Ameliorating Effect of *Mycoleptodonoides aitchisonii* on High-fat Diet-induced Obese Mice

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ABSTRACT: The present study investigated the anti-obesity effects of *Mycoleptodonoides aitchisonii* (MA) in mice fed a high-fat (HF) diet. Two groups were fed either a normal control diet or an HF (45% kcal fat) diet for 12 weeks and three groups were fed an HF diet supplemented with powdered MA (MAP, 1%, 3%, and 5%) for 12 weeks. The anti-obesity effects of MAP supplementation on body weight, fat mass development, and lipid-related markers were assessed. Consumption of an HF diet resulted in increased body weight, serum lipids, relative adipose tissues weight, and liver fat accumulation. However, administration of MAP significantly decreased body weight gain, food intake, food efficiency ratio, hepatic cholesterol level, and adipose tissue weight in a dose-dependent manner. In addition, treatment with MAP significantly reduced the occurrence of fatty liver deposits and steatosis, and inhibited an HF diet-induced increase in adipocyte size. These results suggest that dietary supplementation with MAP exerts anti-obesity effects and indicate that MAP could be used as a functional food to control obesity.

Keywords: *Mycoleptodonoides aitchisonii*, obesity, high-fat diet, adipocyte

INTRODUCTION

Chronic intake of high-fat (HF) diets is strongly associated with the development of a number of metabolic disturbances, including obesity, type-2 diabetes, and cardiovascular disease (1). Obesity is a complex metabolic disorder that is characterized by increases in adipocyte number and size, and is induced by an imbalance between calorie intake and metabolic expenditure (2).

Two types of anti-obesity drugs, orlistat and sibutramine, have been approved by the US Food and Drug Administration for long-term weight control. However, both drugs have side effects, including increased blood pressure, dry mouth, constipation, headache, and insomnia (3,4). Dissatisfaction with the high costs and potentially hazardous side-effects of these drugs has promoted researchers to explore the potential of natural products for treating obesity; such products may provide excellent alternative strategies for developing effective and safe anti-obesity drugs (5,6).

Natural products, including dietary phytochemicals have aroused considerable interest in recent years as potential therapeutic agents to counteract obesity (7,8). Currently, dietary phytochemicals are being studied as potential anti-obesity agents because they may suppress the growth of adipose tissue, inhibit the differentiation of preadipocytes, stimulate lipolysis, and induce apoptosis in existing adipocytes, thereby reducing adipose tissue mass (9).

In recent years, enhanced interest in human health, nutrition, and disease prevention has increased consumer demand for functional foods. Mushrooms are important nutritional and therapeutic items throughout the world. Mushrooms contain substances, mainly polysaccharides, polysaccharopeptides, and polysaccharide proteins, that have well-known antitumor, antiviral, antibacterial, immunomodulatory, and lipid-lowering effects (10-12).

Recently, the cultivation of *Mycoleptodonoides aitchisonii* (M. aitchisonii, MA), a number of the Climacodontaceae family, began in Korea. MA is an edible mushroom commonly used as a natural medicine. Previous studies have demonstrated that the methanol extract of MA inhibits inflammation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) and lowers blood pressure in spontaneously hypertensive rats (13,14). It was also reported that MA increases the presence of nerve growth factor and catecholamines in rat brains (15). In addition, dietary MA...
intake could be a practical approach for the prevention of oxidative stress-related disease and carcinogenesis because MA contains anti-oxidative and phase II enzymes (16). However, no studies have been performed to evaluate the anti-obesity properties of MA. In this manuscript, we demonstrate that MA can also be used as a novel anti-obesity food in obese mice fed an HF diet.

MATERIALS AND METHODS

*M. aitchisonii* powder

The MA used in this study was successful products of artificial cultivation by the Jeonnam Forest Resource Research Institute (Naju, Korea) (17). The fresh fruiting bodies of MA were freeze-dried and then ground into a powder.

