Anti-obesity effects of MPP, a mixed formulation of platycodin and the *Aureobasidium*-derived β-glucan polycan, in *db/db* mice

Jae-Hak Sohn¹, Hyung-Rae Cho², Sae-Kwang Ku¹,³ and Jae-Suk Choi¹,*

¹Division of Bioindustry, College of Medical and Life Sciences, Silla University, 140 Baegyang-daero, 700 beon-gil, Sasang-gu, Busan 46958, Republic of Korea; ²Glucan corp. #305 Marine Bio-Industry Development Center, Hoengggye-ri 27, Ilgwang-myeon, Gijan-gun, Busan 46048, Republic of Korea; ³Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, 290 Yugok-dong, Gyeongsan-si, Gyeongsanbuk-do 38610, Republic of Korea.

ABSTRACT

The anti-obesity effects of MPP, a formulation composed of polycan (β-glucan produced by *Aureobasidium pullulans*) and platycodin (purified from *Platycodi radix*) at an optimal ratio, were observed in obese *db/db* mice. The animals were treated by MPP at 50, 100, and 200 mg/kg, polycan or platycodin at 100 mg/kg, or metformin at 250 mg/kg via daily oral administration for 28 days. During the experimental period, the changes in body weight, epididymal fat weight, serum leptin and adiponectin levels, adipose adiponectin content, and liver triglyceride content were monitored. Prior to initial administration of the test compounds, control *db/db* mice showed noticeable obesity with severe hyperglycemia compared to *db/m* mice. The epididymal fat weight, serum leptin level, and liver triglyceride contents in the *db/db* control were significantly higher than those in the *db/m* mice, whereas serum and adipose adiponectin levels were significantly lower. At all dosages, MPP attenuated these levels of these obesity-related indicators in a dose-dependent manner. In addition, MPP showed stronger anti-obesity effects compared to those of equal-dose polycan or platycodin administered individually by limiting intestinal fat absorption as an anti-obesity mechanism. Taken together, MPP is expected to be beneficial to patients with obesity or related metabolic diseases such as diabetes.

KEYWORDS: polycan, platycodin, *db/db* mouse, obesity, adipokine, MPP.

INTRODUCTION

The worldwide incidence of obesity continues to escalate despite increased awareness and global efforts to understand and confront its origins. In essence, dysregulated energy homeostasis stems from a reduction in physical activity and an increase in the accessibility of and overindulgence in energy-dense foods, in addition to a myriad of complex genetic, social, and economic factors [1]. Over the past several decades, 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 a result of high-calorie diet intake and physical inactivity [2]. Estimates have suggested that the population suffering from type 2 diabetes may reach over 300 million by 2025 [3]. One of the critical indications of the development of this type of obesity is the increase in the regional distribution of body fat, i.e., abdominal obesity. This is often associated with a variety of atherogenic risk
factors [4], such as 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 from cardiovascular diseases [5]. Vigorous efforts have been made to delineate the relationship between increased adiposity and insulin resistance. In this regard, adipokines secreted by adipocytes may modulate the sensitivity of insulin [1]. The action of these molecules, including leptin [6, 7] and adiponectin [8, 9], activates multiple signaling events after phosphorylation of insulin receptors and several other factors involved in type 2 diabetes [10, 11].

The hyperleptinemic db/db mouse develops obesity and severe type 2 diabetes partly because of a functional defect in the long-form leptin receptor, which plays a significant role in the regulation of food intake and body weight control [12, 13]. This breed of genetically obese mouse has been applied in the testing of various pharmaceutical products, including drugs intended for obesity management [14, 15]. Generally, the anti-obesity properties of the tested products are evaluated based on their effect on body weight, fat weight, and organ or serum lipid profiles of adipokines such as leptin and adiponectin [16, 17].

