Research Article

Antiobesity Effect of a Novel Herbal Formulation LI85008F in High-Fat Diet-Induced Obese Mice

Hak Joo Choi, 1 Hwa Young Kim, 2 and Kyoung Sik Park 3

1 Food Test and Research Center, Daejeon University, Daejeon 34520, Republic of Korea
2 Ju Yeung NS Co., Ltd, Seoul 05854, Republic of Korea
3 Department of Biomedical Science, Cheongju University, Cheongju 28503, Republic of Korea

Correspondence should be addressed to Kyoung Sik Park; pks0322@hanmail.net

Received 18 November 2020; Revised 15 January 2021; Accepted 30 January 2021; Published 9 February 2021

1. Introduction

Obesity is a challenging health problem caused by the interaction of various genetic, dietary, lifestyle, and environmental factors [1]. The comorbidities associated with obesity include diabetes, hypertension, and cardiovascular diseases. Excess weight also has an enormous impact on the social, financial, and psychological status of obese individuals, which may contribute to the development of depression [2].

There are a variety of choices for obesity treatment, including lifestyle changes, exercise, dietary control, weight-loss surgery, and prescription weight-loss medications [3]. Medications can facilitate weight loss in obese persons. The first class of medications developed for weight control causes symptoms that mimic the sympathetic nervous system, resulting in the major side effect of this class of medications as high blood pressure. These medications also lower appetite and increase a sensation of fullness. Another class of antiobesity medications suppresses appetite by increasing the concentration of neurotransmitters at the synaptic cleft, where hunger and fullness are regulated by brain neurotransmitters such as dopamine, serotonin, and norepinephrine [4]. Unfortunately, these weight-loss medications are known to have significant adverse effects including headache, insomnia, irritability, nervousness, abdominal pain, and diarrhea [5, 6]. In the face of adverse effects of synthetic drugs, the potential of natural products with antiobesity activity is under exploration [7]. Natural products including crude extracts and isolated pure natural compounds may serve as antiobesity agents via a variety of mechanisms including suppression of adipose tissue growth, inhibition of adipocyte differentiation, stimulation of lipolysis, and induction of apoptosis in existing adipocytes, thereby reducing adipose tissue mass [8].

LI85008F is composed of the extracts of three medicinal plants, Moringa oleifera, Murraya koenigii, and Curcuma


2.1. Test Material. LI85008F, manufactured by Laila Nutraceuticals (Vijayawada, India) and provided by Ju Yeong NS Co., LTD. (Seoul, South Korea), is a novel herbal formulation composed of an aqueous ethanol extract of Moringa oleifera leaves, an aqueous alcohol extract of Murraya koenigii leaves, and Curcuma longa rhizome extract standardized to 95% total curcuminoids, mixed at a ratio of 6:3:1, respectively. Fresh plant raw materials were collected from local areas in Andhra Pradesh, India. Their voucher specimens are preserved in the Taxonomy Division at Laila Impex R & D Centre (Vijayawada, India). LI85008F is commercially available as Slendacor® or Slimvance®.

2.2. Animals and Experimental Diets. Four-week-old male C57BL/6 mice (Raonbio, Yongin, South Korea) were maintained under a 12-hour light-dark cycle in a temperature- and humidity-controlled room during the experimental period. The mice were given laboratory pelleted food and water ad libitum. Following one week of acclimatization, 20 mice were randomly divided based on their body weight into four groups (n = 5) as follows: the normal group received a chow diet (ENVIGO, Huntingdon, UK); the control group was fed with a 60 kcal% fat high-fat diet (HFD; Research Diet Inc, Burnswick, USA); and the two experimental groups were provided with a HFD supplemented with 250 and 500 mg/kg of LI85008F, respectively. Mice allocated to the experimental groups were orally administered with LI85008F for 13 weeks. The body weight and feed consumption of each mouse were measured once a week and daily throughout the study duration, respectively. All animal care and use protocols following NIH guidelines were approved by the Institutional Animal Care and Use Committee (IACUC) of Daejeon University (approval No. DJuARB2020-003).

