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Article

Anti-obesity Effect of *Monascus pilosus* Mycelial Extract in High Fat Diet-induced Obese Rats

Sang-Il Lee[1], Jae-Won Kim[2], Ye-Kyung Lee[3], Seung Hwan Yang[3], In-ae Lee[3], Joo-Won Suh[3]*, and Soon-Dong Kim[3]*

[1]*Departent of Food, Nutrition and Culinary Arts, Keimyung College, Daegu 704-703, Republic of Korea*
[2]*Faculty of Food Science and Industrial Technology, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea*
[3]*Division of Bioscience and Bioinformatics, College of Natural Science, Myongji University, Cheoin-gu, Yongin, Gyeonggi-Do 449-728, Republic of Korea.*

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This study was carried out to investigate the dietary effects of *Monascus pilosus* mycelial extract on obesity in high-fat with cholesterol-induced obese rat models. It was observed that *M. pilosus* mycelial extract contains 25.85±1.98 mg% of total monacolin K without citrinin by high-performance liquid chromatography (HPLC). The rats were randomly divided into 2 groups; normal control and a high-fat with cholesterol diet group. The high-fat with cholesterol diet group was fed a 5L79 diet with an added 15% lard and 1% cholesterol supplemented diet for 3 weeks for induction of obesity. After induction, obesity was confirmed by checking obesity indexes, the animals were divided into 4 groups (n=5); first, the normal control (NC), and then taken from the obese model of rats, a high-fat with cholesterol diet obesity control group (HF), 0.5% *M. pilosus* mycelial extract supplemented high-fat with cholesterol diet group (MPMs), 2% conjugated linoleic acid supplemented high-fat with cholesterol diet group (CLA) for 7 weeks. Body weight gains, obesity indexes, and body fat contents in the experimental groups (MPMs and CLA) were decreased compared with HF group. Feed Efficiency Ratio (FER) in MPMs was significantly lower than that of HF without change of feed intake. These results suggested that the anti-obesity effects of the *M. pilosus* mycelial extracts (MPMs) could prevent obesity induced by high-fat with cholesterol diet possibly via inhibition of lipid absorption.

Key words: conjugated linoleic acid, high-fat with cholesterol diet, *Monascus pilosus* mycelial extract, obesity, reactive oxygen species scavenging enzyme activities

Introduction

Worldwide, the dietary lifestyle has changed to the western-style diet containing a high level of fat and calories [Won *et al*., 2007]. It is well known that excessive intakes of high caloric western-style food are closely related with metabolic syndrome including obesity, hyperlipidemia, hypertension, atherosclerosis and diabetes [Lew, 1985; Hill *et al*., 1992]. It is well known that obesity is a condition of excessive fat accumulation in the body caused by energy imbalance through a high-calorie diet intake such as high-fat diet, deficient energy consumption, or genetic susceptibility. Many studies suggest that obesity is thought to be a definite risk factor for serious diseases such as cardiovascular disease, diabetes, non-alcoholic fatty liver, certain cancers, dementia, and osteoarthritis [Kopelman, 2000; Wellen and Hotamisligil, 2005]. It is well documented that hypercholesterolemia causes ischemic heart disease and atherosclerosis, and despite the advanced medical technology, cardiovascular diseases have still been one of the most important cause of mortality [Song and Son, 2008].

According to a recent report from the International Obesity Task Force (IOTF), it was estimated that 1.7 billion people, which is a quarter of the world population, must be classified overweight or obese [Friedrich, 2002]. In the past 10 years between 1990 and 2001, obese Koreans over 25 kg/m^2 (BMI) increased 2 folds from 16.7% to 30.6%, respectively [Lee and Cho, 2005]. And by the report of National Health and Nutrition Examination Survey, obese adults over 20 years of age in Korea have increased to 31.7%, annually [Ahn *et al*., 2007].

J. W. Suh* and S. D. Kim* contributed equally.

*Corresponding authors
Phone: 82-31-330-6190; Fax: 82-31-336-0870
E-mail: tele-@hanmail.net (Soon-Dong Kim)
Phone: 82-31-330-6190; Fax: 82-31-336-0870
E-mail: jwsuh@mju.ac.kr (Joo-Won Suh)

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Red mold rice fermented by the species of *Monascus* has been used as a traditional foodstuff for therapy for diarrhea, indigestion, and women's diseases in Asian countries [Ma *et al*., 2000]. Furthermore, it is reported that species of *Monascus* have produced statin related compounds including monacolin K, mevinolin and lovastatin, which inhibit cholesterol biosynthesis [Endo, 1980] and stimulate bone formation and decrease bone fracture [Edwards *et al*., 2000]. Recently, many studies reported that red pigments such as rubropuntain and monascorubin, and yellow pigments such as monascin and ankaflavin, and purple pigment such as rubropunctamine and monascorubramine derived from species of Monascus, [Ma *et al*., 2000; Pyo and Lee 2007] have antibacterial and anticancer effects [Birch *et al*., 1962]. Especially, it is well known that monacolin K have various pharmacological activities on fungal infection, hyperglycemia, hypertension, hypercholesterolemia and certain cancer [Kiyoshi *et al*., 1995; Yasukawa *et al*., 1996; Martinkova *et al*., 1999]. On the other hand, Yu *et al*. [2003] found that has been used to prepare red fermented rice (anka, red koji) have anti-obese effect. It is widely accepted that *Monascus* have various pharmacological effects due to fermentation products by species of *Monascus* on steamed rice [Kim *et al*., 2010]. However, the anti-obesity effect of *Monascus* mycelial extract has not been clearly demonstrated.