β-glucans are fiber-type polysaccharides derived from the cell wall of baker’s yeast, oat and barley fiber, and medicinal mushrooms such as maitake [18]. They are primarily used to enhance the immune system [19, 20] and lower blood cholesterol levels [21, 22]. The effective dosage of β-glucan is over 15 g/day in humans [23] and 1.2 g/day in animals [24, 25]. Some researchers have reported evidence of the anti-obesity and hypolipemic effects of β-glucan extracts from natural plants or mushrooms in animal experiments [24, 25] and human clinical trials [23]. However, the dosage applied in these studies was much higher than the ideal dosage for the development of anti-obesity agents.

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 East Asia. In China and Korea, the fresh roots of P. grandiflorum have been consumed as pickles to prevent obesity [26]. The anti-obesity and hypolipemic properties of platycodin, a main component of Platycodi radix, have been reported [27, 28]. However, the effective anti-obesity dose was much higher than the ideal dosage, which is 0.5 g/L in vitro and at least 244 mg/kg in animals [26].

A number of oral medical formulations, such as thiazolidinediones and metformin, have been developed to treat diabetes by ameliorating insulin resistance. Metformin limits hepatic glucose production by reducing gluconeogenesis [29] and effectively inhibits high-fat-induced obesity in mice [30]. Currently available pharmacological agents for diabetes or related obesity have a number of limitations, such as high rates of secondary failure [31]. Because of these factors, diabetic patients and healthcare professionals are increasingly considering complementary and alternative approaches, including the use of medicinal herbs. However, the development of anti-obesity agents is challenging because their effective dosage is relatively high in animal experiments and human clinical trials. In the present study, the anti-obesity properties of MPP, a compound composed of an optimal proportion of polycan and platycodin, were observed in obese db/db mouse. The effect of MPP was compared with that of individual polycan or platycodin treatment, as well as with that of metformin.

**MATERIALS AND METHODS**

**Animals and husbandry**

Ninety-eight male genetically obese db/db mice with a C57BL/KsJ genetic background and fourteen lean non-diabetic heterozygous db/m mice (seven weeks old upon receipt, Clear, Japan) were involved in the study after seven days of acclimatization. Seven animals were allocated in each polycarbonate cage at 20-25 °C and 40-45% humidity. The animals were housed in a 12/12-hour light/dark cycle and given free access to standard rodent chow (Samyang, Korea) and water. Approximately half of the animals were selected (in each group of seven) and fasting was induced two days before initial drug administration. The mean fasting blood glucose levels two days before initial dosing were 97.29 ± 7.20 (90–112) mg/dL in in db/m mice and 278.95 ± 10.61 (266–305) mg/dL in db/db mice (Table 1). All animal experiments were performed in accordance with the institutional guidelines and
The collected blood samples were deposited into NaF glucose vacuum tubes (Becton Dickinson, USA) and plasma was separated. Blood glucose levels were detected using an automated blood analyzer (Toshiba 200 FR, Japan).

**Body weight change**

Body weight was measured one day before drug administration, at initial dosing, and 1, 7, 14, 21, 27, and 28 days after dosing using an automatic electronic balance (Sartorius Co., Ltd., USA). At dosing and termination (sacrifice), the experimental animals were subjected to overnight fasting for approximately 12 h (water was provided) to reduce error caused by feeding. In addition, body weight gain was calculated as follows.

Weight gain (g) = Difference in weight during observation period (from day 0 to 28 after dosing) (Eq. 1)

**Measurement of epididymal fat weight**

At sacrifice, the epididymal adipose tissues were removed and their weight was calculated. To reduce error caused by individual body weight differences, the relative weight (%) was calculated using body weight at sacrifice and absolute weight as follows.

Relative fat weight (%) = (Absolute epididymal fat weight / Body weight at sacrifice) × 100 (Eq. 2)

**Measurement of serum adiponectin level**

To detect the serum adiponectin level, blood samples were collected at sacrifice from the vena cava after overnight fasting and the serum was separated from the collected blood using general methods. Serum adiponectin level was detected using a commercially available enzyme-linked immunosorbent assay kit (Otsuka Pharm., Japan).

