

## COMBINATION EFFECTS OF B-GLUCAN ORIGINATED FROM *AUREOBASIDIUM* AND PLATYCODIN ON THE OBESE *DB/DB* MOUSE

Jae-Suk Choi<sup>1#</sup>, Joo Wan Kim<sup>2#</sup>, Go-Woon Jung<sup>2</sup>, Dong-Chan Park<sup>2</sup>, Hyung-Rae Cho<sup>2</sup>, Sae Kwang Ku<sup>3\*</sup> and Jae-Hak Sohn<sup>1\*\*</sup>

<sup>1</sup>Division of Bioindustry, College of Medical and Life Sciences, Silla University, 140 Baegyang-daero, 700 beon-gil, Sasang-gu, Busan 617-736, Republic of Korea; <sup>2</sup>Glucan corp. #305 Marine Bio-Industry Development Center, Hoenggye-ri 27, Ilgwang-myeon, Gijan-gun, Busan 619-912, Republic of Korea; <sup>3</sup>Aribio Inc. Byeoksan Digital Valley, Suite 1004, Mullae-dong 5ga 9, Yeongdeungpo-gu, Seoul, 150-095, Korea; <sup>4</sup>Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, 290 Yugok-dong, Gyeongsan-si, Gyeongsanbuk-do 712-715, Republic of Korea

\*\*Corresponding author E-mail: jhsohn@silla.ac.kr, \*Co-corresponding author E-mail: gucci200@hanmail.net

<sup>#</sup>JSC and JWK contributed equally.

### ABSTRACT

To find out the fittest composition, the anti-obesity effects of 11 types of the  $\beta$ -glucan compositions and platycodin (PLAT) (MPP) were observed in the obese *db/db* mouse at 200mg/kg-dosing levels with  $\beta$ -glucan or PLAT single formula, respectively. Test materials were administered orally once a day for 28 days. Each of seven mice per group was selected using blood glucose levels and body weight, and the changes in the body weight, epididymal fat weights, serum adiponectin and leptin levels, adipose adiponectin contents and liver triglyceride contents were monitored. The weights of epididymal fats, serum leptin levels and liver triglyceride contents in *db/db* control were significantly ( $p < 0.01$ ) increased compared to that of *db/m* with significant ( $p < 0.01$ ) decrease in serum adiponectin levels and adipose adiponectin contents. However, these obese changes were dramatically decreased after  $\beta$ -glucan single, PLAT single and some MPP-dosing and the changes related to the obese showed decreased trends along increasing the  $\beta$ -glucan composition. Synergic anti-obesity effects were restricted to the MPP 1:1 formulation compared to those of single formulas, respectively. The synergic anti-obesity effects detected in MPP 1:1 formulation were considered as results of the similar anti-obesity mechanisms of  $\beta$ -glucan and PLAT that inhibit the intestinal absorption of fats.

**Keywords:**  $\beta$ -Glucan, Platycodin, *db/db* mouse, obesity, adipokine, composition.

### INTRODUCTION

All around the world, the incidence of obesity continues to increase, despite increased awareness and global exertions to understand and confront its origins. In essence, dysregulated energy homeostasis stems from a societal reduction in physical activity, an increase in the accessibility of, and overindulgence in, energy-dense foods, combined with a myriad of genetic, social and economic complicating factors (Mitchell *et al.*, 2005). Recently, there has been a worldwide increase in the incidence of obesity associated with a metabolic syndrome known as type-2 diabetes, the development of which seems to be as a result of high-caloric diet intake and physical inactivity (James *et al.*, 2001) and predicted estimates suggest that the population with this syndrome may double to over 300 million by the year 2025 (Zimmet, 2003). One of the important determinants for the development of this obesity might be an increase in the regional distribution of body fat, i.e., abdominal obesity. The latter often shows clustering of atherogenic risk factors (Kunitomi *et al.*, 2002), i.e., hypertension, dyslipidemia, alterations in coagulation and inflammatory cytokine profiles, and hyperinsulinemic insulin resistance.

As a consequence, there is an expected increase in morbidity and mortality of cardiovascular disease (Hida *et al.*, 2005). Vigorous efforts have been made to delineate the relationship between increased adiposity and insulin resistance. In this regard, some molecules so-called adipokine (Mitchell *et al.*, 2005), including leptin (Ebihara *et al.*, 2001; Wolf *et al.*, 2002) and adiponectin (Maeda *et al.*, 2002; Yamauchi *et al.*, 2003), which are secreted by the adipocytes, may modulate the sensitivity of insulin, the action of which activates multiple signaling events after phosphorylation of insulin receptor and several other molecules in type-2 diabetes (Senthil *et al.*, 2001; Sakaue *et al.*, 2003).

The *db/db* mouse is hyperleptinemic and develops obesity and severe type-2 diabetes partly due to a functional defect in the long-form leptin receptor (*Ob-Rb*), which plays a significant role in the regulation of food intake and the control of body weight (Chen *et al.*, 1996; Lee *et al.*, 1996; Madiehe *et al.*, 2002; Sharma *et al.*, 2003). Therefore, this genetically obese mouse has been used in the efficacy test field of various medicines including obesity (Nakagawa *et al.*, 2003; Pittner *et al.*, 2004; Neary *et al.*, 2005). Generally, the anti-obesity effects of test materials have been evaluated based on the body and fat weights, and serum or organ lipid profiles

with various adipokines like leptin and adiponectin (Sahai *et al.*, 2004; Fujita *et al.*, 2005; Mitchell *et al.*, 2005).