2.3. Determination of Body Fat Mass. The body fat mass of mice was measured using dual-energy X-ray absorptiometry (DXA) with an apparatus for small animals (Inalyze, Medicors Inc., Seongnam, Korea). All scans were performed with the animals positioned prone and spread, with tape attached to each limb on the platform. The fat mass percentage was calculated as fat mass divided by total body weight and multiplied by 100.

2.4. Sampling Procedures. At the end of the 13-wk experimental phase, mice fasted overnight were anesthetized and sacrificed. The adipose tissues (epididymal and retroperitoneal fat pad) were harvested, weighed immediately, and stored at −80°C until further analysis.

2.5 Western Blot Analysis. Protein from each retroperitoneal fat sample was extracted with RIPA lysis and extraction buffer (Thermo Fisher Scientific, San Jose, USA) at 4°C. Then, the extracts were centrifuged at 13,200 r/min and 4°C for 30 min, and the supernatants of these tissues were used for western blotting analyses. The protein samples were separated on 10% SDS-PAGE gels and electrophoretically transferred onto PVDF membranes. The membranes were blocked at room temperature with 5% nonfat dry milk in TBST for 1 h and then incubated overnight at 4°C with the indicated primary antibodies (Cell Signaling Technology, Beverly, USA) as follows: AMP-activated protein kinase α (AMPKα; 1:100), acetyl-CoA carboxylase (ACC; 1:1,000), uncoupling protein 1 (UCP1; 1:1,000), CCAAT/enhancer binding protein α (C/EBPsα; 1:1,000), hormone-sensitive lipase (HSL; 1:1,000), peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) (1:1,000), peroxisome proliferator-activated receptor γ (PPARY; 1:1,000), and β-actin (1:2,000). After washing three times with TBST, the blots were hybridized with secondary antibodies (1:10,000) conjugated to horseradish peroxidase. The proteins were visualized by enhanced chemiluminescence (Pierce Biotechnology, Rockford, USA) and analyzed using a Chemi-doc (Bio-Rad Laboratories, Hercules, USA).

2.6 Statistical Analysis. The results were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS 21.0 (SPSS, Chicago, USA). The differences among groups were analyzed using one-way
3. Results and Discussion

3.1. Effect of LI85008F on Body Weight Gain. The body weight and food intake of each mouse were measured on a weekly and daily basis throughout the study duration, respectively. Distinct separation can be seen from week 3 onwards, where the control group which received high-fat diet (HFD) revealed a remarkable increase in body weight when compared with the normal group fed with a regular diet, implying the induction of obesity in animals in the control group. From week 6 onwards, daily administration of test article LI85008F at the dose of each of 250 and 500 mg/kg significantly prevented the body weight gain in both LI85008F-treated groups (control: 17.08 ± 2.53 vs. LI85008F 250: 10.78 ± 4.39, p < 0.05; LI85008F 500: 9.37 ± 2.95, p < 0.05) (Figure 1).

3.2. Effect of LI85008F on Whole-Body Fat Mass. To determine whether the observed decrease in body weight by treatment of LI85008F was due to a reduced accumulation of fat, we estimated whole-body fat mass by conducting dual-energy X-ray absorptiometry (DXA) with an apparatus for small animals (Figure 2).

The mice in the control group had a significantly higher whole-body fat mass at the end of the 13-wk intervention period than in mice fed with a regular diet. In contrast, the daily oral administration of the test material LI85008F at the dose of each of 250 and 500 mg/kg resulted in a significantly reduced whole-body fat mass compared to that of the control group (control: 16.20 ± 3.18 vs. LI85008F 250: 11.51 ± 2.16, p < 0.05; LI85008F 500: 11.19 ± 2.63, p < 0.05) (Figure 3(a)). In addition, the fat mass percentage was calculated as fat mass divided by total body weight. In parallel with whole-body fat mass, significant decreases in fat mass percentage were found in mice treated with LI85008F at the dose of 500 mg/kg compared with the control group (control: 36.90 ± 2.95 vs. LI85008F 500: 31.07 ± 6.20, p < 0.05) (Figure 3(b)).