**Measurement of adiponectin content in epididymal adipose tissues**

Adipose tissue adiponectin levels were determined by western blot as previously described [16]. The extracted epididymal adipose tissues were homogenized in phosphate-buffered saline containing 0.5% sodium deoxycholate. The homogenates were incubated for 24 h at 37 °C. After incubation, the homogenates were centrifuged at 15,000 × g for 10 min. The fat cake was removed by suction, and adipose tissue extracts (supernatants) were subjected to western blot. Aliquots of the tissue

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>db/m mice (Sham)</td>
<td>97.29 ± 7.20</td>
</tr>
<tr>
<td>db/db control (Control)</td>
<td>279.43 ± 12.71*</td>
</tr>
<tr>
<td>Polycan: Single formulation 200mg/kg</td>
<td>278.43 ± 11.47*</td>
</tr>
<tr>
<td>Platycodin: Single formulation 200mg/kg</td>
<td>276.86 ± 6.99*</td>
</tr>
<tr>
<td>MPP</td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>278.71 ± 10.98*</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>281.86 ± 16.23*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>278.43 ± 5.22*</td>
</tr>
<tr>
<td>Metformin 250mg/kg</td>
<td>279.29 ± 10.55*</td>
</tr>
</tbody>
</table>

n = 7; (Mean ± S.D.); All test articles were orally dosed once a day for 28 days at 5 ml/kg using distilled water as vehicle; *p < 0.01 compared to that of db/m mice by MW test.

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approved by the Institutional Animal Care and Use Committee of Silla University (Busan, Korea; approval no. SUACUC-2016-017).

**Drug preparation and administration**

Polycan, a β-glucan from *Aureobasidium pullulans* SM-2001 (a UV-induced mutant of *A. pullulans*, half of the dry material is -1,3/1,6-glucans) [32], was obtained from Glucan Corp. (Busan, Korea). Platycodin was extracted and purified from *Platycodi radix* [33]. MPP was prepared with a 1:1 mixture of polycan and platycodin. Metformin was purchased from Sigma (USA). All test materials were stored in a refrigerator at 4 °C in the absence of light with protection against degeneration. The drugs were dissolved or suspended in distilled water and administered daily for 28 days by oral gavage using a sonde attached to a 3-mL syringe containing the drug. MPP was administered at 50, 100, and 200 mg/kg. Polycan and platycodin were administered separately at 100 mg/kg, and metformin was administered at 250 mg/kg or 5 mL/kg.

**Measurement of blood glucose level**

To detect the blood glucose levels, blood was collected from the orbital plexus two days before initial drug administration after overnight fasting.
 extracted (10 μg of protein) prepared in sodium dodecyl sulfate sample buffer were incubated for 5 min at 100 °C. Denatured proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Bio-Rad Lab., USA). The membranes were incubated with monoclonal mouse adiponectin antibodies (Chemicon International, USA) at a dilution of 1:10,000 for 12 h at room temperature and then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibody (DAKO, USA) at a dilution of 1:5,000 for 1 h at room temperature. After incubation, the membranes were soaked in enhanced chemiluminescence detection reagents (Amersham, UK). The membranes were exposed to an X-ray film and adiponectin protein was visualized. The signals from the X-ray film were quantified using a DMI CCD image analyzer system (DMI, Korea). Adiponectin protein contents per 10 μg of adipose tissue protein in db/m and all dosage groups were normalized to those of the db/db control group in the same assay and expressed as the percentage of the value of the control group.

Measurement of serum leptin level
To detect the serum leptin level, blood samples were collected at sacrifice from the vena cava after overnight fasting and serum was separated from the collected blood using general methods. Serum leptin levels were detected using a commercially available radioimmunoassay kit (Linco Research, USA) as previously described [17].

