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Reduction of Mouse Body Fats by Water Extract of *Pleurotus ostreatus*

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Abstract

Body fat-reducing ability of oyster mushroom (*Pleurotus ostreatus*) water extract (OMWE) was investigated for mice by supplying it drinking water. OMWE (2.95% solid content) was prepared by extracting a low grade of the mushroom at 120°C for 10 min. The solid material of OMWE was composed of 65.2% reducing sugar, 0.23% crude fat, 0.5% total protein, 1.2% ash and 32.9% others. OMWE was appropriately diluted with drinking water. Seventy two male ICR mice (25±1 g, 7~8 weeks of age, 6 mice/cage, 18 mice/treatment) housed in polycarbonate cages containing β-chips were adopted in a temperature- and humidity-controlled facility with free access to water and diet. One week later, the mice were subjected to one of the treatments for 36 days: 0 (control), 10, 50 and 100% OMWE. Drinking water with or without OMWE was supplied twice (40 ml each, 80 ml in total) daily per cage. Body weight and feed intake were recorded every three days. At the end of the experiment, mice were sacrificed to determine the chemical composition (fat, protein, ash and water). Body weight of mice treated with OMWE (10, 50 and 100%) at day 36 was 35.9, 35.9 and 35.5 g per mouse, respectively, and not significantly reduced as compared to that (36.5 g/mouse) of control mice. Average body fat of 0, 10, 50 and 100% OMWE-treated mice was 14.3, 13.1, 10.7 and 12.0%, respectively. Body fat reduction by 50% OMWE treatment was 25.2% ($p<0.05$), relative to control. OMWE did not affect feed intake. The contents of body protein and ash were increased with respect to body fat decrease, while water content was not changed much. These results suggest that OMWE could reduce body fat of the mice without body weight change, giving the best effect by 50% OMWE.

Key words: oyster mushroom, oyster mushroom water extract, body fat reduction

INTRODUCTION

The oyster mushroom (*Pleurotus ostreatus*: OM), belonging to *Trichromataceae*, is one of the most popular mushrooms consumed by Korean people. Production of this mushroom is over 60,000 tons per year, which is greater than that of the other mushrooms produced in Korea (1). Consequently, this overproduction results in a drop in prices, especially in the case of low grade OM. To overcome this price drop, the overproduced mushroom or low grade mushroom could be processed as extracts by water or solvents to use for health drinks and as dried products to use for food ingredient in functional foods.

OM possesses various biological activities, especially, anticarcinogenic, antioxidant, and immune stimulation activities (2-7). Currently, research on OM focuses on the hypocholesterolemic and hypoglycerolemic agents in animal models (8). It has been reported that fruit and alcohol extract of OM possessed hypocholesterolemic and hypoglycerolemic activities in the Syrian hamster (9,10) and rat (11), respectively. The reason for these activities of OM was related to the activation of lipases to increase the oxidation of fats and the reduction of HMG-CoA reductase to decrease cholesterol concentration (12,13). Several biological activities of alcohol extract of OM have been reported in the literature (9); however, research on body fat reduction by oyster mushroom water extract (OMWE), which is far more easier to manufacture, cheaper and safer than other processed products, is not shown in the literature.

In the present study, body fat-reducing ability of OMWE in mice was investigated by providing it through drinking water for 36 days. OMWE was prepared from low grade OM by extracting it at 120°C for 10 min. Body fat of mice given OMWE was reduced by as much as 25.2%, relative to control mice.

MATERIALS AND METHODS

Materials

OMWE (2.95% solid content) was prepared from a low grade of OM cultivated at the HK Mushroom Culture Co. (Chinju, Korea) by autoclaving at 120°C for 10 min. ICR mice (male, 6~7 weeks of age), Chow diet, and β-chip were supplied from Hyochang Science (Taegu, Korea). Other chemicals used were greater than reagent grade.

Animal experiment

Seventy-two male ICR mice (25.0±1 g, 7~8 weeks of

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age) were randomly housed by body weight in polycarbonated cage (6 mice/cage, 18 mice/treatment) containing β-chips. Mice were adopted in temperature- and humidity-controlled facility with free access to water and lab. chow diet. One week later, the mice were subjected to one of the following treatments for 36 days: 0 (control), 10, 50, and 100% OMWE diluted with drinking water. Drinking water was supplied to the control mice. Drinking water with or without OMWE was supplied twice (40 ml each and 80 ml total) daily per cage. Body weight and feed intake were recorded every three days. At the end of the experiment, the mice were sacrificed to determine their chemical compositions (fat, protein, ash, and water).

Analysis of some chemical composition of mouse body and OMWE

Frozen empty mouse carcass and frozen OMWE samples were completely freeze-dried using a Bondiro freeze drier (Ilshin Engineering, Korea). Crude fat of the samples was extracted by refluxing in petroleum ether (50°C) using a Soxhlet apparatus.