$\beta$ -glucan is a fiber-type complex sugar (polysaccharide) derived from the cell wall of baker's yeast, many medicinal mushrooms and barley and oat fiber. The two primary uses of  $\beta$ -glucan are to enhance the immune system (Czop, 1986; Estrada *et al.*, 1997) and to lower blood cholesterol levels (Lia *et al.*, 1995; Bell *et al.*, 1999). Although, some reports showing the evidence of anti-obesity and hypolipemic effects of  $\beta$ -glucan extracts from some natural plants or a mushroom in animal experiments (Delaney *et al.*, 2003; Wilson *et al.*, 2004) and human clinical trials (Nicolosi *et al.*, 1999), the dosage showing anti-obesity or hypolipemic effects were much higher than the ideal dosage to develop as anti-obesity agent. The effective dosage of  $\beta$ -glucan is over 15g/day in human subjects (Nicolosi *et al.*, 1999) and 1.2g/day in animals (Delaney *et al.*, 2003; Wilson *et al.*, 2004). In this study,  $\beta$ -glucan purified from a UV-induced mutant of *Aureobasidium pullulans* (half of the dry material is -1,3/1,6-glucans; Seo *et al.*, 2002) was used.

Platycodi radix, the roots of *Platycodon grandiflorum* (Jacq.), has been used traditionally as an expectorant and a remedy for bronchitis, tonsillitis, laryngitis and suppurative dermatitis in China, Korea and Japan. In China and Korea, the fresh roots of *P. grandiflorum* have been eaten as pickles for preventing obesity (Han *et al.*, 2000). It has been reported that platycodin (PLAT), the main component of Platycodi radix (Han *et al.*, 2002), shows the anti-obesity and hypolipemic effects (Han *et al.*, 2000, 2002; Zhao *et al.*, 2005). However, the effective dose induced anti-obesity was much higher than the ideal dosage to develop as anti-obesity agent; 0.5g/L in vitro and at least 244mg/kg in animals (Han *et al.*, 2000).

Until now, numerous natural or biosynthesized materials have shown anti-obesity effect. However, it is very difficult to develop an anti-obesity agent because they have relatively high efficacy dosage on the animal experiments or human clinical trials. In this study, to reduce the efficacy dosage on the anti-obesity, we intended to the mixed  $\beta$ -glucan with PLAT. To find out the fittest composition, the anti-obesity effects of 11 types of the  $\beta$ -glucan compositions and PLAT (MGP) were observed in the obese *db/db* mouse at 200mg/kg-dosing levels with  $\beta$ -glucan or PLAT single formula, respectively.

## MATERIALS AND METHODS

**Animals and husbandry:** One hundred ninety-six male genetically obese *db/db* mice, with a C57BL/KsJ genetic background, and fourteen their lean non-diabetic heterozygous littermates, *db/m* mice (7-wk old upon receipt, Clear, Japan) were used after acclimatization for 7 days. Animals were allocated seven per polycarbonate

cage in the humidity (40-45%) and temperature (20-25°C) controlled room. Light: dark cycle was 12hr: 12hr, and water and standard rodent chow (Samyang, Korea) were supplied free to access. About half of animals were selected (7 per group) with fasting blood glucose levels at 2 days before initial dosing with body weight at initial dosing. Mean fasting blood glucose levels in *db/m* mice at 2 days before initial dosing was  $99.43 \pm 10.86$ mg/dl, and  $269.88 \pm 12.42$ mg/dl in *db/db* mice (Table 1). All animal care and use were in accordance with the institutional guidelines and approved by the Institutional Animal Care and Use Committee of Silla University (Busan, Korea; approval no. SUACUC-2016-018).

**Preparations and administration of drugs:**  $\beta$ -glucan (Glucan Corp., Korea),  $\beta$ -glucan of the UV-induced mutant of *A. pullulans* (Seo *et al.*, 2002), and PLAT were used in this study.  $\beta$ -glucan and PLAT were stored in a refrigerator at 4°C to protect from light and degeneration.  $\beta$ -glucan (2.5% solution) was diluted in distilled water and PLAT was dissolved, and dosed by oral gavage using a zonde attached to 3 ml syringes containing test material at dosage 200mg/kg levels once a day for 28 days. The administered dose, formulation of MGP, and the schedules are shown in Table 2.

**Measurement of blood glucose level:** For detecting the blood glucose levels, blood was collected at two days before initial dose from orbital plexus after overnight fasting, and collected blood was deposited into NaF glucose vacuum tube (Becton Dickinson, USA) and plasma was separated. Blood glucose levels were detected using automated blood analyzer (Toshiba 200 FR, Japan).

**Body weight changes:** Changes in body weight were calculated at one day before dosing, and at initial dosing of test articles, 1, 7, 14, 21, 27 and 28 days after dosing (at sacrifice) using an automatic electronic balance (Sartorius Co., Ltd., USA). At dosing and at a termination, experimental animals were fasted overnight (even no water for about 12hr) to reduce the erratum arouse from feeding. In addition, body weight gains were calculated for the following periods.