Measurement of liver triglyceride content
Liver samples (50–70 mg) were homogenized in 50 mM Tris-HCl buffer at pH 7.4, containing 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, and 1 μM phenylmethylsulfonyl fluoride. Triglyceride content in the liver homogenates was measured using a spectrophotometric kit (Thermo DMA, USA) as previously described [17].

Statistical analysis
All data were expressed as the mean ± standard deviation (n = 7). Statistical analysis was conducted using Mann-Whitney U-Wilcoxon Rank Sum W test with SPSS for Windows (Release 6.1.3., SPSS Inc., USA). The inhibition rate compared to that of the control was calculated to compare the difference in the efficacy of the test compounds between the db/m and control groups (Eq. 3), and between the control and experimental groups (Eq. 4).

Percentage changes vs. sham (%) = [(Data from vehicle control – Data from sham) / Data from sham] × 100 (Eq. 3)
Percentage changes vs. vehicle control (%) = [(Data from test group – Data from vehicle control) / Data from vehicle control] × 100 (Eq. 4)

RESULTS
Changes in body weight
Before initial drug administration, db/db mice showed pronounced obesity compared to db/m mice. The body weight of the control mice was significantly higher (p < 0.01) than that of db/m mice throughout the experimental period and accordingly, the body weight gain increased significantly (p < 0.01). However, significant decreases in body weight were observed from 21 or 27 days after individual administration of polycan (p < 0.01) and platycodin (p < 0.05) at all dosages. In addition, the body weight gains in all dosage groups were significantly decreased (p < 0.01) compared to that of the control. In particular, MPP reduced the increase in body weight compared to that of the control in a dose-dependent manner (Table 2).

The body weight of the control mice during the dosage period showed a change of 63.97% compared to that of the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -45.54% and -35.00%, respectively. In addition, MPP at 50, 100, and 200 mg/kg induced changes of -40.61%, -45.22%, and -55.73% compared to the control, respectively, whereas metformin led to a change of -55.25%.

Changes in epididymal fat weight
Significantly higher absolute and relative epididymal adipose tissue weights (p < 0.01) were detected in the control mice compared to those in the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -45.54% and -35.00%, respectively. In addition, MPP at 50, 100, and 200 mg/kg induced changes of -40.61%, -45.22%, and -55.73% compared to the control, respectively, whereas metformin led to a change of -55.25%.

Changes in epididymal fat weight
Significantly higher absolute and relative epididymal adipose tissue weights (p < 0.01) were detected in the control mice compared to those in the db/m mice. Reduced fat weight (p < 0.01 or p < 0.05) was detected in all dosage groups compared to that of the control, and the increase in fat weight was attenuated by MPP in a dose-dependent manner (Table 3).

The absolute epididymal adipose tissue weight of the control mice during the dosage period showed a change of 527.14% compared to that of the db/m
Table 2. Changes in body weight and body weight gains after 28 days of test article administration.

