice were randomly divided into seven drinking treatments (six replicate each treatment) based on the initial BW. Treatments are as follows: control (CON), normal tap water, MC 1, CON with 1% MC aqueous extract, MC 2, CON with 2% MC aqueous extract, CZ 1, CON with 1% CZ aqueous extract, CZ 2, CON with CZ aqueous extract (2%), MCZ 1, CON with 1% MC aqueous extract and 1% CZ aqueous extract, MCZ 2, CON with 2% MC aqueous extract and 2% CZ aqueous extract. During the entire period, the CZ 1, MCZ 1, and MCZ 2 significantly decreased ($p < 0.05$) gain to feed than CON. The CON significantly higher ($p < 0.05$) water intake than other treatments on d 0 to 14. The MCZ 1 significantly decreased ($p < 0.05$) relative (ratio of absolute organ weight to BW) organ weights, including retroperitoneal white adipose tissue (RWAT) weight and inguinal white adipose tissue (IWAT) weight, compared to CON. In conclusion, our study suggests that there was no significant difference in the anti-obesity effects between MC and CZ, and MCZ 1 has synergistic effects by regulating adipose tissue.

Keywords: Anti-obesity, C57BL/6N mice, *Chrysanthemum zawadskii* var. *latilobum*, *Momordica charantia*, Water intake

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Introduction

Momordica charantia (MC), commonly known as bitter melon, is a member of the Cucurbitaceae family. MC, which contains polysaccharides, saponins, and polyphenols, has been used as a traditional anti-diabetic remedy for many years in countries such as China (Shih et al., 2008; Esther et al., 2019; Zhu et al., 2022). Several studies have indicated that the compounds in MC alleviated

oxidative stress, inflammation, and diabetes caused by obesity in rats (Rodrigues et al., 2005; Xu et al., 2015; Raish et al., 2018; Žiberna et al., 2021). *Chrysanthemi zawadskii* var. *latilobum* (CZ) is a perennial herb that contains terpenoids, essential oils, flavonoids, and polysaccharides in the Asteraceae family (Chang and Kim, 2012; Li et al., 2014). The CZ is widely distributed in Asia and northeastern Europe. It has been reported that CZ has anti-inflammatory, antioxidant, anti-diabetic, and anti-obesity effects (Wu et al., 2011; Kim et al., 2018).

Obesity, defined as a disease, is a serious public health issue and plays a critical role in the pathogenesis of type 2 diabetes, coronary heart disease, cancer, and respiratory complications (WHO, 2018; Kopelman, 2020; Le et al., 2023). Various treatments, including synthetic drugs, have been developed for anti-obesity. However, continuous reports of side effects of these treatments, such as heart disease, stroke, and psychological symptoms, have been reported (Ioannides-Demos et al., 2011; Szymanski et al., 2014; Sun et al., 2016). Thus, herbs, which are known to have lower toxicity and fewer complications, are receiving more attention as therapeutic agents compared to synthetic drugs (Khusro et al., 2013). Previous studies reported that MC and CZ extract decreased body weight gain (BWG) and obesity-induced skeletal muscle atrophy in mice fed a high-fat diet (HFD), respectively (Wang and Ryu, 2015; Yoon et al., 2019; Yoo et al., 2020). These observations indicate that MC and CZ extracts may be good candidates for the control of obesity.

Numerous studies have been conducted to investigate the anti-obesity effects of diets containing MC or CZ extracts (Senanayake et al., 2004; Lee and Kim, 2020). However, there is a lack of comparative and synergistic research on the anti-obesity effects of these two herbal extracts. Therefore, in this study, we compared the anti-obesity effects of MC and CZ and investigated whether a solution containing both MC and CZ extracts has synergistic effects on the treatment of obesity in HFD-induced obese mice.

Materials and Methods

Ethics approval and consent to participate

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-2204-23-02).

