Antiobesity Effect of Gynostemma pentaphyllum Extract (Actiponin): A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective: The effects of actiponin was investigated, a heat-processed Gynostemma pentaphyllum extract, on body weight, fat loss, and metabolic markers of Korean participants in a 12-week, randomized, double-blind, placebo-controlled clinical trial.

Design and Methods: Obese participants (BMI ≥ 25 kg m⁻² and WHR ≥ 0.90 for male or WHR ≥ 0.85 for female) who had not been diagnosed with any disease and met the inclusion criteria were recruited for this study. The 80 subjects were randomly divided into actiponin (n = 40, 450 mg day⁻¹) and placebo (n = 40) groups. Outcomes included measurement of efficacy (abdominal fat distribution, anthropometric parameters, and blood lipid profiles) and safety (adverse events, laboratory test results, electrocardiogram data, and vital signs).

Results: During 12-week of actiponin supplementation, total abdominal fat area, body weight, body fat mass, percent body fat, and BMI were significantly decreased (P < 0.044, P < 0.05, P < 0.0001, P < 0.0001, and P < 0.05, respectively) in the actiponin group compared to the placebo group. No clinically significant changes in any safety parameter were observed.

Conclusion: Our study revealed that actiponin is a potent antiobesity reagent that does not produce any significant adverse effects. These results suggest that actiponin supplementation may be effective for treating obese individuals.

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Introduction

According to the World Health Organization, overweight and obesity are defined as abnormal or excessive fat accumulation that increases the risk of type 2 diabetes, cardiovascular disease, and several types of cancers (1,2). A number of promising antiobesity drugs are developed each year with demonstrable efficacy in cell lines and animal models. However, only a few of these reagents enter and stay in the market because most are associated with serious side effects.

Only a few antiobesity drugs have been approved by the United States Food and Drug Administration for long-term use. One of these is orlistat (Xenical) that reduces intestinal fat absorption by inhibiting pancreatic lipase (3). Another, sibutramine (Meridia), decreases appetite by inhibiting the deactivation of the neurotransmitters in the brain but was withdrawn from the United States and Canadian markets in October 2010 because of increased risk of cardiovascular disease (4,5). Because of potential adverse side effects, it is recommended that anti-obesity drugs only be prescribed for

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treating obesity when the benefits of the treatment clearly outweigh its risks. Moreover, most of functional foods that are generally considered safe have not been scientifically validated for treating obesity and their effects are not significant (6,7).

AMP-activated protein kinase (AMPK) is an important intracellular sensor. This enzyme regulates whole-body and cellular energy balance in response to energy supply and demand. AMPK has been implicated in metabolic diseases such as obesity, type 2 diabetes, and dyslipidemia since its activation increases fat oxidation and glucose uptake while inhibiting fat and cholesterol synthesis (8-10). This kinase is a heterotrimeric enzyme comprised of a catalytic (α1 or α2) and two regulatory (β1 or β2 and γ1, γ2, or γ3) subunits (10). The activation of AMPK is mainly achieved via Thr172 phosphorylation in the α subunit by LKB1 kinase or Ca2+/calmodulin-dependent protein kinase kinase (8-11). Upon activation, AMPK phosphorylates downstream targets and inhibits ATP-consuming anabolic pathways including fatty acid and cholesterol synthesis. At the same time, catabolic pathways that produce ATP, such as glycolysis, fatty acid oxidation, and glucose uptake, are stimulated (9,12).

Gynostemma pentaphyllum (G. pentaphyllum) is a herbaceous vine of the family Cucurbitaceae (cucumber or gourd family) that is indigenous to and widely used in Asian countries including Korea, China, and Japan as traditional medicines or tea. Total extracts or saponins from this plant have been shown to exert a wide range of beneficial effects such as reducing cholesterol and blood glucose levels, strengthening immunity, and inhibiting cancer growth (13-16). These activities likely overlap with diverse downstream effects on AMPK activation (9). In vitro studies revealed that damulin A and B, two dammarane-type saponins purified from the leaves of G. pentaphyllum, are able to increase the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC) through which β-oxidation can be stimulated (17).