Therefore, we investigated the anti-obesity effect of *M. pilosus* mycelial ethanol extract on the body weight gain, obesity indexes, and serum, hepatic and fecal lipid contents in the high-fat with high cholesterol diet induced obese rats and compared with commercial CLA (conjugated linoleic acid).

Materials and Methods

Strains and growth conditions. The strain, *M. pilosus* KCCM 60084, used for the experiments was purchased from Korean Culture Center for Microorganisms (KCCM, Seoul, Korea).

The strain was maintained on Mizutani medium (5.0 g glucose, 2.0 g peptone, 0.8 g KH_2PO_4, 0.05 g $MgSO_4 \cdot 7\ H_2O$, 0.2 g CH_3COOK, 0.1 g NaCl made up in 100 mL distilled water, pH 6.0) [Youn *et al*., 2003] slanted at 10°C and transferred monthly. Seed cultures were prepared by transferring a loopful of spore from the Mizutani agar slanted into a 500-mL Erlenmeyer flask containing 100 mL basal Mizutani medium. The cultures were incubated at 30°C for 3 days at 150 rpm. After that 5% inoculum (v/w) was transferred for solid state fermentation.

Quantitative analysis of monacolin K and citrinin contents. Monacolin K in the *M. pilosus* mycelial was extracted with 75% ethanol by the method of Roman and Vladimir [1993]. After centrifuging for 10 min at 10,000 rpm, and filtering through a 0.45 μm filter, the supernatant was directly applied for high-performance liquid chromatography (HPLC) analysis of monacolin K and citrinin analysis was carried out as described by Reinhard and Zimmerli [1999]. HPLC analysis was carried out using Agilent Technologies series 1200 HPLC (UV-237 detector; Palo Alto, CA) equipped with a Waters 120 ODS-AP column (5 μm, 4.6×150 mm).

Standard monacolin K (lovastatin lactone form, Sigma-Aldrich, St. Louis, MO) was dissolved in 75% ethanol solution and acid form of standard monacolin K was dissolved in 2 mL of 75% ethanol solution, and let stand for 30 mins at room temperature after addition of 0.5 mL of 0.05 N sodium hydroxide solution. The concentration of acid and lactone form of monacolin K in sample were calculated using formula from the standard calibration curve; y=69576.81x−19.36 and y=54904.07x+15.96, respectively. The 1 mg of citrinin (Sigma-Aldrich) was dissolved in 1 mL of methanol solution. The concentration of citrinin in sample was calculated using formula from the standard calibration curve; y=81097.48× −15.97.

Animal experiment and diets. Male Sprague-Dawley rats (150±5 g) were supplied by Oriental Co. Ltd. (Busan, Korea). The rats were fed standard diet (5L79 diets, PMI Nutrition, Brentwood, MO) and acclimatized to the environment for 1 week before commencement of the experiments. The rats were randomly divided into 2 groups, normal control (n=5) and obese group (n=15). The high-fat and high-cholesterol diet group was fed a standard pellet chow with an added 15% lard and 1% cholesterol supplemented diet for 3 weeks for induction of obesity. After obesity was confirmed by checking obesity indexes, the animals were divided into 3 groups (5/group), high-fat (HF) and obese group diet, 0.5% mycelial ethanol extract mixed with high-fat and high-cholestrol diet (MPN), 2% conjugated linoleic acid (Natural ASA, Sandvika, Norway) high-fat and high-cholestrol diet (CLA) group for 7 weeks as given in Table 2. The rats were individually housed in stainless steel wire-bottom cages in a room maintained at 20±2°C and 60±5% relative humidity. The room was exposed to alternating 12-h periods of light and dark. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the Korea food and drug administration (KFDA) guidelines.

Feed Intakes, weight gain, and feed efficiency ratio. Body weight and feed intake were measured every day at the same hour during all experimental periods. The feed efficiency ratio (FER) was calculated as daily weight gain (g)/daily dietary intake (g).

Obesity index. In order to calculate the obesity index, the length from the tip of the nose to the anus and body weight of rats were measured every week according to Kim and Sung [2001] during experimental periods. The Rõhrer and Lee index

[Dubuc, 1981], and TM index [Tsuchimoto *et al*., 1992] were calculated for the obesity index. And the content of body fat was estimated using TM index [Tsuchimoto *et al*., 1992].