3.3. Effect of LI85008F on Fat Mass of Adipose Tissue. After daily administration of test article LI85008F for 13 weeks, mice fed with HFD exhibited a significant increase in both the epididymal and retroperitoneal fat pad weights in comparison with the normal group. A significant decrease in epididymal fat pad weight was observed in all animals that received LI85008F at the dose of 250 mg/kg or 500 mg/kg for 13 weeks in a dose-dependent manner in comparison with the control group (control: 2.03 ± 0.16 vs. LI85008F 250: 1.70 ± 0.37, p < 0.05; LI85008F 500: 1.20 ± 0.21, p < 0.05) (Figure 4(a)). In retroperitoneal fat pad weight, there was a significant reduction in the group receiving 500 mg/kg of LI85008F compared to the control group (control: 0.68 ± 0.12 vs. LI85008F 500: 0.45 ± 0.08, p < 0.05) (Figure 4(b)).

3.4. Effect of LI85008F on the Expression of Adipogenic and Lipogenic Markers in Adipose Tissue. To further elucidate the mechanism underlying the blockade of fat accumulation by LI85008F supplementation, the expression of the key markers of adipogenesis C/EBPα and PPARγ in retroperitoneal fat pads was examined by western blot analysis. The adipogenesis process is tightly controlled by C/EBPα, which promotes the adipogenic pathway through activation of PPARγ [16]. As shown in Figure 5(a), the expression of these two adipogenic markers was suppressed in LI85008F-treated mice compared to HFD-fed control mice. Moreover, the production level of a key lipogenic enzyme, ACC, which is regulated by C/EBPα or PPARγ [17], was measured. The intake of LI85008F for 13 weeks significantly decreased the expression level of ACC in a dose-dependent manner (Figure 5(b)).

3.5. Effect of LI85008F on the Expression of Thermogenic and Lipolytic Markers in Adipose Tissue. It has been reported that phosphorylation of AMPKα triggers the induction of gene expression of thermogenesis markers PGC1α and UCP1 [18]. Therefore, we evaluated the effect of LI85008F supplementation on the expression level of AMPKα, PGC1α, and UCP1 in retroperitoneal fat pads of HFD-fed mice. The oral administration of LI85008F for 13 weeks significantly up-regulated the production level of AMPKα, PGC1α, and UCP1 in a dose-dependent manner (Figure 6(a)). In addition, the alterations in the production level of lipase HSL involved in lipolysis by LI85008F intake were also investigated. LI85008F-treated mice showed higher protein expression of HSL than HFD-fed control mice (Figure 6(b)), implying that LI85008F can be acting on the lipolytic pathway in HFD-induced obese mice.

Obesity can be defined as an increase in body weight beyond the limits of physical requirements, which results from an excessive accumulation of fat. Interestingly, in a previous clinical study [15], LI85008F supplementation for 8 weeks resulted in a significant body weight reduction in overweight or obese adults compared to those in the placebo group (p < 0.001), which is parallel with the suppressive effect of LI85008F on HFD-induced body weight gain in mice observed in this study (Figure 1). Furthermore, we have shown that LI85008F treatment significantly reduces whole-body fat mass and fat mass percentage using DXA (Figures 3(a) and 3(b)), most likely through the inhibition of fat accumulation in adipose tissue including epididymal and retroperitoneal fat pads (Figures 4(a) and 4(b)).

The current study demonstrated that LI85008F suppressed adipogenesis and decreased lipid accumulation by inhibiting the expression of genes involved in adipogenesis and lipogenesis in adipose tissue (Figures 5(a) and 5(b)), which is consistent with the previous report [9] that LI85008F deactivates the key adipogenic transcription factors C/EBPα and PPARγ in 3T3-L1 adipocytes.

