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Anti-obesity Effects of *Sparassis crispa* on High-fat Diet-induced Obese Mice

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The present study investigated the anti-obesity effects of *Sparassis crispa* (SC) on mice fed a high-fat (HF, 45 kcal% fat) diet. Mice were fed either a normal control diet and an HF diet or an HF diet supplemented with SC (1%, 3%, and 5%) for 12 weeks. The consumption of an HF diet compared to the NC group resulted in increases in body weight, the food efficiency ratio (FER), retroperitoneal and subcutaneous fat weights, cholesterol and triglyceride levels, fecal fat, and liver lipids. However, the administration of SC significantly decreased body weight gain, food intake, FER, cholesterol and triglyceride levels, and liver lipids in a dose-dependent manner. In particular, treatment with 5% SC significantly reduced the occurrence of fatty liver deposits and steatosis, which are associated with the increased adipocyte size in mice fed an HF diet. Therefore, these results suggested that dietary supplementation with SC exerts anti-obesity effects and could be used as a functional food to control obesity.

Key words : High fat-diet, obesity, *sparassis crispa*, steatosis

Introduction

Obesity is a chronic global health issue associated with coronary heart disease, diabetes, hypertension, fatty liver, kidney disease, certain cancers, osteoarthritis, disability and mortality [4, 20]. In general, it is accepted that obesity results from an imbalance between energy intake and expenditure, and is characterized by increased fat accumulation in adipose tissue and elevated lipid concentrations in the blood [32]. Various investigations revealed that high intake of dietary fat could result in increased body weight and glucose metabolism disorder [1, 22]. Two types of anti-obesity drugs, orlistat and sibutramine, have been approved for long-term weight control by the U.S. Food and Drug Administration, but both drugs have side effects including increased blood pressure, dry mouth, constipation, headache, and insomnia [12, 25]. Recently, because of dissatisfaction with the high costs and potentially hazardous side-effects, the search for new drugs capable of reducing and regulating serum cholesterol and triglyceride levels has gained momentum over the years, resulting in numerous reports on significant activities of natural agents [34]. Plant products are frequently considered to be less toxic and freer from side effects than synthetic agents. These properties have led to the discovery of new therapeutic agents including antioxidants, hypoglycemics, and hypolipidemics [5, 10].

Sparassis crispa (*S. crispa*, SC) is a mushroom, commonly called cauliflower mushroom in English, hanabiratake in Japanese. Nowadays, this mushroom is very popular among consumers because it is sweet, tender, and rich in nutrients. SC has various medicinal properties and contains large amounts of β-1,3-D-glucan, i.e. about 43.6% of its dry weight [8]. The primary structure of β-glucan isolated from SC was a 6-branched 1, 3-β-glucan, having one branch in every third main-chain [27]. SC has been reported to have many biological effects such as tumor suppression [8], anti-allergy [33], and wound-healing [13], as well as enhancements in hematopoietic responses [7]. In a clinical trial, β-glucan itself had strong anticancer effects on patients with lung, stomach, colon, breast, and prostate cancers [21]. However, no studies have been performed to elucidate the anti-obesity properties of SC. The selected dosages were based on the previous study [3], which showed anti-obesity effect of edible mushrooms. In the present study, we determined SC's pharmacological effects via dietary integration with 1%, 3%, and 5% supplementation on mice

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fed with a high-fat (HF) diets.

Materials and Methods

Sample collection

S. crispa was provided by Jeonnam Forest Resource Research Institute (Naju, Korea). It was freeze-dried and then ground into a powder.

Animal experiments

C57BL/6 mouse is quite susceptible to obesity on an HF diet [2]. In this present study, 6 week-old male C57BL/6 mice were obtained from DaHanBioLink Co., Ltd. (Eumseong, Korea). They were individually housed in stainless steel cages in a room maintained at 22±2°C with 50-55% relative humidity and 12 hr of light/dark cycle (light on at 08:00). The animals were fed a pelletized chow diet for 1 week. Then, they were randomly divided into 5 dietary groups (n=6). Two groups were fed either a normal control (NC) diet or a high-fat (HF, 45 kcal% fat) diet [11]. The other three groups were given an HF diet supplemented with SC (1%, 3%, and 5%). The composition of the experimental diet was based on the AIN-93 semisynthetic diet [23] (Table 1). The mice were allowed free access to food and water during the 12-week experimental period. Food consumption checked twice a week and weight gain were measured weekly. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Chungnam National University (CNU-00028).