Rõhrer index={Body weight (g)/Naso-anal length (cm)^3}×10^3
Lee index={Body weight (g)$^{1/3}$/Naso-anal length (cm)}×10^3
TM index=Body weight (g)/Naso-anal length (cm)$^{2.823}$×10^3
Body fat content=0.581×TM index-22.03

Preparation of analytical samples. After 7 weeks on the experimental diets, rats were fasted for 24 h, and blood was collected from the abdominal aorta while being under anesthetic with ether. The liver was exhaustively perfused with cold physiological saline solution through the portal vein and quickly removed. Liver was rinsed with cold physiological saline and homogenized with 0.25 M sucrose by using a biohomogenizer. The collected blood was centrifuged at 2,500 rpm for 10 mins at room temperature and the separated serum and hepatic homogenate were kept frozen at −70°C.

Biochemical Analysis. Content of triglyceride, total cholesterol and (High-density lipoprotein (HDL)-cholesterol in serum were measured by using commercial kit reagents (AM 157S-K, AM 202-K, AM 203-K, Asanpharm Co., Seoul, Korea). The content of Low-density lipoprotein (LDL)-cholesterol was calculated by using the method of Friedewald *et al*. [1972]. The Atherogenic index (AI) was calculated from (total cholesterol-HDL-cholesterol)/total-cholesterol]. Hepatic and oven-dried fecal lipid was extracted by the method of Folch *et al*. [1957]. After removal of extracted solvent, the hepatic and fecal total lipid was measured according to the procedure of Frings and Dunn [1970]. And the hepatic and fecal triglyceride and cholesterol were measured by using commercial kit (AM 157S-K, AM 202-K, Asanpharm Co.).

Statistical analysis. Samples were measured in triplicate and the main treatment effects were analyzed by Duncan's multiple range test after one-way analysis of variance (ANOVA) using the general linear model procedure of SPSS (Statistical Package for Social Sciences, Ver. 12, SPSS Inc., Chicago, IL) software package. The significance level was set at $p<0.05$. Data were expressed as means ± SD.

Results and Discussion

The content of monacolin K and citrinin. Figure 1 show the HPLC chromatograms of the active acid form and non-active lactone form of monacolin K standard (A), citrinin standard (B), and freeze dried *M. pilosu*s mycelial ethanol extract (C). And Table 1 shows contents of monacolin K and citrinin in *M. pilosus* mycelial ethanol extract (MPM).

It is revealed that retention time (Rt) of active form and non-active form of monacolin K standard is 19 min and 21 min, respectively, and Rt of citrinin standard is 14 min. The active form content of monacolin K (3.3 mg%, dry basis) in MPM sample is lowered than that of non-active form (25.85 mg%, dry basis), and the content of total monacolin K is 25.85 mg% (dry basis) (Fig. 1). However, the content of citrinin in MPM was not detected (Table 1).

It is reported that active acid form of monacolin K coexisted with non-active form in red-yeast rice [Juzlova *et al*., 1996].

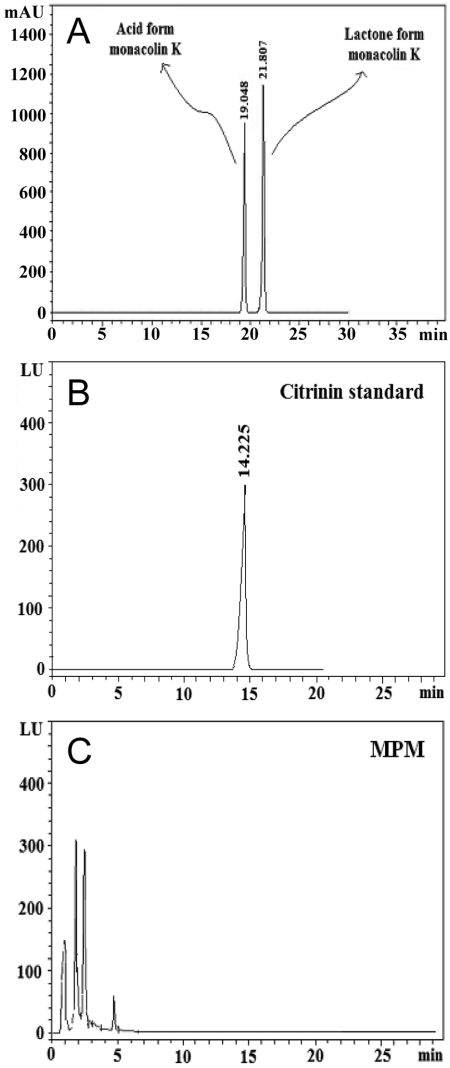

Fig. 1. HPLC chromatograms of standard monacolin K (A), citrinin (B), and *M. pilosus* mycelial ethanol extract (MPM; C). The compounds were identified by comparing the retention time (RT) with that of the standard compound.