Weight gains (g) = During observation periods (From Day 0 to Day 28 of test article-dosing) (Equation-1)

**Measurement of epididymal fat weights:** At sacrifice, the epididymal adipose tissues were removed and, the weight was calculated at g levels, and to reduce the erratum rose from individual body weight differences, the relative weight (%) was calculated using body weight at sacrifice and absolute weight as the following equation

Relative fat weight (%) = [(Absolute epididymal fat weight / Body weight at sacrifice)  $\times$  100] (Equation-2)

**Measurement of serum adiponectin levels:** For detecting the serum adiponectin levels, blood was

collected at sacrifice from vena cava after overnight fasting and serum was separated with general methods from collected blood. Serum adiponectin levels were detected using a commercially available ELISA kit (Otsuka Pharm., Japan)

**Measurement of adiponectin contents in epididymal adipose tissues:** Adipose tissue adiponectin levels were determined by Western blot analysis as described previously (Fujita *et al.*, 2005). The removed epididymal adipose tissues were homogenized in PBS containing 0.5% sodium deoxycholate. Homogenates were incubated for 24h at 37°C. After incubation, the homogenates were centrifuged at 15,000g for 10 min. The fat cake was removed by suction, and adipose tissue extracts (supernatants) were used for Western blot analysis. Aliquots of the tissue extracts (10µg of protein) prepared in SDS sample buffer were incubated for 5 min at 100°C. Denatured proteins were separated by SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Lab., USA). The membranes were incubated with 1:10,000 dilution of mouse anti-mouse adiponectin monoclonal antibody (Chemicon International, USA) for 12h at room temperature and then incubated with 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG antibody (DAKO, USA) for 1h at room temperature. After incubation, the membranes were soaked in chemiluminescence solution using ECL Western blotting detection reagents (Amersham, UK). The membranes were exposed to X-ray film, and the adiponectin protein was thus visualized. The signals from X-ray film were quantified using DMI CCD image analyzer system (DMI, Korea). Adiponectin protein contents per 10 µg of adipose tissue protein in *db/m* and all dosing groups were normalized to those of *db/db* control group in the same assay and expressed as the percentage of the value of control group.

**Measurement of serum leptin levels:** For detecting the serum leptin levels, blood was collected at sacrifice from vena cava after overnight fasting and serum was separated with general methods from collected blood. Serum leptin levels were detected using a commercially available radioimmunoassay kit procedure (Linco Research, USA) as previously (Sahai *et al.*, 2004).

**Measurement of liver triglyceride contents:** Liver samples (50-70mg) were homogenized in 50 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl, 1 mM EDTA, and 1 µM PMSF. Triglyceride content in the liver homogenates was measured using a spectrophotometric kit procedure (Thermo DMA, USA) as described previously (Sahai *et al.*, 2004).

**Statistical analyses:** All data was calculated as Mean ± S.D. (n=7). Statistical analyses were conducted using

Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) with SPSS for Windows (Release 6.1.3., SPSS Inc., USA). The inhibition rate compared to that of control was calculated to help the understanding of the efficacy of test materials on differences between *db/m* and control (Equation-3) and between control and test groups (Equation-4).

Percentage Changes vs Sham (%) =  $\frac{[(\text{Data of vehicle control} - \text{Data of Sham}) / \text{Data of Sham}] \times 100}{\text{Equation-3}}$

Percentage Changes vs vehicle control (%) =  $\frac{[(\text{Data of test groups} - \text{Data of vehicle control}) / \text{Data of vehicle control}] \times 100}{\text{Equation-4}}$

## RESULTS

**Changes and gains in body weight:** Before initial dosing, *db/db* mice showed marked obesity compared to that of *db/m*. Significant ( $p < 0.01$ ) increase in body weight throughout the experimental periods in control compared to that of *db/m* was recorded and the body weight gains during dosing periods non-significantly increased. However, significant ( $p < 0.01$  or  $p < 0.05$ ) decrease in body weights was observed at 27 days after dosing with β-glucan and PLAT single formula-dosing groups, respectively. Additionally, the body weight gains in the β-glucan single-dosing group were significantly ( $p < 0.01$ ) decreased compared to that of control, and in PLAT single-dosing group, they were non-significantly decreased. The body weight gains were gradually decreased along with the increase of the β-glucan composition in all MPP-dosing groups. However, none of the MPP formulations showed favorable effects on the decrease and gains of body weight compared to the β-glucan single-dosing group except for MPP 1:1, in which the highest inhibition effects on the body weight gains was detected in this study (Table 3 and 4).

In control, the body weight gains during dosing periods showed 39.10% changes vs *db/m*, and they showed % changes vs vehicle control as -62.52, -34.80, -80.31, -62.33, -43.59, -40.15, -39.58, -34.99, -36.14, -39.58, -46.65, -49.90 and -60.61% in β-glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

**Changes in the epididymal fat weights:** A significant ( $p < 0.01$ ) increase in absolute and relative epididymal adipose tissue weights was detected compared to that of *db/m*. However, significant ( $p < 0.01$  or  $p < 0.05$ ) decrease of fat weights was detected in all dosing groups compared to that of control except for PLAT single dosing and MPP 1:8 and 1:10 formulation dosing groups showed non-significant decreases in the fat weights. The fat weights gradually decreased along with the increase of β-glucan composition but they did not show favorable

effects compared to that of  $\beta$ -glucan single dosing group except for MPP 1:1, in which the highest inhibition effects on the increase of fat weights were detected in this study (Table 5).