Table 1. Composition of the experimental diets

Contents (g/kg)	NC[1)]	HF[2)]	SC[3)]		
			1%	3%	5%
Casein	200	200	200	200	200
Corn starch	457	260	250	230	210
Sucrose	200	200	200	200	200
Soybean oil	43	25	25	25	25
Lard	0	215	215	215	215
Cellulose	50	50	50	50	50
Choline bitatrate	2	2	2	2	2
L-cystine	3	3	3	3	3
AIN-93 mineral mix	35	35	35	35	35
AIN-93 vitamin mix	10	10	10	10	10
Powdered *S. crispa*	-	-	10	30	50
Total grams (g)	1000	1000	1000	1000	1000
Calories from fat (%)	10	45	45	45	45

[1)]Normal control diet
[2)]High-fat diet
[3)]High-fat diet with powdered *S. crispa*

Collection of serum, organs, and feces

At the end of the experiments, all animals were induced to fast for 12 hr. All mice were 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 and stored at -70°C until analysis. Selected organs, the liver, kidneys, spleen, brain, heart, testes, and visceral fat pad were weighed. Feces were collected during the final 3 days using metabolic cages, and dried feces were used for fecal lipid analysis.

Analytical procedures

The concentrations of total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) in serum were determined using a commercial kit (Asan Pharmaceutical, Seoul, Korea). Hepatic and dried fecal lipid extractions were determined using a modified Tsuchida *et. al.* method [29]. Briefly, hepatic and dried fecal lipids were extracted by chloroform and methanol (2:1, v/v). The extract was dried under N_2 and weighted.

Histopathology

Liver and epididymal adipose tissues were preserved in a 10% buffered formaldehyde solution. They were processed into paraffin blocks, sectioned at a nominal 5 μm, mounted on glass microscope slides and stained with hematoxyline and eosin using the autostainer (Autostainer XL, Leica, Germany).

Statistical analysis

All the results were expressed as means ± SD. All data were analyzed using the SPSS statistical software package, version 20. Differences between groups were analyzed using one-way ANOVA followed by Duncan's multiple range tests. A difference of $p<0.05$ was regarded as being statistically significant.

Results and Discussion

Body weight, food intake, and food efficiency

Mean weights of the various groups were presented in Table 2. Initial body weights of experimental diet groups were similar in all groups, however, after 12 weeks, there was a significant body weight increase in the HF diet group.

Table 2. Effect of powdered *S.crispa* supplementation on body weight gain, food intake, and food efficacy ratio in mice fed with a high-fat diet

Group[1)]	Initial weight (g)	Final weight (g)	Food intake (g/day)	FER[2)] (%)
NC	24.75±3.71[1)]	33.08±3.58[b]	2.78±0.08[b]	3.57±0.11[c]
HF	23.45±0.90	40.15±6.09[a]	3.10±0.09[a]	6.40±0.14[a]
1% SC	23.08±0.86	37.75±5.38[a]	2.66±0.05[bc]	6.57±0.19[a]
3% SC	23.00±1.79	32.58±3.35[b]	2.50±0.08[c]	4.57±0.10[b]
5% SC	23.08±0.92	30.75±3.00[b]	2.46±0.08[c]	3.70±0.12[c]

[1)]Values are expressed as means ± SD (n=6).
[2)]FER (food efficiency ratio) = (body weight gain / food intake) × 100
[3)]Different superscripts in the same column indicate significant differences between the groups (p<0.05).

Consistent with previous study [26], the HF diet used in this study was effective in promoting obesity, which was demonstrated by increased adipose tissues in association with higher body weight. On the other hand, SC supplementation with an HF diet significantly suppressed body weight gain and food intake dose-dependently. The food efficiency ratio (FER) in the HF group was significantly higher than that of the NC group. 1% SC diet did not affect FER, however, the FER of 3% and 5% SC supplemented groups were significantly decreased compared to the HF group. It seems to be due to a high content of β-glucan causing swollen or viscous activity [31]. β-glucan increases postprandial fullness and reduces food intake via increasing the viscosity of the bowel content. Consequently, food rich in dietary fibers may assist body weight management.

Organs weight

Organ weights were expressed as relative weight per body weight (mg / 100 g body weight) (Table 3). Relative weight of the liver increased in the HF group more than in the NC group, but not significantly. The 3% SC group showed a significant decrease compared to the HF group. This might be explained that long-term ingestion of an HF diet leads to dyslipidemia, increased liver mass and hepatic steatosis [19]. The weights of the spleen and heart did not differ between groups. The brain, kidney, and testicle weights were significantly lower in the HF group than in the NC group. However, the 3% and 5% SC supplemented groups had significantly increased brain, kidney, and testicle tissue weights compared to the HF group.