<table>
<thead>
<tr>
<th>Body weight</th>
<th>At Dosing\textsuperscript{1)}</th>
<th>Day 1</th>
<th>Weeks after dosing</th>
<th>At Sacrifice\textsuperscript{1)}</th>
<th>Body weight gains during dosing (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 week</td>
<td>2 weeks</td>
<td>3 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Sham</td>
<td>22.09 ± 1.06</td>
<td>24.03 ± 1.39</td>
<td>24.33 ± 1.05</td>
<td>26.07 ± 1.03</td>
<td>27.97 ± 0.98</td>
</tr>
<tr>
<td>Control</td>
<td>40.26 ± 1.10\textsuperscript{*}</td>
<td>42.66 ± 0.82\textsuperscript{*}</td>
<td>44.01 ± 1.07\textsuperscript{*}</td>
<td>45.93 ± 1.01\textsuperscript{*}</td>
<td>48.41 ± 1.38\textsuperscript{*}</td>
</tr>
<tr>
<td>Polycan</td>
<td>40.19 ± 1.07\textsuperscript{*}</td>
<td>42.40 ± 0.99\textsuperscript{*}</td>
<td>44.33 ± 0.79\textsuperscript{*}</td>
<td>45.83 ± 1.02\textsuperscript{*}</td>
<td>47.23 ± 1.10\textsuperscript{*}</td>
</tr>
<tr>
<td>Platycodin</td>
<td>40.03 ± 1.57\textsuperscript{*}</td>
<td>42.07 ± 1.23\textsuperscript{*}</td>
<td>43.69 ± 1.11\textsuperscript{*}</td>
<td>45.43 ± 0.91\textsuperscript{*}</td>
<td>47.53 ± 1.31\textsuperscript{*}</td>
</tr>
<tr>
<td>MPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>40.50 ± 1.31\textsuperscript{*}</td>
<td>42.37 ± 1.56\textsuperscript{*}</td>
<td>44.41 ± 1.56\textsuperscript{*}</td>
<td>45.83 ± 2.01\textsuperscript{*}</td>
<td>47.34 ± 2.07\textsuperscript{*}</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>40.30 ± 0.99\textsuperscript{*}</td>
<td>42.10 ± 1.00\textsuperscript{*}</td>
<td>44.11 ± 1.34\textsuperscript{*}</td>
<td>46.01 ± 1.52\textsuperscript{*}</td>
<td>46.53 ± 1.74\textsuperscript{*}</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>40.53 ± 1.42\textsuperscript{*}</td>
<td>42.44 ± 1.29\textsuperscript{*}</td>
<td>44.10 ± 1.22\textsuperscript{*}</td>
<td>45.51 ± 1.60\textsuperscript{*}</td>
<td>46.19 ± 1.29\textsuperscript{*,##}</td>
</tr>
<tr>
<td>Metformin</td>
<td>40.19 ± 1.04\textsuperscript{*}</td>
<td>42.37 ± 1.09\textsuperscript{*}</td>
<td>43.80 ± 1.13\textsuperscript{*}</td>
<td>45.23 ± 1.23\textsuperscript{*}</td>
<td>45.60 ± 1.36\textsuperscript{*,#}</td>
</tr>
</tbody>
</table>

n = 7; (Mean ± S.D., g); \textsuperscript{1)}Overnight fasted; \textsuperscript{*}p < 0.01 and \textsuperscript{**}p < 0.05 compared to that of sham by MW test; \#p < 0.01 and \textsuperscript{**,##}p < 0.05 compared to that of control by MW test.
mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -23.32% and -27.33%, respectively. MPP at 50, 100, and 200 mg/kg induced changes of -22.59%, -45.22%, and -47.00% compared to the control, respectively, whereas metformin led to a change of -48.52%.

In terms of the relative epididymal adipose tissue weight, the control mice showed a change of 249.62% compared to the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -15.86% and -21.98%, respectively. MPP at 50, 100, and 200 mg/kg induced changes of -16.67%, -26.28%, and -41.36% compared to the control, respectively, whereas metformin led to a change of -42.69%.

**Changes in adipose tissue adiponectin content**

Significantly lower epididymal adipose tissue adiponectin contents (p < 0.01) were detected in the control mice compared to that in the db/m mice. Augmented adipose tissue adiponectin content (p < 0.01) was revealed in all dosage groups compared to that of the control, whereby MPP induced a dose-dependent increase (Table 4).

The epididymal adipose tissue adiponectin content of the control mice showed a change of -49.58% compared to that of the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -24.29% and -19.97%, respectively. In addition, MPP at 50, 100, and 200 mg/kg induced changes of -27.57%, -30.25%, and -33.67% compared to the control, respectively, whereas metformin led to a change of -44.26%.

**Changes in serum leptin level**

Significantly elevated serum leptin levels (p < 0.01) were identified in the control mice compared to those in the db/m mice. Lower serum leptin levels (p < 0.01 or p < 0.05) were observed in all dosage groups compared to that of the control, where MPP caused a decline in serum leptin level in a dose-dependent manner (Table 5).