In control, the absolute epididymal adipose tissue weights showed 582.53% changes *vs db/m*, and they showed % changes *vs* vehicle control as -16.15, -12.40, -25.82, -11.52, -11.56, -10.72, -9.40, -10.15, -11.30, -12.36, -14.83, -15.00 and -15.40% in  $\beta$ -glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

In control, the relative epididymal adipose tissue weights showed 582.06% changes *vs db/m*, and they showed % changes *vs* vehicle control as -17.76, -12.00, -27.00, -11.15, -13.21, -12.36, -9.63, -11.03, -12.37, -13.64, -15.87, -16.69 and -16.83% in  $\beta$ -glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

**Changes in the serum adiponectin levels:** A significant ( $p < 0.01$ ) decrease of serum adiponectin levels was detected in control compared to that of *db/m*. However, significant ( $p < 0.01$  or  $p < 0.05$ ) increases in serum adiponectin levels were detected in all dosing groups including  $\beta$ -glucan and PLAT single dosing groups compared to that of control. The serum adiponectin levels gradually increased along with the increase of  $\beta$ -glucan composition but they did not show favorable effects compared to that of  $\beta$ -glucan single dosing group except for MPP 1:1 and 10:1 formulation groups, in which the favorable effects on the serum adiponectin levels were detected. Among 11 MPP formulation groups, MPP 1:1 formulation showed favorable effects on inhibition of the decrease of serum adiponectin levels (Table 6).

In control, the serum adiponectin levels showed -43.91% changes *vs db/m*, and they showed % changes *vs* vehicle control as 17.74, 15.87, 35.57, 15.41, 14.26, 12.75, 13.94, 13.19, 12.63, 13.50, 14.29, 15.19 and 23.15% in  $\beta$ -glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

**Changes in the adipose tissue adiponectin contents:** A significant ( $p < 0.01$ ) decrease in epididymal adipose tissue adiponectin contents was detected in control compared to that of *db/m*. However, significant ( $p < 0.01$  or  $p < 0.05$ ) increase of adipose tissue adiponectin contents was detected in all dosing groups including the  $\beta$ -glucan and PLAT single dosing groups compared to that of control. The adipose tissue adiponectin contents gradually increased along with the increase of  $\beta$ -glucan composition but they did not show favorable effects

compared to that of  $\beta$ -glucan single dosing group except for MGP 1:1 formulation group, in which favorable effects on the inhibition of the decrease in adipose tissue adiponectin contents were, detected (Table 6).

In control, the epididymal adipose tissue adiponectin contents showed -63.60% changes *vs db/m*, and they showed % changes *vs* vehicle control as 37.57, 24.57, 71.71, 38.57, 28.86, 27.71, 26.71, 26.43, 26.57, 27.71, 29.71, 30.29 and 35.57% in  $\beta$ -glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

**Changes in the serum leptin levels:** A significant ( $p < 0.01$ ) increase in serum leptin levels was detected in control compared to that of *db/m*. However, significant ( $p < 0.01$  or  $p < 0.05$ ) decreases in serum leptin levels were detected in all dosing groups including the  $\beta$ -glucan and PLAT single dosing groups compared to that of control. The serum leptin levels were gradually decreased along with the increase of  $\beta$ -glucan composition but they did not show favorable effects compared to that of  $\beta$ -glucan single dosing group except for MPP 1:1 formulation group, in which favorable effects on the inhibition of the increase in serum leptin levels was detected (Table 7).

In control, the serum leptin levels showed 523.15% changes *vs db/m*, and they showed % changes *vs* vehicle control as -17.31, -9.64, -26.25, -14.15, -13.44, -13.28, -12.81, -9.64, -11.94, -12.73, -13.60, -15.42 and -16.44% in  $\beta$ -glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

**Changes in the hepatic triglyceride contents:** A significant ( $p < 0.01$ ) increase of liver triglyceride contents was detected in control compared to that of *db/m*. However, significant ( $p < 0.01$  or  $p < 0.05$ ) decreases of liver triglyceride contents were detected in all dosing groups including the  $\beta$ -glucan and PLAT single dosing groups compared to that of control. The liver triglyceride contents gradually decreased along with the increase of  $\beta$ -glucan composition but they did not show favorable effects compared to that of  $\beta$ -glucan single dosing group except for MPP 1:1 formulation group, in which favorable effects on the inhibition of the increase of liver triglyceride contents were, detected (Table 8).

In control, the liver triglyceride contents showed 61.07% changes *vs db/m*, and they showed % changes *vs* vehicle control as -16.92, -15.52, -28.19, -16.69, -14.76, -13.95, -13.98, -15.70, -14.88, -16.10, -15.93, -15.11 and -16.07% in  $\beta$ -glucan single formulation, PLAT single formulation, MPP 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 2:1, 4:1, 6:1, 8:1 and 10:1 formulated groups, respectively.

**Table 1. Fasting blood glucose levels of animals used in this study at 2 days before initial dosing.**

Group	Blood glucose levels (mg/dl)
<i>db/m</i> mice	99.43±10.86
<i>db/db</i> control	267.43±18.04*
β-glucan: Single formulation	272.86±20.14*
PLAT: Single formulation	266.29±17.12*
MPP (β-glucan : PLAT)	
= 1:1	271.57±16.25*
= 1:2	266.57±12.11*
= 1:4	271.29±7.91*
= 1:6	271.71±8.98*
= 1:8	267.71±11.53*
= 1:10	269.71±16.07*
= 2:1	270.14±11.26*
= 4:1	270.57±8.72*
= 6:1	269.71±9.76*
= 8:1	271.86±9.58*
= 10:1	270.86±8.17*

n=7; (Mean ± S.D.); \* p<0.01 compared to that of *db/m* mice by MW test

**Table 2. Group ID and Composition of test articles used in this study.**

Group	Actual dosage (mg/kg)	
	β-glucan	PLAT
<i>db/m</i> Normal littermates	0	0
Control	0	0
β-glucan: Single formulation	200	0
PLAT: Single formulation	0	200
MPP (β-glucan : PLAT)		
= 1:1	100	100
= 1:2	66.66	133.34
= 1:4	40	160
<i>db/db</i> = 1:6	28.57	171.43
= 1:8	22.22	177.78
= 1:10	18.18	181.82
= 2:1	133.34	66.66
= 4:1	160	40
= 6:1	171.43	28.57
= 8:1	177.78	22.22
= 10:1	181.82	18.18

All test articles and vehicle were dosed by gastric gavage for 4 weeks at 200mg/kg/10ml in distilled water.