We recently reported that, the ability of a G. pentaphyllum leaf ethanol extract to activate AMPK is increased by autoclaving with increasing the levels of AMPK activators damulin A and B (18). In both cultured HepG2 and L6 myotube cells, this heat-processed G. pentaphyllum extract named “actiponin” dose-dependently increases the expression of key factors that regulate fat oxidation and adaptive thermogenesis while decreasing the expression of lipogenic transcription factors (18). Furthermore, oral administration of actiponin reduces body fat mass in ob/ob mice by stimulating AMPK and ACC phosphorylation in the soleus muscle (18). Although the antiobesity activities of G. pentaphyllum extract have been demonstrated in vitro and in vivo (17,18), the effects of this plant extract on humans are unclear. Therefore, we performed the first clinical trial for evaluating the antiobesity effects of actiponin on human subjects. The objective of the present study was to document the effect of 12-week actiponin supplementation on body fat composition (particularly abdominal fat) in obese Korean participants using a randomized, double-blind, placebo-controlled protocol.

Methods

Study design

This study was a 12-week, randomized, double-blind, placebo-controlled clinical trial followed by a 3-week screening period. Participants who responded to solicitation and met entry criteria during a telephone screening interview were scheduled for a baseline visit. Evaluation during baseline visit, physical examination, electrocardiogram, and screening blood parameter tests were conducted in all participants within 3-week from initial screening. A random number between 1 and 80 was generated for each subject and the enrolled participants were scheduled for their first visit and randomly assigned to either the actiponin (n = 40) or placebo (n = 40) group. Actiponin/placebo tablets were given to the participants for every 4-week.

During the 12-week intervention period, participants were asked to continue their usual diets and not to take any other functional foods or dietary supplements. Anthropometric, computed tomography (CT), biochemical parameters, vital signs, and nutrient intake of both groups were measured before and after the intervention period. During the run-in phase of the trial, all participants were instructed to maintain their normal diet and physical activity. Every fourth week the participants were asked to report for assessment of any adverse events or changes in training, lifestyle, or eating patterns; and to evaluate tablet compliance.

Participants

The study participants were recruited during 2009 at the Clinical Trial Center for Functional Foods of Chonbuk National University Hospital (Jeonju, Republic of Korea). A total of 105 participants agreed to participate in the study. Only individuals who were obese (BMI ≥ 25 kg m−2 and WHR ≥ 0.90 for male or WHR ≥ 0.85 for female) according to Asia-pacific guideline and had not been diagnosed with any other diseases were included in this study. To meet guidelines for evaluating the efficacy of functional foods from the Korea Food and Drug Administration (19), extremely obese participants (BMI ≥ 30 kg m−2) were not included in this investigation.

Altogether, 80 participants met the study criteria (age, 40.08 ± 10.60 years; weight, 74.58 ± 9.19 kg; BMI, 27.53 ± 1.22 kg m−2) and were randomly divided into two groups (n = 40 each) given either actiponin (450 mg day−1) or a placebo (450 mg day−1). Exclusion criteria for the study were as follows: (a) significant variation in weight (more 10%) in the past 3 months; (b) a history of cardiovascular disease including arrhythmia, heart failure, or myocardial infarction, and use of a pacemaker; (c) a history of conditions that could interfere with the test products or impede their absorption such as gastrointestinal disease (Crohn’s disease) or experienced surgery (caesarean or enteroccele surgery); (d) participation in another clinical trial within the past 2 months; (e) abnormal hepatic function; (f) a history of renal disease (e.g., acute or chronic renal failure and nephritic syndrome); (g) undergoing antipsychotic drug therapy within past the 2 months; (h) laboratory tests results as well as medical or psychological conditions that could interfere with successful participation in the study as judged by the investigators; (i) pregnancy or breast feeding; (j) a history of alcohol or substance abuse; and (k) allergies or hypersensitivity to any of the ingredients in the test products. All participants provided written informed consent before the investigation commenced. The study protocols were approved by the Functional Foods Institutional Review Board of Chonbuk National University Hospital.