The serum leptin level of the control mice showed a change of 700.51% compared to that of the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -27.57%, -30.25%, and -33.67% compared to the control, respectively, whereas metformin led to a change of -44.26%.

**Changes in hepatic triglyceride content**

Significantly higher liver triglyceride contents (p < 0.01) were detected in the control mice compared to that in the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of 28.67% and 26.86%, respectively. In addition, MPP at 50, 100, and 200 mg/kg induced changes of 29.71%, 37.84%, and 43.90% compared to the control, respectively, whereas metformin led to a change of 42.26%.

**Changes in serum adiponectin level**

Significantly lower serum adiponectin levels (p < 0.01) were observed in the control mice compared to those in the db/m mice. Elevated serum adiponectin levels (p < 0.01 or p < 0.05) were detected in all dosage groups compared to that of the control, and the reduction in serum adiponectin level was inhibited by MPP in a dose-dependent manner (Table 4).

The serum adiponectin level of the control mice showed a change of -52.57% compared to that of the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -23.32% and -27.33%, respectively. MPP at 50, 100, and 200 mg/kg induced changes of -22.59%, -45.22%, and -47.00% compared to the control, respectively, whereas metformin led to a change of -48.52%.
Inhibitory effects of β-glucan and platycodin in obese mice

**Table 4.** Changes in serum adiponectin level and fat adiponectin contents after 28 days of test article administration.

<table>
<thead>
<tr>
<th>Adiponectin</th>
<th>Serum adiponectin level (μg/ml)</th>
<th>Fat adiponectin contents (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>27.95 ± 2.86*</td>
<td>272.86 ± 25.29</td>
</tr>
<tr>
<td>Control</td>
<td>14.09 ± 2.80*</td>
<td>100.00 ± 0.00*</td>
</tr>
<tr>
<td>Polycan</td>
<td>18.13 ± 1.43*</td>
<td>129.00 ± 14.57*</td>
</tr>
<tr>
<td>Platycodin</td>
<td>17.88 ± 1.18*</td>
<td>123.14 ± 9.25*</td>
</tr>
<tr>
<td>MPP 50 mg/kg</td>
<td>18.28 ± 1.04*</td>
<td>131.29 ± 8.62*</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>19.42 ± 0.72*</td>
<td>141.86 ± 11.81*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>20.28 ± 1.30*</td>
<td>151.71 ± 16.14*</td>
</tr>
<tr>
<td>Metformin</td>
<td>20.05 ± 1.22*</td>
<td>152.57 ± 10.24*</td>
</tr>
</tbody>
</table>

n = 7; (Mean ± S.D.); *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 5.** Changes in serum leptin levels and liver triglyceride contents after 28 days of test article administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum leptin levels (ng/ml)</th>
<th>Liver triglyceride contents (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>2.81 ± 0.43</td>
<td>30.97 ± 1.44</td>
</tr>
<tr>
<td>Control</td>
<td>22.53 ± 2.47*</td>
<td>49.81 ± 6.79*</td>
</tr>
<tr>
<td>Polycan</td>
<td>17.06 ± 1.12*</td>
<td>40.37 ± 2.37*</td>
</tr>
<tr>
<td>Platycodin</td>
<td>18.03 ± 1.21*</td>
<td>41.73 ± 5.60*</td>
</tr>
<tr>
<td>MPP 50 mg/kg</td>
<td>16.33 ± 1.00*</td>
<td>39.46 ± 3.13*</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>15.71 ± 2.94*</td>
<td>37.59 ± 2.83*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>14.94 ± 1.67*</td>
<td>35.93 ± 3.49*</td>
</tr>
<tr>
<td>Metformin</td>
<td>12.56 ± 1.39*</td>
<td>35.40 ± 3.02*</td>
</tr>
</tbody>
</table>

n = 7; (Mean ± S.D.); *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.