As a master energy sensor, AMPK plays a key role in integrating hormones, nutrients, and stress signals to maintain whole-body energy homeostasis [19]. AMPK is activated by allosteric stimulation in response to an...
Figure 1: The effect of LI85008F on weekly body weight (a) and body weight gain (b) in mice for 13 weeks of treatment. Values are expressed as means ± SD (n = 5). The data were analyzed using ANOVA followed by Duncan’s test. "#" and "∗" indicate the significant difference (p < 0.05) between normal vs. control group and control vs. LI85008F supplemented group, respectively.

Figure 2: Representative images from DXA scans for mice in each group after 13 weeks of treatment.

Figure 3: The effect of LI85008F on whole-body fat mass (a) and fat mass percentage (b) in mice for 13 weeks of treatment. Values are expressed as means ± SD (n = 5). The data were analyzed using ANOVA followed by Duncan’s test. "#" and "∗" indicate the significant difference (p < 0.05) between normal vs. control group and control vs. LI85008F supplemented group, respectively.
Figure 4: The effect of LI85008F on epididymal (a) and retroperitoneal fat pad weight (b) in mice for 13 weeks of treatment. Values are expressed as means ± SD (n = 5). The data were analyzed using ANOVA followed by Duncan’s test. # and * indicate the significant difference (p < 0.05) between normal vs. control group and control vs. LI85008F supplemented group, respectively.

Figure 5: Continued.
Figure 5: The effect of LI85008F on the expression level of adipogenic (a) and lipogenic markers (b) in retroperitoneal fat pads of mice for 13 weeks of treatment. Specific bands were quantified and are presented as bar graphs. Values are expressed as means ± SD (n = 5). The data were analyzed using ANOVA followed by Duncan’s test. # and * indicate the significant difference (p < 0.05) between normal vs. control group and control vs. LI85008F supplemented group, respectively.

Figure 6: Continued.
increased AMP/ATP ratio or mitochondria activity changes [20]. In skeletal and cardiac muscle, AMPK activation leads to the induction of gene expression of thermogenesis markers PGC1α and UCP1 [18]. In the present study, we demonstrated that LI85008F supplementation elevated AMPKα production level and induced the subsequent activation of PGC1α and UCP1 expression, leading to higher energy expenditure and lower body weight (Figure 6(a)).

HSL, the major enzyme catabolizing triglycerides (TGs), induces lipolysis after phosphorylation of serine residues, leading to translocation of itself to the lipid droplets and markedly enhancing lipolysis [21]. As shown in Figure 6(b), LI85008F treatment to HFD-induced obese mice led to upregulate the expression of HSL involved in lipolysis pathways.

Based on these findings, we propose that LI85008F exerts antiobesity activities through a variety of mechanisms including suppression of adipogenesis, inhibition of lipogenesis, stimulation of lipolysis, and upregulation of thermogenesis and energy expenditure in adipose tissue, thereby reducing adipose tissue mass, whole-body fat mass, and body weight gain in HFD-induced obese mice.

### 4. Conclusions

The present study demonstrated that LI85008F administration for 13 weeks significantly suppressed HFD-induced body weight gain and reduced whole-body fat mass, as well as fat weight of adipose tissue including epididymal and retroperitoneal fat in HFD-induced obese mice. These findings may be associated with significant decreases in the expression level of adipogenesis and lipogenesis proteins including C/EBPα, PPARγ, and ACC, as well as marked upregulation in the production level of thermogenesis and lipolysis markers such as AMPKα, PGC1α, UCP1, and HSL induced by LI85008F treatment in HFD-induced obese mice.

### Data Availability

All data generated or analyzed during this study are available and included in this article.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

### Acknowledgments

The current study was financially supported by Ju Yeong NS Co., Ltd. (Seoul, South Korea).

### References

[1] R. N. Dickerson, “Metabolic support challenges with obesity during critical illness,” *Nutrition*, vol. 57, pp. 24–31, 2019.

[2] M. S. Faith, M. Butryn, T. A. Wadden, A. Fabricatore, A. M. Nguyen, and S. B. Heymsfield, “Evidence for prospective associations among depression and obesity in population-based studies,” *Obesity Reviews*, vol. 12, no. 5, pp. e438–e453, 2011.
[3] B. M. Wolfe, E. Kvach, and R. H. Eckel, "Treatment of obesity," *Circulation Research*, vol. 118, no. 11, pp. 1844–1855, 2016.