Fatty acid methylesters from the fat were prepared with HCl-catalyzed transesterification (14) and analyzed by GC (Hewlett Packard Series II, USA). The GC was equipped with FID and Supelcowax-10 silica fused capillary column (60 m $\times$0.32 mm, i.d., $\times$0.25 μm film thickness). Oven temperature was increased from 180°C to 210°C with a rate of 3°C/min for 15 min. Temperatures of the detector and injection port were maintained at 250°C and 240°C, respectively.

Total protein content was determined by the Bio-rad standard method, using bovine serum albumin (BSA) as a standard (15). Ash content was determined by ashing samples in a muffle furnace (800°C) according to the conventional method. Reducing sugar content of samples hydrolyzed with 6N HCl solution for 6 hr in a refluxing apparatus was measured by the Somogyi method (16).

RESULTS

Chemical composition of OMWE

Chemical composition of the freeze-dried OMWE (2.95% solid) appeared to be 0.23% total fat, 65.2% reducing sugar, 0.5% crude protein, 1.2% ash and 32.9% others. Fatty acid composition is shown in Table 1. Of the fatty acids, oleic acid content (617.3 μg/g) was predominant. The ratio of saturated to unsaturated fatty acid was close to 1. Interestingly, substantial amount (148.2 μg/g) of conjugated linoleic acid (CLA) was contained in the lipid sample.

Reduction of body weight

Fig. 1 shows cumulative body weights of mice treated with various concentrations of OMWE (0, 10, 50, and 100%) in drinking water for 36 days. All OMWE treatments reduced the cumulative body weight over a period of time, but significant differences were not shown among treatments. At day 36, the average body weight of the mice treated with 0, 10, 50, and 100% OMWE was 36.5, 35.9, 35.9 and 35.5 g per mouse, respectively, which was not affected by OMWE treatments.

Table 1. Fatty acid composition of the OMWE[1)]

Fatty acid	Concentration[2)] (μg/g dry weight)
C13:0	13.9
C14:0	78.8
C15:0	27.3
C16:0	458.3
C18:0	250.6
C18:1	617.3
C18:2	180.5
C18:3	128.8
CLA[3)]	148.2
C19:0	321.4
Total amount (μg/g dry weight)	2,225.1

[1)]Analytical condition of fatty acid was shown in the method section. OMWE obtained from low grade of OM by extraction at 120°C for 10 min.
[2)]Composition was calculated based on each peak area shown on GC chromatogram.
[3)]c9,t11 and t10,c12 isomer of conjugated linoleic acid (CLA, C18:2).

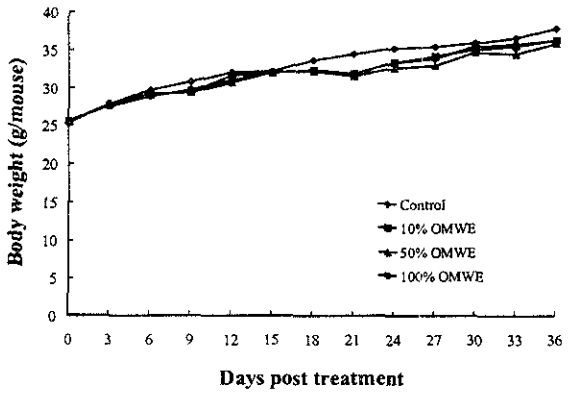

Fig. 1. Effect of the body weight of mice treated with various concentration of OMWE. Each point was an average body weight of 18 mice (6 mice/cage). Standard deviation (SD) of each point was less than 5% of the average value.

Fig. 2 shows food intake (g/mouse/3 days) of mice affected by OMWE over a period of 36 days. Up to 27 days after OMWE treatments, food intake was slightly lower than that of control mice, after which it was reversed. Food intake of mice treated with OMWE was not significantly different from that of control mice.

Body composition

Table 2 shows the body composition of the empty carcass of mice treated for 36 days with OMWE (0, 10 and 50 and 100%) in drinking water. Body weight gain was not significantly reduced by OMWE treatments (Fig. 1); however, OMWE treatments reduced total body fat content from 14.3% in the control to 10.7% in 50% treatment. Body fat reduction was not dose dependant. Only significant difference in body fat content (25.2% reduction), relative to the control, was seen in mice treated with 50% OMWE ($p<0.05$), not with 10%

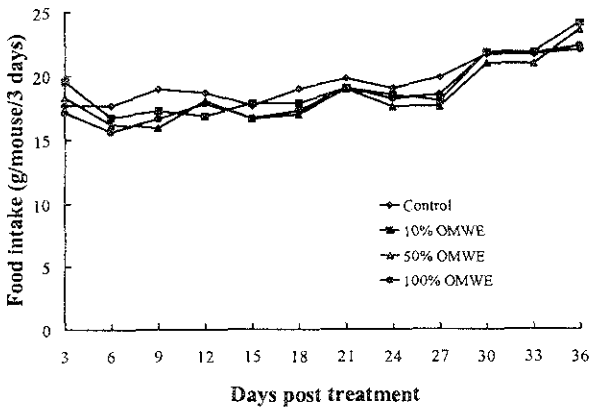

Fig. 2. Effect of the food intake of mice treated with OMWE. Each point was an average food intake of 18 mice (6 mice/cage). Standard deviation (SD) of each point was less than 5% of the average value.

or 100% OMWE. Interestingly, OMWE increased protein and ash contents. Mouse treated for 36 days with 10, 50, and 100% OMWE received 1.39, 6.96 and 13.92 g solid materials, equivalent to 0.88, 4.38 and 8.77 g reducing sugar, respectively. Hence, body fat reduction by OMWE did not correlate to the amount of reducing sugar intake.