Table 1. Monacolin K and citrinin contents of *M. pilosus* mycelial ethanol extracts mg% (dry basis)

Monacolin K			Citrinin
Acid form	Lactone form	Total	
3.38±0.82$^{b,1)}$	22.47±1.65^a	25.85±1.98^a	ND$^{2)}$

$^{1)}$Values are mean ± standard deviations of triplicate determinations, and different superscripts within a row (a-b) indicate significantly different at $p<0.05$.
$^{2)}$Not detected.

Table 2. Compositions of high-fat diet used to induce in laboratory animals

Ingredients	Experimental groups			
	NC	HF	MPM	CLA
Pellet stew (5L79 diets)[1)]	100	83.75	83.25	81.75
Lard	-	15.00	15.00	15.00
Cholesterol	-	1.00	1.00	1.00
Sodium chlorate		0.25	0.25	0.25
Freeze dried ethanol extracts of MPM	-	-	0.50	-
CLA	-	-	-	2.00

NC, normal control group; HF, high fat and high cholesterol diet group; MPM, freeze dried *M. pilosus* mycelial ethanol extracts 0.5% supplemented diet group; CLA, conjugated linoleic acid 2% supplemented diet group.
[1)]The diets for animal experiments manufactured in the PMI Nutrition, LLC, PO Box 19798, Brentwood MO 63144, USA. Guaranteed analysis: crude protein, 18%; crude fat, 5%; crude fiber, 5%; ash, 8%.

Furthermore, non-active form is converted to active form by cellular enzyme, and active form of monacolin K inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG) Co-A reductase which is rate-limiting enzyme in cholesterol biosynthesis [Manzoni & Rollini, 2002]. The above results show that MPM does not contain citrinin, which is strictly prohibited in foods.

Weight gain, feed intakes, FER, and fecal weight. After the induction of obesity, animals were fed with experimental diet (Table 2) for 7 weeks, and subsequently, body weight gain, feed intakes, FER, and fecal weight were examined (Fig. 2 and Table 3). Body weight in NC was gradually increased according to the experimental period. While, body weight in HF was rapidly increased during 4 week, those of HF were gradually increased after 4 weeks. Although, body weight in CLA was lower than that of HF during 5 weeks, those of CLA was similar with HF at 7 week. Whereas body weight in MPM was similar with CLA during 2 weeks and then that was significantly retarded after 2 weeks. And that was similar with NC at 7 week. In addition, mean weight gain per day in MPM (3.09 g) and CLA (4.38 g) were lower 31.03% and 2.24%, respectively than those of HF (4.48 g), However, mean feed intakes per day in HF and MPM (23.01~24.91 g) were not significant compared to the NC (29.60

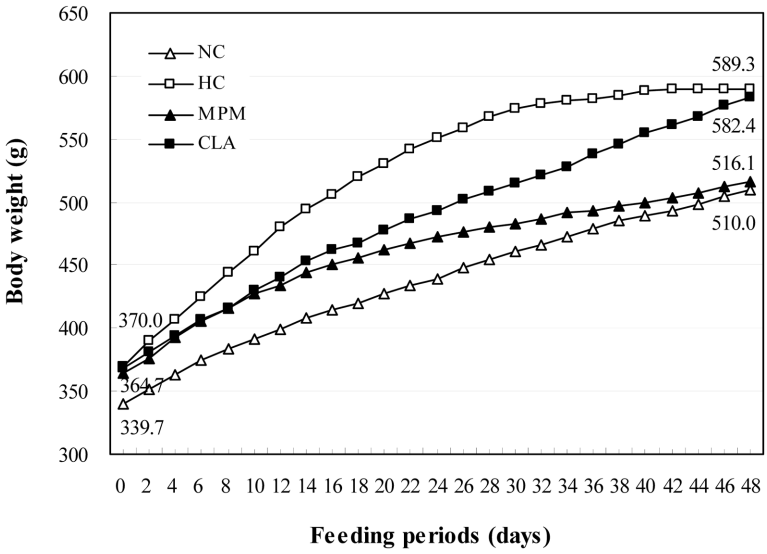

Fig. 2. Effects of *M. pilosus* mycelial ethanol extracts on the changes in body weight of obese rats fed high fat with cholesterol diets for 7 weeks. Abbreviations: NC; normal control group, HF; high fat and high cholesterol diet group, MPM; freeze dried ethanol extracts of *M. pilosus* mycelium (MPM) 0.5% supplemented diet group, CLA; conjugated linoleic acids 2% supplemented diet group. Values are mean of 5 rats.