Preparation of *Momordica charantia*, *Chrysanthemi zawadskii* var. *latilobum* extract

The MC and CZ underwent hot water extraction at 70℃ and 95℃, respectively, for 4 hours. Following this, the MC and CZ extracts were stored at room temperature before being centrifuged at 3,000 rpm for 10 minutes. Subsequently, separate portions of the liquid components were filtered through Whatman No. 4 filter paper (Cytiva, USA). The extraction yield of MC was approximately 10% (weight/weight), and the extraction yield of CZ was approximately 50% (weight/weight).

Experimental design and diets

A total of 84 eight-week-old C57BL/6N male mice (DBL Co., Ltd., Korea) with an initial body weight (BW) of 28.11 ± 1.39 g was used in this study. All mice were fed the HFD with normal tap water *ad libitum* for two weeks to induce obesity (diet-induced obesity, DIO) before the start of the experiments. Afterward, they were randomly divided into seven drinking

treatments based on the initial BW. All treatments were fed the HFD for d 28. Treatments are as follow: control (CON), normal tap water, MC 1, CON with 1% MC aqueous extract, MC 2, CON with 2% MC aqueous extract, CZ 1, CON with 1% CZ aqueous extract, CZ 2, CON with CZ aqueous extract (2%), MCZ 1, CON with 1% MC aqueous extract and 1% CZ aqueous extract, MCZ 2, CON with 2% MC aqueous extract and 2% CZ aqueous extract. Each treatment had 6 replicates with two mice per cage. The HFD contains 60% kcal from fat content and was purchased from commercial company (DooYeol Biotech, Korea) (Table 1). All mice were housed in a room maintained at 25 ± 2℃ and a relative air humidity of 55 ± 5% and controlled 12-hour light/dark cycle, with free access HFD and water.

Table 1. Compositions of high-fat diets.

Item	Content
Ingredients (%)	
Casein	25.84
L-cystine	0.39
Maltodextrin	16.15
Sucrose	8.89
Cellulose, BW 200	6.46
Soybean oil	3.23
Lard	31.66
Mineral mix[y], Pr-Cel	1.29
DiCalcium phosphate	1.68
Calcium carbonate	0.71
Potassium citrate	2.13
Vitamin mix[z]	1.29
Choline bitartrate	0.26
Calculated value (kcal %)	
Protein	26.2
Carbohydrate	26.3
Fat	34.9

[y] Provided per kg of complete diet: Na, 1.0 g; Cl, 1.6 g; Mg, 0.5 g; S, 0.33 g; Mo, 1.6 mg; Cr, 2.0 mg; Fe, 37 mg; Mn, 59 mg; I, 0.2 mg; Fl, 0.9 mg; Se, 0.16 mg; Zn, 29 mg.
[z] Provided per kg of complete diet: vitamin A, 4,000 IU; vitamin D_3, 1,000 IU; vitamin E, 50 IU; menadione, 0.5 mg; vitamin B_{12}, 10 μg; folic acid, 2 mg; niacin, 30 mg; pantothenic acid, 16 mg; vitamin B_6, 7 mg; vitamin B_2, 6 mg; vitamin B_1, 6 mg.

Growth performance

Growth performance was measured by BW, BWG, feed intake (FI), and gain to feed (G : F) ratio. The BW, BWG, FI, and G : F ratio were recorded at the start of the experiment at day 0, 14, and 28. The BWG was calculated as the BW of the previous time point was subtracted from the BW of the current time point. The residual amount was subtracted from the diet amount to calculate FI. The G : F ratio was calculated by dividing BWG by FI. After the adaption period, water intake was recorded for each interval from day 0 to 14, day 14 to 28, and day 0 to 28.