DISCUSSION

OMWE exhibited a body fat-reducing effect in mice (Table 2), which was in the order of 50, 100 and 10% OMWE treatments, while the contents of body protein and ash increased in OMWE treatments. Significant body fat reduction (25.2% reduction, $p<0.05$) was seen only in mice treated with 50% OMWE, relative to the control mice.

The reason why OMWE exhibits the body fat-reducing activity is not known at this time. However, considering biological activities of OM published in the literature, the reason for the body fat-reducing activity can be deduced from reports that fruit body or alcohol extract of OM activated lipases to increase oxidation of fats, resulting in a decrease in triacylglycerol in the blood of the Syrian hamster and rat (9-11). It is interesting to note that OMWE contained a substantial amount of CLA which exhibited body fat reduction activity in animal experiments (17,18). Further research is needed to clarify whether CLA is one of the parameters of the observed OMWE's bodyfat-reducing activity.

β-D-glucan in the OMWE may prevent fat absorption from the gut so as to reduce body fat accumulation. OM contained several types of β-D-glucan, a polymer of β-D-glucose which is a reducing sugar (19). β-D-glucan possesses the inhibitory function of lipid or cholesterol absorption from the gut (20). We did not determine the β-D-glucan content in the sample, but more than 65% of the solid content of the sample was a reducing sugar derived from β-D-glucan. Thus, the glucan in the sample might function as an inhibitor for the absorption of lipids in the gut.

Body fat-reducing effect of OMWE was not proportional to the OMWE concentrations in drinking water. The best effect was seen in the 50% OMWE treatment group. The effect of 100% OMWE treatment was better than that of 10% OMWE. This effect might be due to the rejection response of mice by the unpleasant taste from 100% OMWE. Drinking water (80 ml per day for each cage) supply was enough for the mice, but not excessive. Mice subjected to 100% OMWE treatment sipped the drinking water, but mice subjected to 10 or 50% OMWE treatment drank the drinking water, suggesting that mice showed a rejection response against 100% OMWE. Hence, 100% OMWE treatment gave the mice stress, which might cause hormone imbalance related to the metabolism of body fats.

Effect of body fat reduction by OMWE was derived from the complexity of all constituents of the samples, not from only a specific chemical constituent. Thus, OMWE can be extrapolated to human use as a body fat-reducing agent, since it is easier to prepare than any other processed mushroom products. Furthermore, concentration of OMWE can not be a problem, since humans are much more tolerant to unpleasant taste which might be originated from the high concentration of OMWE. This study might also provide an opportunity for farmers involved in mushroom cultivation to raise their incomes by utilizing the low grade mushroom that has been worthless.

In conclusion, OMWE exhibited a body fat-reducing effect in mice, while the contents of protein and ash of the mice were concomitantly increased. The best effect in the reduction of mouse body fat was seen in the 50% OMWE treatment group. Mice exhibited rejection response against 100% OMWE, due to the unpleasant taste originated from it. OMWE can be extrapolated to human use as a body fat-reducing agent. These results may also provide an opportunity for farmers involved in mushroom cultivation to raise their incomes

Table 2. Body composition of mice treated with OMWE for 36 days

Treatment	ECW[1] (g/mouse)	Body fat (%)	Protein (%)	Ash (%)	Water (%)	Others[2] (%)
Control	33.5 ± 0.8^{a}[3]	14.3 ± 0.7^{c}	16.8 ± 1.7^{a}	3.1 ± 0.1^{a}	63.3 ± 1.5^{a}	2.5^{a}
10% OMWE	32.0 ± 0.3^{a}	13.1 ± 0.2^{c}	17.5 ± 0.9^{a}	3.3 ± 0.2^{a}	62.2 ± 2.8^{a}	3.9^{a}
50% OMWE	31.2 ± 0.4^{a}	10.7 ± 0.4^{a}	19.6 ± 0.8^{b}	3.9 ± 0.3^{b}	63.1 ± 1.7^{a}	2.7^{a}
100% OMWE	32.5 ± 0.6^{a}	12.0 ± 0.3^{b}	18.8 ± 0.6^{a}	3.3 ± 0.2^{a}	63.8 ± 1.2^{a}	2.1^{a}

[1]Empty carcass weight of mice at day 36.
[2]Others was calculated by subtracting total content (%) of body fat, protein, ash, and water from 100%.
[3]Different superscript letters in the same column meant significantly different at $p<0.05$ by Duncan's multiple test.

by utilizing low grade mushrooms.

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