**Table 3. Changes of body weight after 28 days of test article administration**

Body weight	At Dosing <sup>1)</sup>	Day 1	Weeks after dosing				At Sacrifice <sup>1)</sup>
			1 week	2 weeks	3 weeks	4 weeks	
Sham	22.21±1.66	24.66±1.56	25.54±1.76	27.34±1.92	28.27±1.64	29.63±1.61	27.59±1.60
Control	39.74±0.74*	42.49±1.18*	43.97±1.67*	44.99±1.78*	46.56±1.57*	49.37±2.12*	47.21±1.48*
β-glucan <sup>1)</sup>	40.00±1.00*	42.63±1.25*	43.47±1.15*	44.33±1.21*	44.97±2.11*	44.91±1.95* <sup>#</sup>	42.80±1.92* <sup>#</sup>
PLAT <sup>1)</sup>	40.04±1.59*	42.43±1.39*	44.03±1.74*	45.66±1.83*	46.96±1.91*	46.87±1.88* <sup>##</sup>	44.91±2.00* <sup>##</sup>
MPP							
= 1:1	40.01±1.25*	42.50±1.12*	43.53±2.02*	43.41±1.93*	43.74±1.91* <sup>##</sup>	44.10±1.44* <sup>#</sup>	41.49±1.72* <sup>#</sup>
= 1:2	39.80±1.06*	42.73±1.18*	44.16±0.86*	45.71±1.00*	45.71±1.11*	45.37±1.16* <sup>#</sup>	42.61±1.33* <sup>#</sup>
= 1:4	39.49±1.75*	42.17±1.63*	44.07±1.31*	45.41±1.18*	46.06±0.86*	46.30±1.00* <sup>#</sup>	43.70±1.54* <sup>#</sup>
= 1:6	39.73±1.04*	42.31±1.61*	43.51±1.25*	45.26±1.31*	46.00±1.58*	46.60±1.69* <sup>##</sup>	44.20±2.32* <sup>##</sup>
= 1:8	39.64±1.30*	42.54±1.43*	44.53±1.70*	45.73±1.71*	46.43±1.98*	46.44±1.86* <sup>##</sup>	44.16±1.72* <sup>##</sup>
= 1:10	39.79±1.26*	42.06±1.80*	44.39±1.51*	45.61±1.75*	46.69±1.85*	46.97±1.62* <sup>##</sup>	44.64±1.63* <sup>##</sup>
= 2:1	39.61±1.73*	42.06±2.03*	43.66±1.50*	45.04±1.41*	45.74±1.33*	46.70±2.10* <sup>##</sup>	44.39±2.41* <sup>##</sup>
= 4:1	39.91±1.15*	42.87±1.81*	45.09±2.47*	45.91±2.16*	46.60±1.91*	46.63±1.72* <sup>##</sup>	44.43±2.15* <sup>##</sup>
= 6:1	39.67±2.18*	42.17±1.72*	44.03±1.84*	44.29±2.66*	45.20±3.07*	46.17±2.12* <sup>##</sup>	43.66±1.94* <sup>#</sup>
= 8:1	39.86±1.26*	42.30±0.99*	43.87±1.23*	44.63±1.15*	45.37±1.14*	46.10±1.98* <sup>##</sup>	43.60±2.46* <sup>##</sup>
= 10:1	39.73±1.31*	42.26±1.88*	43.84±1.29*	44.69±1.19*	45.10±1.01*	45.14±1.27* <sup>#</sup>	42.67±1.42* <sup>#</sup>

n=7; (Mean ± S.D., g); <sup>1)</sup> Overnight fasted; <sup>2)</sup> Single formula; \* p<0.01 compared to that of sham by MW test; # p<0.01 and ## p<0.05 compared to that of control by MW test.

**Table 4. Changes of body weight gains during 28 days of test article administration**

Group	Body weight gains during dosing (g)
<i>db/m</i> Sham	5.37±1.75
Control	7.47±1.79
β-glucan <sup>1)</sup>	2.80±2.13 <sup>#</sup>
PLAT <sup>1)</sup>	4.87±3.01

MPP ( $\beta$ -glucan : PLAT)	
= 1:1	1.47±1.62 <sup>*,#</sup>
= 1:2	2.81±1.61 <sup>**,#</sup>
= 1:4	4.21±1.60 <sup>##</sup>
= 1:6	4.47±1.64 <sup>#</sup>
= 1:8	4.51±1.94 <sup>##</sup>
= 1:10	4.86±1.17 <sup>##</sup>
= 2:1	4.77±1.87 <sup>##</sup>
= 4:1	4.51±2.00 <sup>##</sup>
= 6:1	3.99±1.91 <sup>#</sup>
= 8:1	3.74±1.91 <sup>#</sup>
= 10:1	2.94±2.19 <sup>#</sup>

n=7; (Mean  $\pm$  S.D., g); <sup>1)</sup> Single formula; \* p<0.01 and \*\* p<0.05 compared to that of sham by MW test; # p<0.01 and ## p<0.05 compared to that of control by MW test.