Test supplement

Actiponin powder containing 1.1% (w/w) damulin A (Figure 1) was prepared by TG Biotech (Daegu, Republic of Korea) as previously described (18). Briefly, dried leaves of G. pentaphyllum underwent extraction with 50% ethanol. The extracts were then concentrated.
under reduced pressure, autoclaved at 121°C for 4 h, and dried using a spray dryer. Actiponin was administered to the study subjects as tablets (225 mg actiponin and 275 mg diluting agents in a 500 mg tablet). Traditionally, a daily dose of 3–9 g dried G. pentaphyllum leaves is recommended for human subjects as folk medicine or tea. We therefore decided to administer a daily dose of 450 mg day⁻¹ actiponin in our clinical trial based on the following calculation: daily dosage of G. pentaphyllum leaf (3 g day⁻¹) × (3 mg day⁻¹) × product yield (15%) of the extract powder (actiponin) from the dried plant = 450 mg day⁻¹. The energy contents (3.07 kcal), flavor, and appearance of the actiponin and placebo tablets were identical (Table 1).

All participants were instructed to take two tablets per day (one tablet after breakfast and one after dinner). Actiponin and placebo tablets were packaged in an indistinguishable manner and labeled with the study subject’s number. Participants were instructed to bring all remaining supplements at each visit and were withdrawn from the study if supplement consumption was <70% of the recommended dose.

Efficacy outcome measurements
Total 80 participants met the study criteria were asked to visit the clinic once in every 4 weeks (0th, 4th, 8th, and 12th wk of the study period) for a total of five clinical visits including the initial screening. During each visit, current supplementation use was reviewed and symptoms or side effects were recorded. During the screening visit, demographic and lifestyle information was collected (gender, age, alcohol consumption, and smoking habits). A medical history was taken and a urine pregnancy test was conducted.

The following parameters were assessed. Abdominal fat distribution was measured and analyzed using CT (Somatom Sensation 16 MDCT; Siemens, Germany) before and after the 12-week intervention period. Body weight, BMI, body fat mass, percent body fat, and lean body mass were measured using Inbody 3.0 (Biospace, Seoul, Korea) during each visit. Blood samples were collected after a minimum 12 h of fasting during the initial screening as well as on the 8th- and 12th-week of the intervention period to obtain lipid metabolism profiles [total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), free fatty acids, apolipoprotein AI (apoAI),and apolipoprotein B (apo B)]. Blood samples were analyzed with Hitachi 7600-110 analyzer (Hitachi High-Technologies, Japan) using standard methods in the biochemical laboratory of Chonbuk National University Hospital.

Safety and dietary evaluations
Safety of the extract was assessed by the following procedures. Electrocardiogram, hematology, and laboratory tests were conducted during the screening, 8th-, and 12th-week intervention periods (WBC, RBC, and platelet counts; hemoglobin, hematocrit, total protein, albumin, ALT, AST, BUN, and creatinine levels). Pulse rate and blood pressure were measured at every visit after a 5-min rest using IntelliVue MP70 (Philips, Netherlands). A personal report was also recorded at these times. We kept subjects maintaining their usual diet and activity, and all participants completed a dietary record at each visit to the clinic during the intervention period in order to evaluate their energy intake and diet quality. Dietary intake data were analyzed by the same dietitian using CAN-pro 3.0 software (The Korea Nutrition Society, Seoul, Korea).