The liver triglyceride content of the control mice showed a change of 60.84% compared to that of the db/m mice. Compared to the control, individual polycan and platycodin treatment resulted in changes of -18.96% and -16.23%, respectively. In addition, MPP at 50, 100, and 200 mg/kg induced changes of -20.79%, -24.55%, and -27.87% compared to the control, respectively, whereas metformin led to a change of -28.94%.

**DISCUSSION**

It is well known that some β-glucans exhibit hypolipemic and anti-obesity effects [24, 25]. The mechanism by which β-glucans elicit these effects is not fully understood, but several hypotheses have been proposed [34]. One favored hypothesis is that β-glucans inhibit 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 into bile acids [34]. Another hypothesized mechanism in the literature is the effect of short chain fatty acids (SCFAs) on cholesterol metabolism. SCFAs are products of the colonic bacterial fermentation of dietary fibers including β-glucans. Several studies have suggested that the suppressive effect of certain dietary fibers on plasma cholesterol content was partly due to the inhibition of cholesterol biosynthesis caused by SCFAs [35, 36]. The anti-obesity and hypolipidemic effects of platycodin have also been reported [27, 28], and it is generally recognized that the anti-obesity activities are mediated by pancreatic lipase [37, 38]. Platycodin inhibited the intestinal absorption of dietary fats by inhibiting the activity of pancreatic lipase [27]. However, the dosage of individual β-glucan and platycodin administration showing anti-obesity or hypolipemic effects was much higher than the ideal dosage for the development of anti-obesity agents. MPP, a combination of polycan (β-glucan produced by Aureobasidium pullulans SM-2001) and platycodin at an optimal composition ratio, showed more favorable anti-obesity effects compared to that of individual polycan or platycodin [33].

In the present study, the anti-obesity effects of MPP were observed in obese db/db mouse at 50, 100, and 200 mg/kg. The effects were compared to those of 250 mg/kg metformin, which ameliorated insulin resistance and inhibited insulin-associated obesity. All tested compounds were administered orally daily for 28 days in obese db/db mice and the changes in body weight, epididymal fat weight, serum leptin and adiponectin levels, adipose
adiponectin content, and liver triglyceride content were monitored. Prior to initial dosing of the test compounds, db/db control mice exhibited distinct obese states with severe hyperglycemia compared to db/m mice (normal littermates). The epididymal fat weight, serum leptin level, and liver triglyceride content in the db/db mice were elevated compared to those in the db/m mice (p < 0.01), whereas the serum adiponectin and adipose contents were reduced (p < 0.01). However, these effects were mitigated by metformin, polycan, platycodin, and MPP at all doses applied. MPP attenuated the changes in the obesity-related indicators and showed dramatic anti-obesity effects in a dose-dependent manner compared to equal doses of polycan or platycodin. In addition, 200 mg/kg MPP showed similar or slightly weaker anti-obesity effects compared to those of metformin at 250 mg/kg.