Adipose tissue weight

As shown in Table 4, there was no difference in epididymal adipose tissue weight among groups. An HF diet ingestion for 12 weeks significantly increased retroperitoneal and subcutaneous adipose tissues by 166% and 208%, compared to the NC group, respectively. However, SC supplementation decreased the amount of adipose tissue compared to the HF group in a dose-dependent manner. Particularly, retroperitoneal and subcutaneous adipose tissue were decreased by 67% and 47% in the 5% SC group compared to the HF group, respectively, and were similar to those in the NC group. Consuming an HF diet increases adipocytes size and number, and changes fat deposition as compared to a balanced diet [30]. Moreover, it was reported that despite only an acute exposure to the HF diet for 9 days, these animals gained more weight and adipose tissue than the control diet [17].

Table 3. Effect of powdered *S. crispa* supplementation on organ weights fed with a high-fat diet

(g/100 g b.w.)	NC	HF	1% SC	3% SC	5% SC
Liver	2.94±0.05[ab]	3.23±0.15[a]	3.20±0.11[ab]	2.77±0.93[b]	3.14±0.17[ab]
Spleen	0.29±0.06	0.21±0.00	0.23±0.05	0.23±0.02	0.25±0.02
Heart	0.39±0.02	0.32±0.01	0.34±0.03	0.39±0.03	0.36±0.07
Brain	0.96±0.06[a]	0.78±0.03[b]	0.80±0.06[b]	0.97±0.04[a]	1.08±0.04[a]
Kidney	1.04±0.09[a]	0.86±0.02[bc]	0.77±0.04[c]	0.95±0.05[ab]	1.00±0.03[a]
Testicles	0.63±0.04[a]	0.52±0.03[b]	0.50±0.02[b]	0.66±0.02[a]	0.72±0.04[a]

[1)]Values are expressed as means ± SD (n=6).
[2)]Different superscripts in the same column indicate significant differences between the groups (p<0.05).

Table 4. Effects of powdered *S.crispa* supplementation on white adipose tissue weight in mice fed with a high-fat diet (g/ 100 g b.w.)

Group	Epididymal fat	Retroperitoneal fat	Subcutaneous fat	Adipocyte size % of NC
NC	4.06±1.07	1.44±0.50^{b}	2.12±0.70^{c}	100.00±13.84^{b}
HF	4.88±0.73	2.39±0.49^{a}	4.40±1.06^{a}	126.24±24.37^{a}
1% SC	4.60±1.09	2.07±0.64^{ab}	3.72±1.20^{ab}	131.38±21.98^{a}
3% SC	5.01±0.78	2.02±0.41^{ab}	2.42±0.45^{ab}	141.71±24.44^{a}
5% SC	4.81±1.49	1.60±0.50^{ab}	2.08±0.68^{ab}	73.52±18.83^{c}

[1)]Values are expressed as means ± SD (n=6).
[2)]Different superscripts in the same column indicate significant differences between the groups (p<0.05).

Serum lipid profiles

Table 5 displayed serum TC, TG, and HDL-C level in different groups. The HF diet group had elevated serum TC and TG level by 11% and 22% compared to the NC group, respectively. The 3% and 5% SC diet significantly lowered the serum TC level by 25% and 38% compared to the HF group, respectively. Moreover, SC supplementation with an HF diet significantly reduced serum TG level by 28%, 42%, and 44% compared to the HF group, respectively. There was no significant difference between the NC and HF group concerning HDL-C level. The 1% SC group showed the highest HDL-C value, while the 5% SC group exhibited the lowest HDL-C serum value. This result is inconsistent with Lee *et al.* [15]. However, Tirupathi Pichiah *et. al.* reported that obese mice fed 60 kcal% fat diet showed increased HDL-C serum value than those of normal control diet group [28]. It was also reported that no significant alteration of the HDL-C level in the experiment to take soup containing 30 g dried oyster mushrooms on a daily basis for 21 days [24]. There maybe an indication that a higher dosage or oral administration route could improve HDL-C level. The hypocholesterolemic action of edible mushrooms has been reported in the early work [9]. The formation of viscous gels from soluble dietary fiber such as glucans might contribute to inhibiting cholesterol and triglycerol absorption [16]. Thus, SC may have an anti-obesity effect through the suppression of dyslipidemia and hepatostea tosis in obese mice.