Statistical analysis
Statistical analyses were performed using SAS software, version 9.2 (SAS Institute, USA). Data are presented as the mean ± standard error (S.E.) to detect a 0.3 kg (S.D. = 0. 60 kg) difference in body weight between groups with 80% power and a two-tailed z-value of 0.05 (20). The appropriate sample size of each group in the study was determined

| Component | Test tablet Content (mg) | Placebo tablet Content (mg) |
|-----------|-------------------------|-----------------------------|
| G. pentaphyllum extract powder | 225 | 350 |
| Maltodextrin | 187.5 | 96 |
| Microcrystalline cellulose | 74 | 24 |
| Silicon dioxide | 5 | 10 |
| Niacin | 2.5 | 1.5 |
| Magnesium Stearate | 6 | 5 |
| Total | 500 | 500 |
to be 32 participants, allowing a 20% drop-out rate. Intention-to-treat (ITT) analysis included all randomized participants who received at least one dose of the actiponin or placebo tablet. Efficacy and safety parameters of the ITT group were analyzed. A chi-square test was performed to determine differences in the frequencies of categorized baseline variables between the groups. A paired Student’s $t$ test was used to assess differences in continuous measures before and after the 12-week intervention period. A linear mixed-effects model was applied to repeated measures data for each continuous outcome. Data were adjusted according to gender. Fixed effects included treatment group, treatment visit, and interaction between treatment group and visit. A $P$ value <0.05 was considered statistically significant.

**Results**

**Participants**

Among the 105 participants screened, 25 were excluded due to anthropometric characteristics and/or laboratory test results consistent with the exclusion criteria. The remaining 80 participants fulfilled the study criteria and were divided equally into the actiponin and placebo groups. Four participants from the actiponin group and two from the placebo group withdrew from the study due to personal reasons. Ultimately, 74 participants (36 actiponin and 38 placebo group members) were able to finish the study (Figure 2).

**Dietary assessment**

No significant differences in dietary intake (calorie, carbohydrate, protein, and fat) were observed between the groups during the intervention period (Supporting Information Table S1).

**Participant characteristics**

General characteristics of the participants are shown in Table 2. There were no significant differences in baseline characteristics such as age, height, weight, and BMI between the actiponin and placebo groups.

**Abdominal fat**

Changes in abdominal fat (total abdominal fat, abdominal visceral fat, and abdominal subcutaneous fat) before and after the 12-week intervention period were analyzed (Table 3 and Figure 3). After 12-week of intervention, statistically significant differences were found in the total abdominal fat area (actiponin group: $-20.90 \pm 8.29$ cm$^2$, placebo group: $-2.87 \pm 3.73$ cm$^2$, $P = 0.044$). Areas of abdominal visceral (actiponin group: $-11.70 \pm 5.65$ cm$^2$, placebo group: $-2.92 \pm 2.21$ cm$^2$, $P = 0.146$) and subcutaneous fat (actiponin group: $-20.90 \pm 8.29$ cm$^2$, placebo group: $-2.87 \pm 3.73$ cm$^2$, $P = 0.044$). Areas of abdominal visceral (actiponin group: $-11.70 \pm 5.65$ cm$^2$, placebo group: $-2.92 \pm 2.21$ cm$^2$, $P = 0.146$) and subcutaneous fat (actiponin group: $-20.90 \pm 8.29$ cm$^2$, placebo group: $-2.87 \pm 3.73$ cm$^2$, $P = 0.044$).

**TABLE 2 Demographic characteristics of the study participants**

|                | Actiponin group $(n = 40)$ | Placebo group $(n = 40)$ | $P^{b}$ |
|----------------|-----------------------------|--------------------------|---------|
| Age (years)    | 40.10 ± 1.53                | 40.05 ± 1.83             | 0.983   |
| Height (cm)    | 165.78 ± 1.36               | 162.90 ± 1.41            | 0.146   |
| Weight (kg)    | 76.56 ± 1.36                | 73.41 ± 1.55             | 0.126   |
| BMI (kg m$^{-2}$) | 27.80 ± 0.19               | 27.55 ± 0.20             | 0.364   |
| Sex            |                             |                          |         |
| Male           | 22 (55)$^{c}$               | 10 (25)                  | 0.006$^{d}$ |
| Female         | 18 (45)                     | 30 (75)                  |         |

$^{a}$All values are presented as the mean ± S.E.
$^{b}$Derived from an independent student’s $t$ test. No significant differences between the two groups were observed.
$^{c}$Derived from a chi-square test. Statistically significant compared to the placebo group ($P < 0.05$).
Obesity

Significant differences were observed between the two groups. The C-reactive protein levels tended to decrease in the actiponin group, although no statistically significant differences were found between the actiponin and placebo groups. In addition, there were no differences in total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels between the two groups.