Animal experiments

Six-week old male C57BL/6 mice were obtained from DaHanBioLink Co., Ltd. (Eumseong, Korea). Mice were individually housed in stainless steel cages in a room maintained at 22±2°C with 50~55% relative humidity and a 12-h light/dark cycle (beginning at 08:00). All mice were fed a pelleted chow diet for 1 week. They were then randomly divided into 5 dietary groups (n=6). The mice in the first group were fed a normal control (NC) diet. The mice in the second group were fed a high-fat (HF, 45% kcal fat) diet. The mice in the remaining groups were fed an HF diet supplemented with 1% powdered MA (MAP), 3% MAP, or 5% MAP. The composition of the experimental diet was based on the AIN-93 semisynthetic diet (Table 1). The mice were allowed free access to food and water during the 12-week experimental period. Food intake was recorded twice a week and weight gain were measured weekly. At the end of the experimental period, all animals were fasted for at least 12 h and then anesthetized by carbon dioxide. Blood was collected using a polyethylene tube with no heparin and centrifuged at 1,000 g for 15 min at 4°C to obtain the serum. The serum was stored at −70°C until analysis. The livers were surgically excised and rinsed with physiological saline. The adipose tissues (epididymal, retroperitoneal, and subcutaneous) were surgically excised and immediately weighed. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Chungnam National University.

| Table 1. Composition of diets used in the present study |
|--------------------------------------------------------|
| **Contents (g/kg)** | **NC** | **HF** | **MAP** |
|--------------------|--------|--------|---------|
| Casein             | 200    | 200    | 200     |
| Corn starch        | 457    | 260    | 250     |
| Sucrose            | 200    | 200    | 200     |
| Soybean oil        | 43     | 25     | 25      |
| Lard               | 0      | 215    | 215     |
| Cellulose          | 50     | 50     | 50      |
| Choline bitartrate | 2      | 2      | 2       |
| L-cystine          | 3      | 3      | 3       |
| Mineral mix        | 35     | 35     | 35      |
| Vitamin mix        | 10     | 10     | 10      |
| Powdered MAP       | 10     | 30     | 50      |
| Total (g)          | 1,000  | 1,000  | 1,000   |
| Calories from fat (%) | 10   | 45    | 45      |

NC, normal control diet; HF, high-fat diet; MAP, high-fat diet with powdered *M. aitchisonii*.

Hepatic analytical procedure

Livers were homogenized in a glass Teflon homogenizer containing 10 volumes of homogenization buffer (12.5 mM sodium phosphate buffer pH 7.0, 400 mM NaCl) and then centrifuged at 1,000 g for 10 min at 4°C. Hepatic lipid extractions were measured according to the method of Folch et al. (18). Briefly, hepatic lipids were extracted by chloroform and methanol (2:1, v/v). The extract was dried under N₂ gas. Hepatic malondialdehyde (MDA) and cholesterol assays were performed on the supernatant fraction of each homogenate. MDA content was measured using the thioarbituric acid reactive substances (TBARS) assay of Uchiyama and Mihara (19). Hepatic cholesterol was determined using a commercial kit (Asan Pharmaceutical, Seoul, Korea).

Serum lipids

The serum concentrations of total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL) were determined using a commercial kit (Asan Pharmaceutical).

Histopathology

Liver and epididymal tissues were preserved in a 10% buffered formaldehyde solution. The preserved tissues were processed into paraffin blocks, sectioned at a nominal 5 μm, mounted on glass microscope slides and stained with hematoxylin and eosin. Epididymal adipose cell size was determined by measuring the diameters of 50 randomly selected adipose cells from light microscope images (100× magnification) of epididymal adipose tissue sections.

Statistical analysis

All results are expressed as means±SD. Data were analyzed by one-way ANOVA followed by Duncan’s test for multiple comparisons. A difference of *P*<0.05 was statistically significant.

RESULTS AND DISCUSSION

In order to study the anti-obesity effect of MA, we fed an
HF diet to C57BL/6 mice for 12 weeks. The change in body weight and food intake during the experimental feeding is shown in Table 2. The body weight of the HF groups was significantly greater (21%) than that of the NC group. However, supplementation of an HF diet with 1\textasciitilde{}5% MAP significantly suppressed body weight gain by 13.5\textasciitilde{}34.45% in a dose-dependent manner. Food intake and energy intake were higher in the HF group than in the NC group, but were significantly reduced in MAP-treated groups compared to the HF group ($P < 0.001$). Despite a higher energy intake, food efficiency ratio (FER) of the 3% and 5% MAP groups was significantly decreased compared to the NC group ($P < 0.001$). These results indicate that MA mushrooms contain a food intake-suppressing substance. The present results are supported by a previous report that rats did not consume diets containing 5% lyophilized fruiting bodies of several mushrooms (20). Previous report indicate that lectin isolated from *Pleurotus ostreatus* has the most potent a food intake-suppression activity. Moreover, consumption of viscous dietary fibers has been shown to increase sensations of satiety and decrease energy intake at the following meal (21,22). Mushroom cell walls contain a mixture of fibrillar and matrix components, which include chitin and polysaccharides such as $\beta$-glucans and mannans, respectively (23).