Table 3. Effects of *M.pilosus* mycelial ethanol extracts on the weight gain, feed intakes, feed efficiency ratio and weight of feces in obese rats fed high fat with cholesterol diet for 7 weeks

Measurements	Experimental groups			
	NC	HF	MPM	CLA
Initial weight (g)	339.70±9.93[b,2)]	370.00±4.16[a]	364.70±7.93[a]	368.00±13.30[a]
Final weight (g)	510.00±14.30[b]	589.30±16.05[a]	516.10±13.57[b]	582.40±15.36[a]
Weight gain (g/day)	3.48±0.34[b]	4.48±0.41[a]	3.09±0.40[b]	4.38±0.51[a]
Feed intake (g/day)	29.60±2.01[a]	24.91±2.11[b]	23.01±1.15[b]	23.33±1.35[b]
FER[1)]	0.12±0.02[b]	0.18±0.03[a]	0.13±0.01[b]	0.19±0.02[a]
Feces (g/day, wet basis)	10.23±0.60[NS,3)]	9.98±0.39[a]	10.55±0.58[a]	10.08±0.76[a]

[1)]Feed efficiency ratio: daily weight gain/daily feed intake.
[2)]Values are mean ± SD of 5 rats, different superscripts in the same row indicates significant differences (p <0.05).
[3)]NS: not significant.

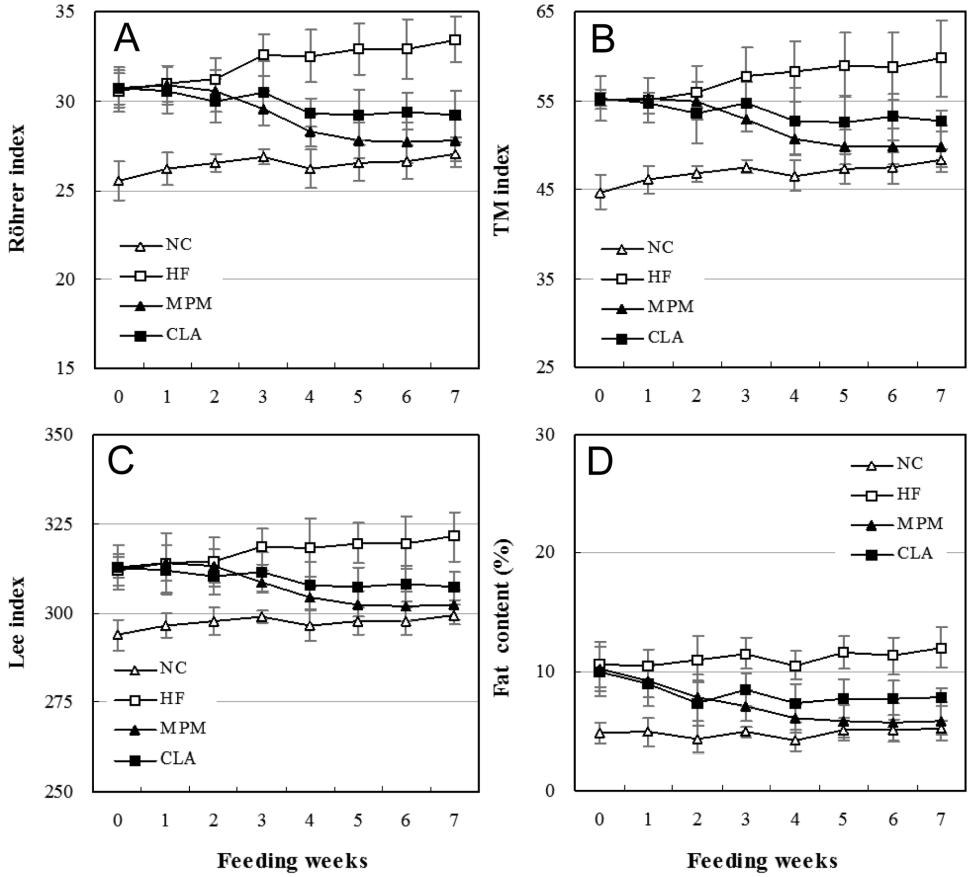

Fig. 3. Changes in Rõhrer index, TM index, Lee index and body Fat content of obese rats fed with *M. pilosus* mycelial ethanol extracts supplemented high fat with cholesterol diets during feeding. Abbreviations: See Table 2. Values are mean ± standard deviations of 5 rats, different superscripts indicates significant differences (p <0.05).

g). While, FER in MPM (0.13) was slightly lower than those of HF and CLA (0.18~0.19), fecal weight in all experimental groups were not significantly different.