Changes in anthropometric parameters (weight, BMI, body fat mass, percent body fat, waist circumference, and hip circumference) during the 4-, 8-, and 12-week intervention periods are shown in Table 4. As a result, decrease in body weight ($P = 0.021$), BMI ($P = 0.029$), body fat mass ($P < 0.0001$), and percent body fat ($P < 0.0001$) in actiponin group was statistically significant. However, when analyzing the changes of anthropometric parameters before and after the 12-week intervention, decrease in waist circumference (actiponin group: $-2.49 \pm 0.35$ cm, placebo group: $-1.33 \pm 0.37$, $P = 0.029$) was also found in the actiponin group. Similarly, body weight, BMI, body fat mass and percent body fat were also significantly reduced in the actiponin group (Supporting Information Table S2).

Changes in lipid metabolism parameters (serum levels of total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, free fatty acids, apo AI, and apo B) during the 4-, 8-, and 12-week intervention periods are shown in Table 5. During 12-week of intervention periods, total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels tended to decrease in the actiponin group. However, no statistically significant differences were found between the actiponin and placebo groups. In addition, there were no differences in free fatty acid, apo AI, or apo B within or between the groups.

Safety analyses

Hematology test results and vital signs of both the actiponin and placebo groups (Tables S3 and S4) were within normal ranges, indicating that actiponin supplementation did not cause any adverse side effects.

Discussion

AMPK has been regarded as an emerging target for drugs designed to combat metabolic diseases including obesity and dyslipidemia (12,21). Activation of AMPK by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) inhibits adipogenesis by downregulating the expression of key transcription factors such as peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT/enhancer binding protein (CEBP)α as well as lipogenic factors including fatty acid binding protein 4 and lipoprotein lipase (22,23). Additionally, AMPK activation directly inactivates ACC through Ser79 phosphorylation, leading to decreased fat synthesis by reducing the production of malonyl-CoA from acetyl-CoA (24,25). Decreased malonyl-CoA concentrations stimulate mitochondrial fatty acid oxidation through increased mitochondrial fatty acid flux by inhibiting carnitine palmitoyl-CoA transferase-1 (12). In agreement with these findings, a thienopyridone family of an AMPK activator, A-769662 was found to improve diabetes, dyslipidemia, and fatty liver while substantially reducing body weight gain (21). Metformin, another AMPK activator, is widely prescribed to treat type 2 diabetes (26). Clinical studies have also demonstrated that metformin significantly decreases body weight (26,27), thus further strengthen the beneficial effects of AMPK activation for treating obesity.

We recently reported that damulin A and B isolated from G. pentaphyllum extract can increase the phosphorylation of AMPK and ACC in L6 myotube cells in dose-dependent manners, thereby increasing β-oxidation (17). Other study using ob/ob mice model also revealed that actiponin, a heat-processed G. pentaphyllum extract enriched with damulin A and B, reduces body weight, body fat mass, and total plasma cholesterol levels with activation of AMPK in their soleus muscle (18). In the current clinical study, participants who completed the 12-week of treatment with actiponin (450 mg/day) showed statistically significant reductions in abdominal fat (total abdominal fat area) and anthropometric parameters (weight, BMI, body fat mass, percent body fat, and waist circumference) without any significant changes in food intake, dietary, or lifestyle compared to the placebo group. These results were similar to our previous observations made in cultured cells and ob/ob mice both in vitro and in vivo (17,18). These observations suggest that AMPK activators, such as damulin A and B, in actiponin might be successfully delivered to peripheral tissues and exert anti-obesity effect in human subjects as similar to the result obtained in rodent model (18), although we did not analyze changes of AMPK activity in human tissues of actiponin and placebo groups.