The white adipose tissue weight was higher in the HF group than it was for in the NC group (Fig. 1). Conversely, when compared to the HF group, the epididymal, retroperitoneal, and subcutaneous fat weights were significantly decreased by 16.5%, 39.1%, and 60.7%, respectively, in the 3% MAP group and significantly decreased by 24.8%, 46.2%, and 67.6%, respectively, in the 5% MAP group.

HF diet ingestion induced fat accumulation in the liver and increased hepatic cholesterol levels. As shown in Table 3, the liver lipid level was increased by 32.8% in the HF group compared to the NC group. However, MAP supplementation significantly reduced hepatic lipid level, by more than 30% compared to the HF group. Notably, hepatic fat accumulation in the MAP treated group was much lower than it was in the NC group. These results suggest that MAP was responsible for sup-

### Table 2. Effect of *M. aitchisonii* on body weight gain, food intake, and food efficacy ratio in mice fed a high-fat diet

| Group$^1$ | Initial weight (g) | Final weight (g) | Food intake (g/d) | Energy intake (kcal/d) | FER$^2$ (%) |
|-----------|--------------------|-----------------|-------------------|------------------------|-------------|
| NC        | 24.75±1.17         | 32.92±3.71$^{(k, l)}$ | 2.80±0.29$^b$     | 10.66±1.12$^c$        | 3.51±0.37$^c$ |
| HF        | 23.45±0.90         | 40.17±3.41$^a$   | 3.11±0.34$^a$     | 14.91±1.64$^a$        | 6.2±0.74$^a$ |
| 1% MAP    | 23.33±1.21         | 34.42±3.38$^b$   | 2.61±0.30$^c$     | 12.40±1.42$^b$        | 5.16±0.52$^a$ |
| 3% MAP    | 23.58±0.49         | 29.50±2.14$^d$   | 2.44±0.34$^d$     | 11.42±1.50$^c$        | 2.92±0.41$^d$ |
| 5% MAP    | 23.42±0.74         | 27.50±1.59$^d$   | 2.33±0.31$^d$     | 10.73±1.41$^c$        | 2.11±0.24$^d$ |

Values are expressed as means±SD (n=6).

$^1$NC, normal control diet; HF, high-fat diet; MAP, high-fat diet with powdered *M. aitchisonii*.

$^2$FER (food efficiency ratio)=(body weight gain/food intake)×100.

$^3$Different superscripts in the same column indicate significant differences between groups ($P<0.05$).

### Table 3. Effect of *M. aitchisonii* on lipid content, cholesterol, and liver TBARS levels in mice fed a high-fat diet

| Group$^1$ | Lipid (mg/g) | Cholesterol (mg/dL) | TBARS$^2$ (mmole/mg protein) |
|-----------|--------------|---------------------|-------------------------------|
| NC        | 142.78±2.64$^a$ | 93.50±4.38$^a$     | 2.89±0.63                     |
| HF        | 189.62±39.87$^a$ | 139.36±16.40$^a$  | 3.27±0.63                     |
| 1% MAP    | 123.15±16.10$^b$ | 91.30±11.70$^c$   | 2.98±0.35                     |
| 3% MAP    | 126.72±39.63$^a$ | 80.00±12.86$^c$   | 2.67±0.68                     |
| 5% MAP    | 124.88±21.18$^c$ | 70.00±18.04$^d$   | 2.53±0.19                     |

Values are expressed as means±SD (n=6).

$^1$NC, normal control diet; HF, high-fat diet; MAP, high-fat diet with powdered *M. aitchisonii*.

$^2$TBARS, thiobarbituric acid reactive substances.