Many studies reported that conjugated linoleic acid (CLA), which is widely distributed in nature, could have anti-mutagenicity, anti-cancer, anti-hypertension and anti-dibetic effect, and anti-obese effect via inhibition of body fat [Ip and Scimeca 1997; Munday *et al.,* 1999; Park *et al.,* 1999]. On the other hand, immunostimulatory response growth factor-induced catabolic response is decreased by CLA [Miller *et al.,* 1994]. Decreased catabolic response may stimulate growth and FER [Benson *et al.,* 1993], but body weight and FER animals fed CLA supplemented diet was higher than that of normal diet group animal [Park *et al.,* 1999]. Moreover, dietary effects of CLA were different according to the sex, age and experimental period [Sisk *et al* 2001]. In this study, dietary effect of body weight reduction in CLA is in similar with Park *et al.* [1999]. Furthermore, these results indicated that higher reduction rate of body weight in MPM group without significantly affecting feed intakes may be caused by improvement of indigestion and anti-obesity effect [Li *et al.,* 1998]. However, more detailed work is required on antiobese effect of the *M. pilosus* mycelial extract *in vivo* systems to determine their exact mechanism of action.

Obesity indexes. Figure 3 show changes in Rõhrer index, TM index, Lee index, and Fat content of obese rats fed with MPM supplemented high fats with cholesterol diets during 7 weeks feeding. In this study, we observed Rõhrer index (over 30), Lee index (over 300) and TM index (over 55) in animals fed with high fat with cholesterol diet for 3 weeks for the induction of obesity.

It is well known that obesity is confirmed in the case of a Rõhrer index of over 30, a Lee index of over 300, and a TM index of over 55 [Kim and Sung, 2001]. Therefore, we could confirm that the experimental animals had become an obesity model by high-fat with high-cholesterol diet feeding for the 3 weeks. In this experimental condition, we observed the dietary effects of experimental diets on the obese animal model.

In the initial experiments, the Rõher index in all experimental groups was not significantly different. After 7 weeks, the Rõher index of HF (33.43) was higher than that of NC (22.53). Furthermore, that of MPM (27.79) and CLA (29.23) was lower than that of HF. Moreover, the TM index in NC before experimental diet feeding was 44.70, that of HF, MPM and CLA was 55.14~55.23. After 7 weeks, the TM index of NC and HF

Table 4. Effects of *M. pilosus* mycelial ethanol extracts on liver weight and adipose tissue lipid content of obese rats fed high fat with cholesterol diet for 7 weeks (g/100 g body weight)

Organs	Experimental groups			
	NC	HF	MPM	CLA
Liver	2.56±0.06[c,1)]	4.35±0.07[a]	3.86±0.30[b]	4.22±0.53[ab]
Perirenal	1.84±0.40[c]	3.78±0.19[a]	2.65±0.48[b]	3.48±0.27[a]
Epididymal	1.61±0.30[b]	2.27±0.21[a]	1.80±0.22[ab]	2.09±0.26[a]

[1)]Values are mean ± SD of 5 rats, different superscripts in the same row (a-c) indicate significant differences (p <0.05).

continuously increased to 48.29 and 59.77, respectively. In contrast, MPM and CLA decreased to 49.89 and 52.67, respectively. On the other hand, the Lee index in NC, and HF, MPM and CLA before experimental diet feeding was 293.81, and 311.97~312.86 respectively. Whereas, that in NC and HF after 7 weeks was increased 299.42 and 321.34 respectively, that in MPM and CLA was decreased 302.23 and 307.39 respectively. Moreover, although slight difference, the content of body fat in all experimental groups showed similar tendency of other obese indexes changes. While, body weight and obese indexes in MPM was reduced in spite of continuous high-fat with high-cholesterol diet, obese indexes in CLA group was reduced without body weight reduction (Fig. 2).

Park and Kang [2003] reported that Rõher index is increased according to duration of high-fat diet and degree of obesity. It is well known that anti-obese effect of CLA is due to increment of heparin-releasable lipoprotein lipase (HSL) activity and stimulation of lipid degradation in adipose tissue [Pariza *et al*., 2000]. It was reported that this effect of CLA is due to reduction of fat cell numbers, body fat, inhibition of preadipocytes growth and differentiation, inhibition of free fatty acid and triglyceride biosynthesis, and increment of energy expenditure in adipose tissues [Bee, 2000]. However, DeLany and West [2000] reported that accumulation of fat and lipid oxidation is increased by CLA. On the other hand, Sisk *et al*. [2001] reported that feed intakes and growth rate in SD female rat was not affected by feeding of 0.5% CLA supplemented diet. In addition, Sisk *et al.* [2001] reported that body fat in SD male rat was not affected by diet of 12% triglyceride supplemented with 1% CLA for 4 weeks. Wahle *et al*. [2004] suggests different effect of body reduction by CLA may be according to the species of animals, and different lipid metabolism in liver and adipose tissues.

Thus above results indicated that reduction of obese indexes, adipose tissues, serum and hepatic lipid profile, and increment of excretion of fecal lipid in MPM group may prevent obesity due to stimulation of lipid degradation and lipid excretion.