During the 12-week intervention periods, some hematologic values (RBC, Hb, Hct, and platelet) in the actiponin group were slightly decreased. Thus, actiponin, a heat-processed G. pentaphyllum extract enriched with damulin A and B, reduces body weight, body fat mass, and total plasma cholesterol levels with activation of AMPK in their soleus muscle. These findings support the potential of actiponin in the treatment of obesity.

**Table 3: Abdominal fat area of the actiponin and the placebo groups measured at 0- and 12-week of the study**

| Parameters                     | Actiponin group (n = 40) | Placebo group (n = 40) |
|-------------------------------|--------------------------|------------------------|
|                               | 0-wk                     | 12-wk                  | Difference | P<sup>b</sup> | P<sup>c</sup> |
| Total abdominal fat (cm<sup>2</sup>) | 332.24 ± 10.65           | 313.15 ± 9.04          | −20.90 ± 8.29 | 0.016      |            |
|                               | 335.36 ± 7.24            | 333.05 ± 8.36          | −2.87 ± 3.73 | 0.447      | 0.044      |
| Visceral fat (cm<sup>2</sup>)  | 106.84 ± 6.61            | 95.97 ± 5.23           | −11.70 ± 5.65 | 0.046      |            |
|                               | 98.78 ± 4.85             | 96.68 ± 4.79           | −2.92 ± 2.21 | 0.195      | 0.146      |
| Subcutaneous fat (cm<sup>2</sup>) | 225.40 ± 8.55           | 215.38 ± 8.06          | −8.70 ± 3.54 | 0.019      |            |
|                               | 236.57 ± 9.08            | 236.37 ± 10.17         | 0.05 ± 3.85 | 0.990      | 0.092      |
| VSR<sup>d</sup>               | 0.51 ± 0.04              | 0.47 ± 0.04            | −0.04 ± 0.02 | 0.075      |            |
|                               | 0.47 ± 0.04              | 0.47 ± 0.04            | −0.00 ± 0.02 | 0.801      | 0.203      |

<sup>a</sup>All values are presented as the mean ± S.E.
<sup>b</sup>Derived from a paired t test. Statistically significant compared to the baseline ($P < 0.05$).
<sup>c</sup>Derived from the linear mixed-effects model adjusted for gender. Statistically significant compared to the placebo group ($P < 0.05$).
<sup>d</sup>VSR, visceral subcutaneous ratio.
decreased, although their values were still in the normal range and no statistically significant difference was found between the actiponin and placebo groups. Further research is required to explain whether this decrease of hematologic values in the actiponin group is caused by pseudoanemia as similar to the condition seen in sports anemia (28) or other unexpected adverse effects of actiponin.

(-)-Hydroxycitrate (HCA) is a major constituent of *Garcinia* (*G.* cambogia) fruit extract, a representative dietary supplement used for weight management due to its ability to inhibit ATP-citrate lyase (EC 4.1.3.8) that catalyzes cytosolic acetyl-CoA production from citrate in the initial fat biosynthesis pathway (29). However, numerous clinical studies have also provided evidences that *G. cambogia* extract (HCA) does not ameliorate obesity (30-34). In addition, potential testicular toxicity of *G. cambogia* extract at high doses was reported in an obese animal model (35). In these regards, it is likely that *G. cambogia* extract (HCA) may not be extremely effective for