**Table 5. Changes on the epididymal fat weights after 28 days of test article administration**

Fat weights	Absolute weight (g)	Relative weight (%)
Sham	0.47±0.26	0.18±0.10
Control	3.24±0.27 <sup>*</sup>	1.22±0.13 <sup>*</sup>
$\beta$ -glucan <sup>1)</sup>	2.71±0.15 <sup>*,#</sup>	1.00±0.09 <sup>*,#</sup>
PLAT <sup>1)</sup>	2.84±0.15 <sup>*,#</sup>	1.07±0.10 <sup>*</sup>
MPP		
= 1:1	2.40±0.34 <sup>*,#</sup>	0.89±0.14 <sup>*,#</sup>
= 1:2	2.86±0.10 <sup>*,#</sup>	1.08±0.04 <sup>*,##</sup>
= 1:4	2.86±0.17 <sup>*,#</sup>	1.06±0.05 <sup>*,##</sup>
= 1:6	2.89±0.11 <sup>*,#</sup>	1.07±0.07 <sup>*,##</sup>
= 1:8	2.93±0.15 <sup>*,##</sup>	1.10±0.09 <sup>*</sup>
= 1:10	2.91±0.15 <sup>*,##</sup>	1.08±0.08 <sup>*</sup>
= 2:1	2.87±0.17 <sup>*,#</sup>	1.07±0.09 <sup>*,##</sup>
= 4:1	2.84±0.15 <sup>*,#</sup>	1.05±0.07 <sup>*,##</sup>
= 6:1	2.76±0.14 <sup>*,#</sup>	1.02±0.05 <sup>*,#</sup>
= 8:1	2.75±0.14 <sup>*,#</sup>	1.01±0.05 <sup>*,#</sup>
= 10:1	2.74±0.12 <sup>*,#</sup>	1.01±0.03 <sup>*,#</sup>

n=7; (Mean  $\pm$  S.D.); Relative liver weight (%) = [(Absolute organ weight / Body weight at sacrifice)  $\times$  100]; <sup>1)</sup> Single formula; \* p<0.01 compared to that of sham by MW test; # p<0.01 and ## p<0.05 compared to that of control by MW test.

**Table 6. Changes of adiponectin after 28 days of test article administration**

Adiponectin	Serum adiponectin level ( $\mu$ g/ml)	Fat adiponectin contents (% control)
Sham	26.91±2.26	271.71±23.74
Control	15.09±1.02 <sup>*</sup>	100.00±0.00 <sup>*</sup>
$\beta$ -glucan <sup>1)</sup>	17.77±1.73 <sup>*,#</sup>	137.57±12.97 <sup>*,#</sup>
PLAT <sup>1)</sup>	17.49±1.97 <sup>*,##</sup>	124.57±10.91 <sup>*,#</sup>
MPP		
= 1:1	20.46±1.82 <sup>*,#</sup>	171.71±18.35 <sup>*,#</sup>
= 1:2	17.42±2.01 <sup>*,##</sup>	138.57±18.21 <sup>*,#</sup>
= 1:4	17.24±1.56 <sup>*,##</sup>	128.86±15.72 <sup>*,#</sup>
= 1:6	17.02±1.45 <sup>*,##</sup>	127.71±11.34 <sup>*,#</sup>
= 1:8	17.20±2.09 <sup>*,##</sup>	126.71±14.63 <sup>*,#</sup>
= 1:10	17.08±1.81 <sup>*,##</sup>	126.43±8.89 <sup>*,#</sup>
= 2:1	17.00±1.42 <sup>*,##</sup>	126.57±17.83 <sup>*,#</sup>

= 4:1	17.13±1.11 <sup>*,##</sup>	127.71±14.75 <sup>*,#</sup>
= 6:1	17.25±1.37 <sup>*,##</sup>	129.71±12.07 <sup>*,#</sup>
= 8:1	17.38±1.33 <sup>*,##</sup>	130.29±8.67 <sup>*,#</sup>
= 10:1	18.59±1.89 <sup>*,#</sup>	135.57±6.70 <sup>*,#</sup>

n=7; (Mean ± S.D.), <sup>1)</sup> Single formula; \* p<0.01 compared to that of sham by MW test; # p<0.01 and ## p<0.05 compared to that of control by MW test.

**Table 7. Changes of serum leptin levels after 28 days of test article administration**

Group	Serum leptin levels (ng/ml)
db/m Sham	2.90±0.24
Control	18.07±1.67 <sup>*</sup>
β-glucan <sup>1)</sup>	14.94±1.29 <sup>*,#</sup>
PLAT <sup>1)</sup>	16.33±1.19 <sup>*,##</sup>
MPP (β-glucan : PLAT)	
= 1:1	13.33±1.95 <sup>*,#</sup>
= 1:2	15.51±0.61 <sup>*,#</sup>
= 1:4	15.64±1.32 <sup>*,#</sup>
= 1:6	15.67±1.14 <sup>*,#</sup>
= 1:8	15.76±1.19 <sup>*,#</sup>
= 1:10	16.33±1.21 <sup>*,##</sup>
= 2:1	15.91±0.76 <sup>*,#</sup>
= 4:1	15.77±1.21 <sup>*,##</sup>
= 6:1	15.61±1.01 <sup>*,#</sup>
= 8:1	15.29±1.01 <sup>*,#</sup>
= 10:1	15.10±0.98 <sup>*,#</sup>

n=7; (Mean ± S.D.), <sup>1)</sup> Single formula; \* p<0.01 compared to that of sham by MW test; # p<0.01 and ## p<0.05 compared to that of control by MW test.