Weight of liver and adipose tissue. Table 4 shows the effects of MPMs on the weight of liver and body fat such as perirenal and epididymal adipose tissue in obese rats fed high fats with cholesterol diet for 7 weeks. The liver weight per body weight (relative liver weight) in HF was markedly higher (1.70 fold) than that of NC, whereas, that in MPM was significantly higher (1.51 folds) than that of NC, these in HF and CLA group higher (1.09~1.13-folds) than that of MPM. Furthermore, the weight of perirenal adipose tissue in HF was markedly higher (2.05 folds) than that of NC. While that in MPM and CLA was higher (1.44~1.89 folds) than that of NC, that in CLA was higher (1.31 folds) than that of MPM. And that of CLA was not significantly different from HF. Additionally, although slight difference of degrees, the weight of epididymal adipose tissue in all experimental groups showed similar tendency to perirenal adipose tissue weight changes.

These results suggest that *M. pilosus* mycelial ethanol extract may ameliorate and/or prevent obesity without inhibition of feed intakes.

Serum lipid profile. Table 5 shows the levels of serum lipid in obese rats fed high fats with cholesterol diet for 7 weeks. The content of serum triglyceride in HF was higher (1.12 folds) compared with NC. While, the level of triglyceride in MPM was lower (24%) than that of NC, those of MPM was not significantly different from CLA. However, that of CLA was not significantly different from NC. Moreover, the level of total cholesterol in MPM and CLA was lower 11.94~20.23% than that of HF, while that of CLA was higher (1.23 folds) than that of NC. However, that of MPM was not significantly different from NC. On the other hand, although slight difference, the levels of LDL-cholesterol and atherogenic index in all experimental groups were similar tendency of total cholesterol changes. On the contrary, HDL-cholesterol in HF was significantly lower (14.64~19.30%) than those of MPM and NC. Whereas, that of MPM was not significantly different from NC and CLA, that of CLA was lower (13.27%) than that of NC.

It was well documented that serum cholesterol and transport of cholesterol (TG) is transported by lipoprotein, and three quarter of serum cholesterol is contained as LDL, which is related to the occurrence of atherosclerosis [Gordon *et al.,* 1981]. Nicolosi *et al.* [1997] reported that intake of CLA may prevent antherosclerosis via inhibition of serum LDL-cholesterol. Furthermore, Lee *et al*. [1994] confirmed that the levels of total cholesterol, LDL-cholesterol and TG, and the ratio of total cholesterol/HDL-cholesterol as atherogenic index in rabbit fed CLA supplemented diet for 12 weeks were markedly decreased

Table 5. Effect of *Monascus pilosus* mycelial ethanol extracts on serum lipids content in obese rats fed high fat with cholesterol diet for 7 weeks

Measurements	Experimental groups			
	NC	HF	MPM	CLA
Triglyceride (mg/dL)	74.59±6.49$^{ab,3)}$	83.79±7.67^{a}	56.70±8.42^{c}	68.89±6.12^{bc}
Total cholesterol (mg/dL)	115.75±6.55^{c}	162.31±7.05^{a}	129.48±7.87^{bc}	142.93±6.53^{b}
HDL-cholesterol (mg/dL)	55.91±2.65^{a}	45.12±2.15^{c}	52.86±1.72^{ab}	48.49±1.47^{bc}
LDL-cholesterol (mg/dL)$^{1)}$	44.92±3.45^{d}	109.43±5.92^{a}	65.28±3.86^{c}	80.66±4.75^{b}
Atherogenic index$^{2)}$	1.07±0.12^{d}	2.60±0.16^{a}	1.45±0.15^{c}	1.95±0.13^{b}

$^{1)}$LDL-cholesterol content=total cholesterol−(triglyceride/5+HDL-cholesterol)
$^{2)}$Altherogenic index=(Total cholesterol−HDL-cholesterol)/HDL-cholesterol
$^{3)}$Values are mean ± SD of 5 rats, different superscripts in the same row (a-b) indicate significant differences (p <0.05).

Table 6. Effect of *M.pilosus* mycelial ethanol extracts on hepatic lipids content in obese rats fed high fat with cholesterol diet for 7 weeks (unit: mg/g tissue)

Measurements	Experimental groups			
	NC	HF	MPM	CLA
Total lipid	64.24±4.96$^{d,1)}$	94.59±5.28^{a}	75.14±2.97^{c}	82.59±3.43^{b}
Triglyceride	32.54±2.49^{c}	46.77±4.31^{a}	37.80±2.32^{b}	40.11±2.90^{ab}
Total cholesterol	10.14±0.68^{d}	32.97±3.31^{a}	16.23±2.33^{c}	23.23±2.32^{b}

$^{1)}$Values are mean ± SD of 5 rats, different superscripts in the same row(a-d) indicate significant differences (p <0.05).