Table 5. Effects of powdered *S.crispa* supplementation on serum lipid profiles of mice fed with a high-fat diet

Group	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-cholesterol (mg/dL)
NC	177.44±8.97^{b}	82.79±1.65^{b}	89.50±11.69^{bc}
HF	197.74±8.16^{a}	101.02±3.19^{a}	93.78±8.11^{b}
1% SC	193.93±5.73^{a}	72.40±1.30^{c}	112.72±10.02^{a}
3% SC	148.21±3.88^{c}	58.21±2.67^{d}	83.51±2.43^{bc}
5% SC	122.56±1.58^{d}	56.77±4.10^{d}	79.21±6.19^{c}

[1)]Values are expressed as means ± SD (n=6).
[2)]Different superscripts in the same column indicate significant differences between the groups (p<0.05).

Fecal weight, fecal fat and liver lipid

As shown in Table 6, the HF group and 1% SC group showed decreased fecal weight compared to the NC group without statistical difference. However, the 3% and 5% SC group had significantly increased fecal weight by 129% and 136% compared to the HF diet, respectively. The mice fed with an HF diet showed a marked increase in the fecal fat compared with the NC group. The results of total fecal weight and fecal fat excretion in this study were also in agreement with previous study conducted on rats [6]. The 24 hr dry fecal weight and fecal fat in this study were proportional to dietary fiber levels. The increase in total fecal weight and fecal fat excretion may have been due to the fat binding capacity of β-glucan. The hypocholesterolemic effect of β-glucan has been explained that its binding with bile acids and their fecal excretion tend to lower cholesterol level in the body [14]. The HF diet ingestion caused the liver to accumulate a higher lipid content. The liver lipid level

Table 6. Effects of powdered *S. crispa* supplementation on fecal weight, fecal lipid, and liver lipid in mice fed with a high-fat diet

Group	Fecal weight (g/day)	Fecal lipid (mg/g)	Liver lipid (mg/g)
NC	0.17±0.01^{ab}	39.27±1.09^{d}	129.22±18.5^{ab}
HF	0.14±0.03^{b}	85.27±1.20^{a}	189.62±15.07^{a}
1% SC	0.14±0.01^{b}	51.75±0.38^{c}	171.78±17.76^{a}
3% SC	0.18±0.02^{a}	65.90±0.82^{b}	151.43±21.60^{ab}
5% SC	0.19±0.01^{a}	83.47±0.32^{a}	102.12±21.49^{b}

[1)]Values are expressed as means ± SD (n=6).
[2)]Different superscripts in the same column indicate significant differences between the groups (p<0.05).

was 1.47 fold than that of the NC group. However, the 3% and 5% SC group inhibited the accumulation of hepatic lipid caused by an HF diet and the 5% SC group showed a markedly lower hepatic lipid level than that of the HF group.

Histopathology

High ratio of fat consumption accompanies excessive growth of adipose tissue in both cell number and cell size, and consequently induces fat accumulation. In the present study, mice fed with an HF diet developed hepatic steatosis (Fig. 1). However, SC supplementation within an HF diet significantly reduced the occurrence of fatty liver deposit and steatosis compared to the HF group. Especially, fat accumulation in 5% SC was almost completely improved comparable to the NC group. Animal studies showed that an HF diet induced fatty liver or steatosis which is characterized by an excess accumulation of lipid, primarily triacylglycerol within hepatocytes [18].

Microscopic epididymal adipose and the size of adipocytes were shown in Fig. 2 and Table 4, respectively. The epididymal adipose cell diameter in the HF group increased to 126.24% compared to the NC group. However, the 5% SC treated group showed the smallest adipocytes among all the groups and decreased the epididymal adipose cell size by 61% compared to the HF group. These results showed that SC efficiently inhibited fat accumulation in liver and epididymal adipocyte tissues. Moreover, our results substantiated the previous study that SC water extract enhanced lipolysis and up-regulated the expression of lipolytic enzymes such as CPT-1 and UCP-2 in differenciated 3T3-L1 cell [15].

In conclusion, the present study first evaluated the effect of SC on anti-obesity function in mice fed with a 45 kcal% HF diet. Treatment with SC improved many parameters of an HF diet-induced obesity. Collectively, inhibition of fat