Relative organ weight

At the end of experiment (day 28), all mice were sacrificed by cervical dislocation. Liver, epididymal white adipose tissue (EWAT), retroperitoneal white adipose tissue (RWAT), and inguinal white adipose tissue (IWAT) were dissected according to the defined anatomical landmarks. The relative (ratio of absolute organ weight-to-BW) organ weights were recorded, and the relative organ weights were calculated using the following formula (1):

Relative organ weight (%) = organ weight (g)/live BW (g) × 100 (1)

Ileal bacteria count

Ileal digesta was collected from section of ileum in conical tubes. From the ileal digesta, 0.1 g was suspended in distilled water, homogenized, and diluted from 10^{-2} to 10^{-5} to count the number of bacteria. Evenly spread 100 μL of the diluted solution on the agar. *Escherichia coli* (*E. coli*) and *Lactobacillus* were analyzed for bacteria. Sorbitol MacConkey agar (KisanBio, Korea) was used for *E. coli* and De Man-Rogosa-Sharpe (MRS) agar (KisanBio, Korea) was used for *Lactobacillus*. *E. coli* was cultured for 24 hours at 37℃, and *Lactobacillus* was cultured for 48 hours at 37℃. Immediately after removal from the incubator, *E. coli* and *Lactobacillus* were counted, and statistical analysis was performed by converting them to log.

Statistical analysis

All data were analyzed to one-way ANOVA using JMP® Pro (version 16.0.0, SAS Institute Inc., USA), using each pen as the experimental unit. Differences between treatment means were determined using Tukey's multiple range test. A probability level of $p < 0.05$ was indicated to be statistically significant.

Results

Growth performance

The effect of herbal extracts in supplementing drinking water on growth performance in C57BL/6N fed HFD are shown in Table 2. There was no significant difference ($p > 0.05$) on BW and FI among treatments. The MCZ 1 had significantly lower ($p < 0.05$) BWG than CON on day 0 to 14. The CON had significantly higher ($p < 0.05$) G : F ratio than the other treatments on day 0 to 14. The CZ 1, MCZ 1 and MCZ 2 had significantly lower ($p < 0.05$) G : F ratio than the CON on day 0 to 28. The CON had significantly higher ($p < 0.05$) water intake than other treatments on d 0 to 14.

Table 2. Effect of herbals in supplementing drinking water on growth performance in C57BL/6N mice fed HFD.

Item	CON	MC 1	MC 2	CZ 1	CZ 2	MCZ 1	MCZ 2	SE	p-value
MC aqueous extract	-	1%	2%	-	-	1%	2%		
CZ aqueous extract	-	-	-	1%	2%	1%	2%		
BW (g)									
Day 0	28.61	28.07	28.01	27.99	28.04	27.95	28.11	0.615	0.991
Day 14	35.99	33.36	33.16	33.08	33.65	32.75	33.13	0.964	0.289
Day 28	40.61	38.22	38.05	37.63	38.53	36.62	37.78	1.156	0.374
Day 0 to 14									
BWG (g)	7.37a	5.29ab	5.14ab	5.09ab	5.61ab	4.80b	5.03ab	0.545	0.036
FI (g)	32.62	34.22	33.34	34.33	34.94	33.60	34.88	1.064	0.700
G : F (g/g)	0.226a	0.150b	0.155b	0.150b	0.160b	0.142b	0.143b	0.014	0.002
Water intake (mL)	45.00a	35.00b	32.50b	37.50b	35.83b	36.67b	32.50b	1.643	< 0.001
Day 14 to 28									
BWG (g)	4.62	4.87	4.90	4.55	4.87	3.87	4.65	0.366	0.463
FI (g)	29.03	30.60	30.18	30.53	31.90	29.43	31.75	0.834	0.159
G : F (g/g)	0.159	0.159	0.163	0.150	0.153	0.132	0.145	0.011	0.517
Water intake (mL)	26.67	29.17	25.83	35.00	30.00	30.33	30.83	2.979	0.127
Day 0 to 28									
BWG (g)	12.00	10.15	10.04	9.64	10.48	8.67	9.68	0.723	0.096
FI (g)	61.65	64.81	63.52	64.86	66.84	63.02	66.63	1.677	0.294
G : F (g/g)	0.195a	0.154ab	0.158ab	0.150b	0.157ab	0.137b	0.144b	0.009	0.005
Water intake (mL)	71.67	64.17	58.33	62.50	65.83	67.00	63.33	3.251	0.058