**FIGURE 3** Representative CT-scan data at 0 and 12-week in a subject from actiponin or placebo group. Differences in abdominal visceral fat (AVF), abdominal subcutaneous fat (ASF), and total abdominal fat (TAF) areas at 0 and 12-week were measured. Each data is expressed as a mean ± S.D. of 5 CT-scan (L1–5) area values.
TABLE 4 Anthropometric parameters of the actipolin and the placebo groups measured at 0-, 4-, 8-, and 12-week of the study\textsuperscript{a}

| Parameters               | Actipolin group (n = 40) | Placebo group (n = 40) |
|--------------------------|--------------------------|------------------------|
|                          | 0-wk        | 4-wk        | 8-wk        | 12-wk       | P\textsuperscript{b} | 0-wk        | 4-wk        | 8-wk        | 12-wk       | P\textsuperscript{b} | P\textsuperscript{b} |
| Body weight (kg)         | 76.56 ± 8.31 | 76.26 ± 8.35 | 75.57 ± 8.18\textsuperscript{c} | 75.21 ± 8.20\textsuperscript{f} | <0.0001 | 73.41 ± 9.79 | 73.53 ± 9.79 | 73.33 ± 9.98 | 73.33 ± 10.17 | 0.187 | 0.021 |
| Body mass index (kg m\textsuperscript{-2}) | 27.80 ± 1.21 | 27.69 ± 1.17 | 27.44 ± 1.28\textsuperscript{c} | 27.31 ± 1.24\textsuperscript{f} | <0.0001 | 27.55 ± 1.27 | 27.61 ± 1.36 | 27.54 ± 1.45 | 27.55 ± 1.54 | 0.159 | 0.029 |
| Body fat mass (kg)       | 22.65 ± 3.12 | 22.28 ± 3.45 | 21.89 ± 3.35\textsuperscript{c} | 21.40 ± 3.62\textsuperscript{f} | <0.0001 | 23.04 ± 3.55 | 23.17 ± 3.61 | 23.15 ± 3.8 | 23.32 ± 3.82 | 0.147 | <0.0001 |
| Percent body fat (%)     | 29.95 ± 5.4  | 29.56 ± 5.73 | 29.31 ± 5.67\textsuperscript{c} | 28.79 ± 5.87\textsuperscript{f} | <0.0001 | 31.76 ± 5.39 | 31.91 ± 5.62 | 31.96 ± 5.68 | 32.13 ± 5.4 | 0.018 | <0.0001 |
| Waist circumference (cm) | 93.69 ± 4.85 | 92.26 ± 5.03\textsuperscript{c} | 91.65 ± 4.62\textsuperscript{c} | 91.07 ± 5.11\textsuperscript{f} | <0.0001 | 92.23 ± 4.03 | 91.57 ± 4.55 | 91.46 ± 4.25\textsuperscript{f} | 91.04 ± 4.50\textsuperscript{f} | 0.0001 | 0.060 |
| Hip circumference (cm)   | 99.85 ± 3.30 | 99.41 ± 3.28 | 99.21 ± 3.39\textsuperscript{c} | 98.85 ± 3.40\textsuperscript{f} | <0.0001 | 99.86 ± 4.28 | 99.81 ± 4.62 | 99.76 ± 4.59 | 99.68 ± 4.83 | 0.257 | 0.127 |

\textsuperscript{a}All values are presented as the mean ± S.E.
\textsuperscript{b}Derived from the linear mixed-effects model adjusted for gender. Statistically significant compared to the placebo group (P < 0.05).
\textsuperscript{c}Multiple comparison by Bonferroni correction. Statistically significant difference compared to baseline (0-wk).

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TABLE 5 Lipid profiles of the actipolin and placebo groups at 0-, 8- and 12-wk\textsuperscript{a} of the study