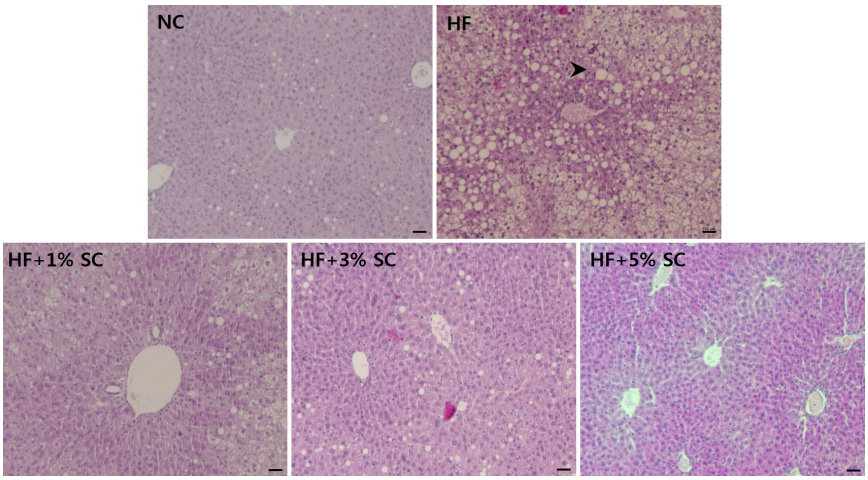

Fig. 1. Hematoxylin and eosin-stained photomicrographs showing the liver. Fat accumulation, indicated by the arrowhead, in the form of large fat droplet is present in liver of mice fed with an HF diet. NC, normal control diet; HF, high-fat diet; SC, high-fat diet with powdered *S. crispa* ; Bar = 100 μm.

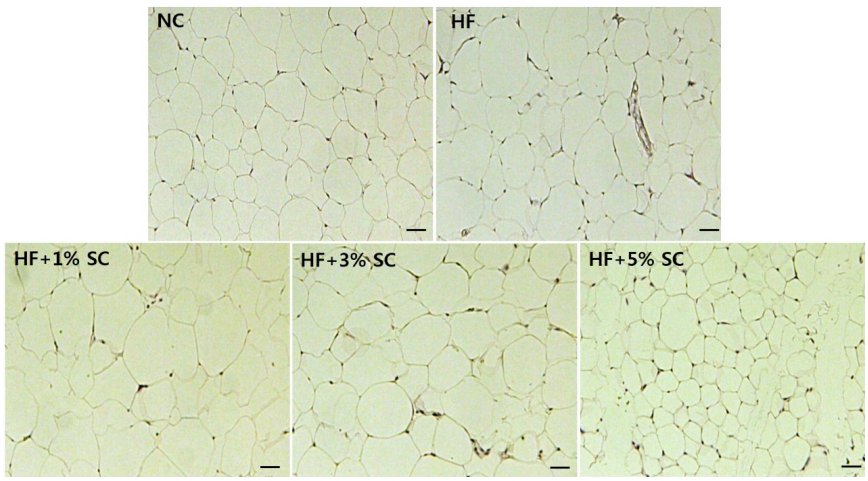

Fig. 2. Hematoxylin and eosin-stained photomicrographs showing the epididymal tissue. NC, normal control diet; HF, high-fat diet; SC, high-fat diet with powdered *S. cripsis* ; Bar = 100 μm.

absorption and fat accumulation by SC are responsible for the reduction of fat accumulation in liver and adipocyte, which leads to recover liver function and lipid metabolism. Therefore, SC appears to exert an anti-obesity effect through fat digestion inhibition.

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초록 : 고지방식이로 유도한 비만 흰쥐에 대한 꽃송이 버섯의 항비만 효과

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본 연구는 고지방식이로 유도한 비만 흰쥐에 대한 꽃송이 버섯의 항비만 효과를 관찰하였다. 6주령 수컷 C57BL/6 마우스를 이용하여 칼로리의 45%를 지방으로 구성한 고지방식이를 이용하여 비만을 유도하였으며, 대조군은 정상식이를 제공하였다. 처리군은 고지방 식이에 꽃송이 버섯 분말을 1%, 3%, 5% 수준으로 첨가하여 12주간 제공하였다. 체중, 식이섭취, 장기무게, 내장지방, 혈청지질, 변무게 및 변지방, 간지방, 조직병리실험을 실시하였다. 고지방식이 섭취군은 체중, 식이섭취, 피하지방 및 복막하지방, 혈청 콜레스테롤 및 중성지방농도, 변지방, 간지방, 부고환지방 조직의 지방세포 크기가 증가하였다. 그러나 고지방식이에 꽃송이 버섯 분말을 첨가한 실험군에서는 체중증가, 식이섭취 및 식이효율, 간 콜레스테롤 함량, 내장지방 무게가 꽃송이 버섯 첨가량에 따라 감소하였다. 특히, 5% 꽃송이버섯 첨가군은 간세포의 지방축적과 지방간 현상이 현저히 개선되었으며, 부고환 지방조직에서의 지방세포 크기도 현저히 감소하였다. 본 연구결과를 통하여 볼 때, 꽃송이 버섯은 뛰어난 항비만 효과를 가지고 있어, 비만 조절을 위한 기능성 식품으로의 이용이 가능할 것으로 사료된다.