**Table 8. Changes of liver triglyceride contents after 28 days of test article administration**

Group	Liver triglyceride contents (mg/g liver)
db/m Sham	30.46±2.26
Control	49.06±5.00 <sup>*</sup>
β-glucan <sup>1)</sup>	40.76±3.07 <sup>*,#</sup>
PLAT <sup>1)</sup>	41.44±2.94 <sup>*,##</sup>
MPP (β-glucan : PLAT)	
= 1:1	35.23±3.64 <sup>**,#</sup>
= 1:2	40.87±1.95 <sup>*,#</sup>
= 1:4	41.81±4.08 <sup>*,##</sup>
= 1:6	42.21±3.51 <sup>*,##</sup>
= 1:8	42.20±3.40 <sup>*,##</sup>
= 1:10	41.36±2.29 <sup>*,#</sup>
= 2:1	41.76±1.79 <sup>*,#</sup>
= 4:1	41.16±2.55 <sup>*,#</sup>
= 6:1	41.24±2.03 <sup>*,#</sup>
= 8:1	41.64±4.40 <sup>*,##</sup>
= 10:1	41.17±4.41 <sup>*,##</sup>

n=7; (Mean ± S.D.), <sup>1)</sup> Single formula; \* p<0.01 and \*\* p<0.05 compared to that of sham by MW test; # p<0.01 and ## p<0.05 compared to that of control by MW test.

## DISCUSSION

It is well known that some of the β-glucans exhibit hypolipemic and anti-obesity effect (Nicolosi *et al.*, 1999; Delaney *et al.*, 2003; Wilson *et al.*, 2004). The mechanism by which β-glucans elicit their hypolipemic and anti-obesity effect is not fully understood, but several hypotheses have been proposed (Anderson, 1987). One favored hypothesis is that β-glucan inhibits the intestinal absorption of bile acids and cholesterol, promoting enhanced fecal excretion of acidic and neutral sterols. A reduction in the enterohepatic circulation of bile acids increases the conversion of cholesterol to bile acids (Anderson, 1987). Another mechanism hypothesized in the literature is the effect of short-chain fatty acids (SCFA) on cholesterol metabolism. SCFA are products of the colonic bacterial fermentation of dietary fiber including β-glucans. Several studies have suggested that the suppressive effect of certain dietary fibers on the plasma cholesterol level was at least partly due to the inhibition of cholesterol biosynthesis caused by SCFA (Hara *et al.*, 1998, 1999). The effect of PLAT as the anti-obesity and hyperlipemia has also been reported (Han *et al.*, 2000, 2002; Zhao *et al.*, 2005), and they are generally known to mediate the anti-obesity activities to the pancreatic lipase (Arai *et al.*, 1997; Ida *et al.*, 1998). PLAT inhibits the intestinal absorption of dietary fat by inhibiting the pancreatic lipase activity (Han *et al.*, 2002). However, the dosage showing anti-obesity or hypolipemic effects of the single use of β-glucan and PLAT is considered as much higher than the ideal dosage to develop an anti-obesity agent.

In this study, to reduce the efficacy dosage on the anti-obesity, we intended to the mixed β-glucan (originated from *Aureobasidium*) with PLAT (extracted and purified from *Platycodi radix*). To find out the fittest composition, the anti-obesity effects of 11 types of the composition of β-glucan and PLAT (MPP) were observed in the obese *db/db* mouse at 200mg/kg-dosing levels with β-glucan or PLAT single formulation. The obese changes detected in control were dramatically decreased after β-glucan single, PLAT single and MPP-dosing, and the changes related to the obese showed decreased trends along the increase of β-glucan composition. However, synergic anti-obesity effects were restricted to the MPP 1:1 formulation compared to those of single formulas, respectively. Only MPP 1:1 formulation showed favorable effects on reducing the obesity compared to that of β-glucan single formulation.

The *db/db* mouse is hyperleptinemic and develops obesity. It has been used in the efficacy test field of various pharmaceuticals including obesity (Nakagawa *et al.*, 2003; Pittner *et al.*, 2004; Neary *et al.*, 2005). The inhibitions of increase in body weight and gains have been regarded as an evidence of treatment of obesity. In this study, among 11 types of MPP formulations, MPP 1:1 formulation showed the favorable efficacy on the inhibition of the increase in body weight, and only one formulation showed the favorable effect compared to that of the  $\beta$ -glucan single-dosing group. Additionally, the increases in the accumulation of adipose tissues are common features in obesity. Adipose tissue is currently known to work not simply as an organ for energy storage, but also as an endocrine and secretory organ (Fujita *et al.*, 2005). Adipose tissues secrete adipokines and changes in the expression, secretion, and action of the adipokines in obesity are possibly implicated in the development of various diseases including insulin resistance (Fujita *et al.*, 2005; Mitchell *et al.*, 2005). In this study, quite similar to those of body weight changes, among 11 types of MGP formulations, MPP 1:1 formulation showed the favorable efficacy on the inhibition of the increase in epididymal fat weights, and only one formulation showed the favorable effects compared to that of the  $\beta$ -glucan single-dosing group.