[4] D. H. Bessesen and L. F. Van Gaal, "Progress and challenges in anti-obesity pharmacotherapy," *The Lancet Diabetes & Endocrinology*, vol. 6, no. 3, pp. 237–248, 2018.

[5] G. Srivastava and C. Apovian, "Future pharmacotherapy for obesity: new anti-obesity drugs on the horizon," *Current Obesity Reports*, vol. 7, no. 2, pp. 147–161, 2018.

[6] A. A. Coulter, C. J. Rebello, and F. L. Greenway, "Centrally acting agents for obesity: past, present, and future," *Drugs*, vol. 78, no. 11, pp. 1113–1132, 2018.

[7] C. Torres-Fuentes, H. Schellekens, T. G. Dinan, and J. F. Cryan, "A natural solution for obesity: bioactives for the prevention and treatment of weight gain. A review," *Nutritional Neuroscience*, vol. 18, no. 2, pp. 49–65, 2015.

[8] C. Fu, Y. Jiang, J. Guo, and Z. Su, "Natural products with anti-obesity effects and different mechanisms of action," *Journal of Agricultural and Food Chemistry*, vol. 64, no. 51, pp. 9571–9585, 2016.

[9] K. Sengupta, T. Golakoti, V. R. Chirravuri, and A. K. Marasetti, "An herbal formula LI85008F inhibits lipogenesis in 3T3-L1 adipocytes," *Food and Nutrition Sciences*, vol. 2, no. 8, 2011.

[10] F. Anwar, S. Latif, M. Ashraf, and A. H. Gilani, "*Moringa oleifera*: a food plant with multiple medicinal uses," *Phytotherapy Research*, vol. 21, no. 1, pp. 17–25, 2007.

[11] C. Waterman, P. Rojas-Silva, T. B. Tumer et al., "Isothiocyanate-rich *Moringa oleifera* extract reduces weight gain, insulin resistance, and hepatic gluconeogenesis in mice," *Molecular Nutrition & Food Research*, vol. 59, no. 6, pp. 1013–1024, 2015.

[12] S. Yadav, V. Vats, Y. Dhunnoo, and J. K. Grover, "Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats," *Journal of Ethnopharmacology*, vol. 82, no. 2-3, pp. 111–116, 2002.

[13] B. B. Aggarwal, S. C. Gupta, and B. Sung, "Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers," *British Journal of Pharmacology*, vol. 169, no. 8, pp. 1672–1692, 2013.

[14] S. C. Gupta, G. Kismali, and B. B. Aggarwal, "Curcumin, a component of turmeric: from farm to pharmacy," *Biofactors*, vol. 39, no. 1, pp. 2–13, 2013.

[15] K. Sengupta, A. T. Mishra, M. K. Rao et al., "Efficacy and tolerability of a novel herbal formulation for weight management in obese subjects: a randomized double blind placebo controlled clinical study," *Lipids in Health and Disease*, vol. 11, p. 122, 2012.

[16] U. A. White and J. M. Stephens, "Transcriptional factors that promote formation of white adipose tissue," *Molecular and Cellular Endocrinology*, vol. 318, no. 1-2, pp. 10–14, 2010.

[17] F. Guebre-Egziabher, P. M. Alix, L. Koppe et al., "Ectopic lipid accumulation: a potential cause for metabolic disturbances and a contributor to the alteration of kidney function," *Biochimie*, vol. 95, no. 11, pp. 1971–1979, 2013.

[18] M. López, R. Nogueiras, M. Tena-Sempere, and C. Diéguez, "Hypothalamic AMPK: a canonical regulator of whole-body energy balance," *Nature Reviews Endocrinology*, vol. 12, no. 7, pp. 421–432, 2016.

[19] D. G. Hardie, F. A. Ross, and S. A. Hawley, "AMPK: a nutrient and energy sensor that maintains energy homeostasis," *Nature Reviews Molecular Cell Biology*, vol. 13, no. 4, pp. 251–262, 2012.