$^3$Different superscripts in the same column indicate significant differences between groups ($P<0.05$).
pression of liver lipid accumulation. The hepatic cholesterol level was increased by 49.1% in the HF group compared to the NC group. 1%, 3%, and 5% MAP treatments dramatically reduced hepatic cholesterol level by 34.5%, 42.6%, and 49.8%, respectively, compared to the HF group. The hepatic TBARS content was higher in the HF group than in the NC group, but not significantly. Previous reports indicate that additional fat from excess dietary lipids translocates to non-adipose tissues, such as liver, skeletal muscle, heart, and pancreatic β-cell, where it exerts toxic effects and triggers organ dysfunction (24). This lipotoxicity may also be exacerbated by impaired β-oxidation of fat within tissues (25).

Serum lipid levels are shown in Table 4. Consumption of an HF diet for 12 weeks resulted in a non-significant increase in serum lipids levels compared to the NC group. However, MAP supplementation caused significant decreases in serum TG and TC levels, compared to the HF group. However, no significant difference in serum HDL concentrations were observed among groups. Edible mushrooms and their constitutive active compounds have been described as having beneficial effects on hyperglycemia and hypercholesterolemia (26,27). Previous studies have suggested that continuous treatment with mushrooms improves the lipid profiles of human and rodents (28,29).

Histopathological analysis revealed the occurrence of macrovesicular steatosis in the hepatocytes of the HF group (Fig. 2). Animal studies indicate that an HF diet induces fatty liver and steatosis, both of which are characterized by an excess accumulation of lipids, primarily triacylglycerol, within hepatocytes (30,31). Triglyceride and free fatty acid accumulation in the liver is associated with non-alcoholic steatohepatitis (NASH), which is characterized by an inflammatory response with evidence of hepatocyte damage and fibrosis that can progress to cirrhosis (32). In the HF group of the present study, single large lipid droplets displaced the nuclei of

Table 4. Effect of *M. aitchisonii* on serum lipid levels in mice fed a high-fat diet

| Group  | TC   | TG    | HDL  |
|--------|------|-------|------|
| NC     | 174.4±20.60a,b   | 82.7±4.05a   | 89.5±11.70   |
| HF     | 197.7±23.01a     | 88.7±18.43a   | 92.7±8.35   |
| 1% MAP | 145.0±22.33c     | 77.2±15.81a   | 88.5±7.61   |
| 3% MAP | 154.2±15.31bc    | 51.2±9.22b    | 89.3±6.20   |
| 5% MAP | 135.9±11.41c     | 60.4±4.76b    | 82.2±6.53   |

Values are expressed as means±SD (n=6).

1) NC, normal control diet; HF, high-fat diet; MAP, high-fat diet with powdered *M. aitchisonii*.

2) TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol.

3) Different superscripts in the same column indicate significant differences between the groups (P<0.05).

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**Fig. 2.** Hematoxylin and eosin-stained photomicrographs of the liver. Fat accumulation in the form of large fat droplets, indicated by the arrowhead, is present in the livers of mice fed a high-fat diet. NC, normal control diet; HF, high-fat diet; MAP, high-fat diet with powdered *M. aitchisonii*. Bar=300 μm.
many hepatocytes. In addition, ballooning degeneration of hepatocytes was apparent, with conspicuous swelling of the cells and cytoplasmic vacuolation. Treatment with MAP significantly reduced the occurrence of fatty liver deposits and macrovesicular steatosis compared to the HF group.

Representative images of epididymal adipose tissues and a graphical representation of epididymal adipocyte size are shown in Fig. 3 and Fig. 4, respectively. The epididymal adipose cell size of the HF group greatly increased to 146.56% of the epididymal adipose cell size of the NC group. However, 1%, 3%, and 5% MAP supplementation significantly decreased epididymal adipose cell size by 22.6%, 18.7%, and 53.61%, respectively, compared to the HF group. Moreover, the adipocytes of the 5% MAP group were the smallest among all treatment groups. From these results, it appears that MAP inhibits the increase of adipocyte size observed in mice fed an HF diet. Previous work indicates that an HF diet is associated with excessive growth of adipose tissue in terms of both cell number and cell size, which consequently induces fat accumulation (33).

In conclusion, the present study is the first to evaluate the anti-obesity function of MAP in mice fed an HF diet. Supplementation with 1% to 5% MAP dramatically improved many parameters of HF diet-induced obesity, leading to better weight control, suppression of hepatosteatosis, and amelioration of the serum lipid profile. Therefore, MAP appears to exert an anti-obesity effect by inhibiting fat digestion.

ACKNOWLEDGMENTS

This study was supported by Jeollanam-do Forest Resource Research Institute, Republic of Korea.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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