Because db/db mice are hyperleptinemic and develop obesity, they have been applied to test the efficacy of various pharmaceutical products including drugs formulated to treat obesity [14, 15]. Prevention of body weight gain has been regarded as an evidence of effective obesity treatment. In the present study, MPP effectively inhibited body weight gain in a dose-dependent manner, with more prominent effects than those of equal-dose polycan or platycodin administrated individually. In obesity, increase in accumulation of adipose tissues is a common feature. Adipose tissues are currently known to function not only as an energy storage, but also as an endocrine and secretory organ [16]. Adipose tissues secrete adipokines, and changes in the expression, secretion, and action of adipokines during obesity are possibly implicated in the development of various conditions including insulin resistance [1, 16]. Similar to the effect on body weight, MPP effectively prevented the increase in epididymal fat weight in a dose-dependent manner, and greater effects were observed compared to those of equal-dose polycan or platycodin administrated individually. These results on body and fat weights reconfirmed the synergic effects of polycan and platycodin. MPP at 200 mg/kg showed similar effects as metformin at 250 mg/kg in inhibiting the body and fat weight increases. Adiponectin, also known as Acrp30, is a novel adipokine that has recently been identified [39, 40]. It is exclusively expressed in adipose tissue [39], and abundantly released into circulating blood [41]. More recently, it has been reported that obesity decreased plasma adiponectin levels in humans [42, 43] and experimental animals [44, 45]. Moreover, hypoadiponectinemia is closely related to insulin resistance [1, 17]. In the present study, hypoadiponectinemia and decreases in adiponectin content in adipose tissue were markedly inhibited by MPP in db/db mice. In addition, MPP showed more prominent effects compared to those of equal-dose polycan or platycodin administrated individually. These changes evidently verified the anti-obesity effects of MPP, and at 200 mg/kg, MPP showed similar effects to those of metformin at 250 mg/kg in preventing the decrease in adiponectin content. Leptin is produced predominantly by adipose tissues and was first explored as a satiety signal that regulates food intake and energy expenditure. Leptin binds to the long-form of the leptin receptor in the hypothalamus to reduce the activity of neuropeptide-Y and agouti-related protein and increase pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript protein neuron activity, effectively reducing appetite and feed intake [46]. Deficiencies in leptin signaling or function in the hypothalamus are thought to contribute to the development of obesity. Hyperleptinemia was markedly decreased by MPP in db/db mice in a dose-dependent manner in this study, and MPP was more effective compared to equal-dose polycan or platycodin administrated individually. These changes clearly revealed that the combination of polycan and platycodin exerted dramatic synergistic effects. In addition, 200 mg/kg MPP showed similar or slightly lower effects compared to those of 250 mg/kg metformin in preventing the increase in serum leptin level. The increase in the circulation of free fatty acids in obesity might lead to insulin resistance and ultimately to diabetes in genetically prone subjects via the mechanism of lipotoxicity [30, 47]. Therefore, liver triglyceride contents should be reduced to treat obesity and prevent obesity-related diabetes. The hepatic triglyceride content in db/db mice was reduced by MPP in a dose-dependent manner, and MPP exerted a stronger effect than that of equal-dose polycan or platycodin administrated individually. These changes signified that MPP has anti-obesity effects and can restrict the process of diabetes. In addition, MPP at 200 mg/kg showed similar effects as those of 250 mg/kg in inhibiting the increase in liver triglyceride level.
Inhibitory effects of \(\beta\)-glucan and platycodin in obese mice

The stronger and more prominent anti-obesity properties of MPP compared to those of the individual administration of polycan or platycodin confirmed the synergistic anti-obesity mechanisms of the two compounds. However, more detailed mechanistic studies are required to further validate these findings.

CONCLUSION

Based on the results, MPP showed more dramatic anti-obesity effects compared to those of equal-dose polycan or platycodin administered individually. These synergistic effects were a result of the similar anti-obesity mechanisms of the individual components in inhibiting intestinal fat absorption. MPP is expected to be beneficial to patients suffering from obesity or related metabolic diseases such as diabetes. In the currently study, relatively favorable effects were also exerted by MPP at 50 mg/kg, the lowest dosage used. A dose of 50 mg/kg in mice is roughly equivalent to 4.17 mg/kg in humans (calculated as 1/12 based on the body surface area) and thus, MPP at 250 mg/day will show anti-obesity effects in a human being with a body weight of 60 kg.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DECLARATION STATEMENT

This study “Anti-obesity effects of MPP, a mixed formulation of platycodin and the \(\text{Aureobasidium}\) derived \(\beta\)-glucan polycan, in \(\text{db/db}\) mice” has not been published or submitted for publication elsewhere, and all the listed authors have read and approved the submitted manuscript. All authors have contributed significantly and all authors are in agreement with the content of the manuscript and agree to the conditions outlined in the copyright assignment form. In addition, this manuscript was revised by an English-speaking consultant.

REFERENCES