compared to the control. And, Park *et al*. [1997] found that CLA may regulate the level of serum TG via increment of carnitine palmitoyltrasferase-I (CPT-I), which is the rate-limiting enzyme of fatty acid β-oxidation in adipose tissue and skeletal muscle. Pariza *et al*. [2000] reported that CLA may inhibit obesity due to reduction of serum TG via induction of heparin-releasable lipoprotein lipase (HSL) and stimulation of norepinephrine-induced lipid degradation in adipose tissue, although action mechanism is not clear. Yu *et al*. [2003] reported that the level of HDL-cholesterol in rat fed 2% red-yeast rice supplemented diet was increased, while the level of LDL-cholesterol and atherogenic index was significantly decreased. And, many studies revealed that the levels of total cholesterol, LDL-cholesterol and TG by the administration of red-yeast rice in clinical test were significantly decreased without side effects [Li *et al.,* 1998; Heber *et al*., 1999]. It is well known that *M. pilosus* could produce monacolin K, which is a strong inhibitor of cholesterol biosynthesis [Kroon *et al.,* 1982]. Especially, it is reported that monacolin K could reduce the level of serum cholesterol in severe hypercholesterolemic patients [Arad *et al*., 1990]. Therefore, above results implied that MPM which contains monacolin K produced by *M. pilosus* could reduce the serum lipid level possibly by the inhibition of HMG-CoA reductase.

Hepatic lipid profile. Table 6 shows the hepatic lipid profile in obese rats fed high fats with cholesterol diet for 7 weeks. The level of hepatic total lipid in HF was markedly higher (1.47 folds) than that of NC. Although, hepatic total lipid level in CLA and MPM was significantly lower (12.69~20.56%) than that of HF, that of MPM was lower than that of CLA. Additionally, that of MPM and CLA was higher (1.17~1.28 folds) than that of NC. And, TG level in HF was higher (1.44 folds) than that of NC. That of MPM was significantly lower (19.18%) than that of HF, while that of MPM was higher (16.16%) than that of NC. On the other hand, that of CLA was slightly lower than that of HF. Moreover, hepatic total cholesterol in HF was extremely high (3.25 folds) than that of NC. Whereas that of CLA and MPM was significantly lower (29.54~50.77%) than that of HF, that of MPM and CLA was markedly higher (1.60~2.29) folds than that of NC and that of MPM was significantly lower (30.13%) than that of CLA.

It is well known that lipid metabolism is highly associated with the liver and insufficient removal of biosynthesized TG in the liver may induce fatty liver via accumulation of TG [Schaefer *et al*., 1995]. These are in agreement with Lee *et al*. [2006] that the levels of hepatic total lipid, cholesterol and TG in rat fed high-fat diet. Results of hepatic TG in this study suggests that *M. pilosus* mycelial ethanol extract may regulate hepatic and serum TG level due to increment of fecal lipid excretion via retardation and/or inhibition of intestinal absorption.

Furthermore, Kang *et al*. [2003] reported that inhibition of bile acid reabsorption may prevent hypercholesterolemia via control of endogenous cholesterol.

Therefore, markedly decreased hepatic total cholesterol content in MPM suggests that *M. pilosus* mycelial ethanol extract could ameliorate the hypercholesterolemia via inhibition of bile acid reabsorption.

Fecal lipid content. Table 7 shows the fecal weight and the

Table 7. Effect of *M. pilosus* mycelial ethanol extracts on weight of feces, total lipid and total cholesterol content in feces of obese rats fed high fat with cholesterol diet for 7 weeks

Measurements	Experimental groups			
	NC	HF	MPM	CLA
Feces (g/day, dry basis)	5.78±0.45[NS,1)]	5.43±0.35[a]	5.06±0.37[a]	5.34±0.32[a]
Total lipid (mg/g dry basis)	91.76±5.84[d,2)]	108.90±8.37[c]	153.12±9.07[a]	132.14±7.58[b]
Total cholesterol (mg/g)	28.54±3.79[d]	44.03±5.83[c]	91.06±6.59[a]	74.60±5.80[b]

[1)]NS: not significant.
[2)]Values are mean ± SD of 5 rats, different superscripts in the same row (a-d) indicate significant differences (p <0.05).

level of fecal lipid in obesity rats fed high fats with cholesterol diet for 7 weeks. Daily fecal weight in all experimental groups was not significantly different. On the other hand, the content of fecal total lipid in HF was higher (1.18 folds) than that of NC. Furthermore, while, the content of fecal total lipid in CLA and MPM was markedly higher (1.21 to 1.41 folds) than that of HF, that of MPM was higher (1.16 folds) than that of CLA. Additionally, the content of fecal total cholesterol in HF was greatly higher (1.54 folds) than that of NC. Whereas, that of CLA and MPM was markedly higher (1.69~2.07) folds than that of HF and that of MPM was significantly higher (1.23 folds) than that of CLA.

These results support that *M. pilosus* mycelial ethanol extract may prevent hyperlipidemia via inhibition of intestinal lipid absorption, and stimulation of fecal lipid excretion.

In conclusion, although the precise action mechanisms of anti-obese effects of MPM diet is not known, our study provides experimental evidence that *M. pilosus* mycelial ethanol extract may regulate obesity via improvement of serum lipid contents in rats fed high-fat with cholesterol diet.

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