Adiponectin (also known as Acrp30) is a novel adipokine that has been recently identified (Scherer *et al.*, 1995; Hu *et al.*, 1996; Maeda *et al.*, 1996; Nakano *et al.*, 1996). It is exclusively expressed in adipose tissue (Maeda *et al.*, 1996) and abundantly released into circulating blood (Arita *et al.*, 1999). Recently, the findings that obesity decreases plasma adiponectin levels have been reported in humans (Arita *et al.*, 1999; Matsubara *et al.*, 2002; Matsubara, 2004) and experimental animals (Hotta *et al.*, 2001; Maebuchi *et al.*, 2003). Moreover, it has been shown that hypoadiponectinemia is closely related to insulin resistance (Mitchell *et al.*, 2005). In this study, the hypoadiponectinemia and decrease of adipose tissue adiponectin contents in *db/db* mice were markedly inhibited by treatment of  $\beta$ -glucan single and PLAT single-dosing and most of MPP formulations-dosing, respectively. These changes are obvious evidence that the  $\beta$ -glucan or PLAT and their combinations have anti-obesity effects. In addition, among 11 types of MPP formulations, MPP 1:1 formulation showed the favorable efficacy on the inhibition of the decrease of tissue and blood adiponectin levels, and only one formulation showed the favorable effect on the both blood and tissue levels compared to that of the  $\beta$ -glucan single-dosing group.

Leptin, produced predominantly by adipose tissue, was first explored as a satiety signal regulating food intake and energy expenditure (Hakansson *et al.* 1996; Mercer *et al.* 1996; Cheung *et al.* 1997; Bjorbaek

and Kahn 2004). Deficiencies in leptin signaling or functioning in the hypothalamus are thought to contribute to the development of obesity. The *db/db* mouse used in this study is hyperleptinemic and develops obesity. The hyperleptinemia in *db/db* mice was markedly inhibited by treatment of  $\beta$ -glucan single and PLAT single-dosing and most of MPP formulations-dosing. These changes are obvious evidence that the  $\beta$ -glucan or PLAT and their combinations have anti-obesity effects. In addition, among 11 types of MGP formulations, MPP 1:1 formulation showed the favorable efficacy on the inhibition of the increase in serum leptin levels, and only one formulation showed the favorable effects compared to that of the  $\beta$ -glucan single-dosing group.

The increase of circulation of free fatty acids in obesity might lead to insulin resistance, and ultimately to diabetes in genetically prone subjects by the mechanism of lipotoxicity (Manco *et al.*, 2004; Park *et al.*, 2005). Therefore, the liver triglyceride contents should be reduced to treat obesity and prevent obese related diabetes. These hepatic triglyceride contents in *db/db* mice were markedly inhibited by treatment of  $\beta$ -glucan single and PLAT single-dosing and most of MPP formulations-dosing. These changes are obvious evidence that  $\beta$ -glucan or PLAT and their combinations have anti-obesity effects and reduce the progress to diabetes. In addition, among 11 types of MPP formulations, MPP 1:1 formulation showed the favorable efficacy on the inhibition of the increase of hepatic triglyceride contents, and only one formulation showed the favorable effects compared to that of the  $\beta$ -glucan single-dosing group.

The favorable anti-obesity effects detected in the MPP 1:1 formulation compared to those of  $\beta$ -glucan and PLAT single-dosing groups is considered as synergic effects as results of the similar anti-obesity mechanisms of  $\beta$ -glucan and PLAT; inhibition of the intestinal absorption of fats. However, the detailed mechanism studies should be done in future to confirm these findings.

Among 11 types of mixed composition and two single formulations, MPP 1:1 composition is considered as the fittest composition for treating obesity. Although the synergic anti-obesity effects detected in MPP 1:1 formulation were considered as results of the similar anti-obesity mechanisms of  $\beta$ -glucan and PLAT that inhibition of the intestinal absorption of fats, the dosage-related test of MPP 1:1 formulation. However, the detailed mechanism studies should be tested further to confirm these findings.

The obesity rate is low in those whose food intake contains an adequate amount of fiber (Kimm 1995). Moreover, inverse correlations have been reported between fiber intake and weight (Alfieri *et al.*, 1995) and body mass index. However, because most clinical experiments do not distinguish between the different types of fiber, it is unclear if a specific fiber,  $\beta$ -glucan in particular, is more or less effective at reducing weight



(Kim *et al.*, 2006). A few clinical studies have examined the effects of  $\beta$ -glucan on body weight. For example, the consumption of pearl barley with a high  $\beta$ -glucan content reduces not only the serum low-density lipoprotein cholesterol but also the visceral fat area (Shimizu *et al.*, 2008). However, no clinical study has examined the effects of  $\beta$ -glucan derived from *Aureobasidium* on body weight and obesity.

Cho *et al.* (2013) investigated the effects of an herbal extract, a mixture of *Scutellariae Radix* and *Platycodi Radix* containing the active ingredients baicalin and saponin [target herbal ingredient (THI)], on lowering body weight in a randomized, double-blind, placebo-controlled trial. However, no clinical study has examined body weight and obesity using platycodin D, a major saponin component of *Platycodi Radix*, which inhibits fat accumulation and adipogenesis in rodents (Zhao *et al.*, 2005; Kim *et al.*, 2009).

Therefore, further clinical studies of body weight and obesity using  $\beta$ -glucan derived from *Aureobasidium*, platycodin, and MPP 1:1 are needed.

**Conflict of interest:** The authors declare that there are no conflicts of interest.

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