HFD, high-fat diet; MC, *Momordica charantia*; CZ, *Chrysanthemum zawadskii* var.*latilobum*; CON, normal tap water; MC 1, CON with 1% MC aqueous extract; MC 2, CON with 2% MC aqueous extract; CZ 1, CON with 1% CZ aqueous extract; CZ 2, CON with 2% CZ aqueous extract; MCZ 1, CON with 1% MC aqueous extract and 1% CZ aqueous extract; MCZ 2, CON with 2% MC aqueous extract and CZ aqueous extract; BW, body weight; BWG, body weight gain; FI, feed intake; G : F, feed efficiency; SE, standard error.
a, b: Means within column with different letters differ significantly ($p < 0.05$).

Relative organ weight

The effect of herbal extracts in supplementing drinking water on relative organ weight in C57BL/6N fed HFD is shown in Table 3. There was no significant difference ($p > 0.05$) in relative liver weight and relative EWAT weight. The MCZ 1 had significantly lower ($p < 0.05$) relative RWAT weight and relative IWAT weight than the CON.

Table 3. Effect of herbals in supplementing drinking water on relative organ weight in C57BL/6N mice fed HFD.

Item (%)	CON	MC 1	MC 2	CZ 1	CZ 2	MCZ 1	MCZ 2	SE	p-value
MC aqueous extract	-	1%	2%	-	-	1%	2%		
CZ aqueous extract	-	-	-	1%	2%	1%	2%		
Liver	4.12	4.26	4.15	4.21	4.02	4.13	4.15	0.150	0.960
EWAT	7.01	6.85	6.98	6.32	7.19	6.41	7.09	0.306	0.320
RWAT	3.50a	2.74ab	2.70ab	3.08ab	2.96ab	2.47b	2.95ab	0.190	0.018
IWAT	7.02a	6.56ab	6.54ab	6.63ab	6.62ab	5.70b	6.06ab	0.267	0.033

HFD, high-fat diet; MC, *Momordica charantia*; CZ, *Chrysanthemum zawadskii* var.*latilobum*; EWAT, epididymal white adipose tissue; RWAT, retroperitoneal white adipose tissue; IWAT, inguinal white adipose tissue; CON, normal tap water; MC 1, CON with 1% MC extract; MC 2, CON with 2% MC extract; CZ 1, CON with 1% CZ extract; CZ 2, CON with 2% CZ extract; MCZ 1, CON with each 1% MC and CZ extracts; MCZ 2, CON with each 2% MC and CZ extracts; SE, standard error.
a, b: Means within column with different letters differ significantly ($p < 0.05$).

Ileal bacteria count

The effect of herbal extracts in supplementing drinking water on ileal bacteria counts in C57BL/6N fed HFD is shown in Table 4. There was no significant difference ($p > 0.05$) in *E. coli* and *Lactobacillus* counts among treatments.

Table 4. Effect of herbals in supplementing drinking water on ileal bacteria counts in C57BL/6N mice fed HFD.

Item (%)	CON	MC 1	MC 2	CZ 1	CZ 2	MCZ 1	MCZ 2	SE	p-value
MC aqueous extract	-	1%	2%	-	-	1%	2%		
CZ aqueous extract	-	-	-	1%	2%	1%	2%		
E. coli	7.18	7.16	7.14	7.12	7.15	7.12	7.15	0.034	0.805
Lactobacillus	6.62	6.66	6.6	6.63	6.78	6.75	6.59	0.122	0.891

HFD, high-fat diet; MC, *Momordica charantia*; CZ, *Chrysanthemum zawadskii* var.*latilobum*; CON, normal tap water; MC 1, CON with 1% MC extract; MC 2, CON with 2% MC extract; CZ 1, CON with 1% CZ extract; CZ 2, CON with 2% CZ extract; MCZ 1, CON with each 1% MC and CZ extracts; MCZ 2, CON with each 2% MC and CZ extracts; *E. coli*, *Escherichia coli*; SE, standard error.