| Parameters                | Actipolin group (n = 40) | Placebo group (n = 40) |
|---------------------------|--------------------------|------------------------|
|                          | 0-wk        | 8-wk        | 12-wk       | P\textsuperscript{b} | 0-wk        | 8-wk        | 12-wk       | P\textsuperscript{b} | P\textsuperscript{b} |
| Total cholesterol (mg dL\textsuperscript{-1}) | 195.50 ± 5.03 | 185.97 ± 4.98 | 185.97 ± 29.9 | 0.055 | 190.40 ± 4.93 | 190.42 ± 5.89 | 190.42 ± 36.3 | 0.865 | 0.461 |
| Triglycerides (mg dL\textsuperscript{-1})     | 134.35 ± 9.60 | 117.28 ± 8.71 | 117.28 ± 52.26 | 0.336 | 136.40 ± 13.88 | 143.03 ± 12.44 | 143.03 ± 76.67 | 0.717 | 0.316 |
| HDL-C (mg dL\textsuperscript{-1})              | 44.43 ± 1.32 | 41.64 ± 1.01 | 41.64 ± 6.05 | 0.018 | 47.35 ± 1.71 | 44.37 ± 1.59 | 44.37 ± 9.78 | 0.052 | 0.946 |
| LDL-C (mg dL\textsuperscript{-1})              | 119.75 ± 4.22 | 111.61 ± 4.11 | 111.61 ± 24.67 | 0.060 | 113.03 ± 4.71 | 107.97 ± 4.88 | 107.97 ± 30.11 | 0.155 | 0.901 |
| FFA (uEq L\textsuperscript{-1})                | 582.48 ± 29.50 | 602.03 ± 31.42 | 602.03 ± 188.53 | 0.592 | 591.83 ± 33.47 | 608.45 ± 27.97 | 608.45 ± 172.39 | 0.223 | 0.197 |
| Apo A1 (g L\textsuperscript{-1})               | 1.41 ± 0.04 | 1.35 ± 0.03 | 1.35 ± 0.18 | 0.165 | 1.43 ± 0.04 | 1.43 ± 0.04 | 1.43 ± 0.25 | 0.158 | 0.202 |
| Apo B (g L\textsuperscript{-1})                | 0.89 ± 0.03 | 0.85 ± 0.03 | 0.85 ± 0.19 | 0.119 | 0.85 ± 0.03 | 0.86 ± 0.03 | 0.86 ± 0.19 | 0.846 | 0.692 |

\textsuperscript{a}All values are presented as the mean ± S.E.
\textsuperscript{b}Derived from the linear mixed-effects model adjusted for gender. No statistically significant differences between the two groups were observed. Multiple comparison by Bonferroni correction. Statistically significant difference compared to baseline (0-wk).
treat obesity and actiponin could be a potential alternative for controlling body fat accumulation and weight.

To the best of our knowledge, the current study is the first to demonstrate that *G. pentaphyllum* extract significantly reduces body weight and fat mass in obese human subjects. Nevertheless, our study has several limitations. First, we only surveyed the subjects’ food intake during the treatment period by reviewing food records. Therefore, the participants’ food intake and activity levels were not accurately controlled and impartially investigated. According to the European Medicines Agency, participants in randomized controlled trials evaluating weight control should adhere to an appropriate weight-reducing diet for a specified minimum period of time (36). All participants should also be given similar instructions, advice, and encouragement with regard to diet, behavior modification, and exercise. In the current study, total cholesterol and LDL-cholesterol levels are decreased after the 12-week intervention although no significant changes were observed between two groups. Megalli et al. (13) demonstrated that *G. pentaphyllum* alleviates hypercholesterolemia in animals and humans. Difference in the results from our current study and Megalli et al. may be due to the subjects’ lipid profiles. Generally, inclusion criteria for clinical trials focused on hyperlipidemia dictate that fasting serum LDL-cholesterol levels should fall between 160 and 250 mg dL\(^{-1}\) (37). However, the LDL-cholesterol baseline levels of participants in our study were within a normal range (\(~117.5\) mg dL\(^{-1}\)), which may be responsible for the absence of a significance difference between the actiponin and placebo groups.

Despite of the limitations mentioned above, data from our study suggest that actiponin is a safe and effective antiobesity agent. In these regards, actiponin is the newly reported edible AMPK activator, thus it will be of interest to investigate long-term effect of actiponin supplementation on improving severe obesity. Combined with life style modification or weight-reducing diet program, the antiobesity effect of actiponin will become more pronounced.

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