Discussion

Obesity is defined as an excessive accumulation of adipocytes in adipose tissue, which can lead to various diseases, including cardiovascular diseases, diabetes, fatty liver disease, and systemic oxidative stress through a variety of biochemical pathways (Saltiel and Olefsky, 2017; Ananthakumar et al., 2020).

The results of our study showed that the addition of MC or CZ to drinking water decreased the G : F ratio of mice fed a HFD from 0 to 14 days. Consistent with this study, Senanayake et al. (2004) reported that the addition of the methanol fraction of MC (1% level in diet) to the diet of hamsters resulted in decreased triglyceride absorption and a lower G : F ratio. Also, Kim et al. (2018) reported that the inclusion of CZ ethanol extract (500 mg/kg BW) in the diet of mice fed a HFD also resulted in a decrease in BW. In this study, over the entire period, the addition of MC or CZ numerically decreased RWAT in mice fed HFD by at least 12% and decreased IWAT by at least 6%. The MC and CZ contain functional substances, such as catechin and quercetin, respectively, which are substances that have anti-obesity effects (Fan et al., 2021; Sharma et al., 2023). Catechin in MC can inhibit the accumulation of epididymal and retroperitoneal fat in mice by non-tissue-specific thermogenesis (Murase et al., 2002; Akhlaghi and Kohanmoo, 2018). Isorhamnetin in CZ can inhibit lipid accumulation by suppressing peroxisome proliferator-activated receptor (PPAR) γ transcriptional activity (Yamauchi et al., 2001; Zhang et al., 2016). Also, the compounds contained in MC and CZ could increase fatty acid oxidation, converting fat into energy by up-regulating adenosine monophosphate-activated protein kinase (Ahn et al., 2008; Hardie et al., 2012; Yu et al., 2013; Seo et al., 2015). However, Bao et al. (2013) reported that supplementation with MC resulted in a significant decrease in adipose tissue compared to mice fed HFD. Previous studies conducted anti-obesity trials for at least 9 to 12 weeks (Huang et al., 2008; Shih et al., 2008; Bao et al., 2013). This measure that our study attributed to having a research period of only 4 weeks which could have potentially led to the insufficient activation of compounds within such a short timeframe. Therefore, this study showed that adding MC and CZ numerically reduced the accumulation of visceral fat, which lowered the G : F ratio, and two herbals had a similar level of anti-obesity effect.

In our study, the MCZ 1 (treatment supplementing 1% of MC aqueous extract and 1% of CZ aqueous extract to drinking water) was lower than treatment supplementing a single herbal extract in BWG, RWAT weight, and IWAT weight in mice fed HFD. In other words, it reports that MC and CZ have a synergistic anti-obesity effect. Through the above-mentioned different mechanisms of catechin (thermogenesis) and quercetin (suppressing PPARγ), MCZ could effectively inhibit the

accumulation of fatty acids within a short period of time. According to Sharma et al. (2015), complex addition showed a greater reduction in body weight and fat weight compared to single addition. This effect is also attributed to the different compounds present in the complex addition. Therefore, the results of this study demonstrated that the herbal extract combination was more effective in combating obesity than single herbal extracts due to synergy. Among them, a mixture of 1% MC and 1% CZ is considered the optimal additive amount for suppressing fat accumulation in a short period of time. However, additional research is required to determine the exact mechanism of these effects.

Conclusion

In conclusion, this study indicated that herbal extracts have anti-obesity effects in mice. According to the results of this study, the combination of 1% MC aqueous extract and 1% CZ aqueous extract added to drinking water showed synergistic effects on decreasing BWG, G : F ratio, relative IWAT, and relative RWAT. Therefore, supplementing drinking water with 1% of MC aqueous extract and 1% of CZ aqueous extract was considered the most effective supplementation amount. However, it seems that additional studies are needed to investigate the functional substance of each additive and the basic mechanism of herbals against obesity.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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