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Effects of Onion (*Allium cepa*) Skin Extract on Pancreatic Lipase and Body Weight-related Parameters

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Abstract The aim of the present study was to assess the effects of onion (*Allium cepa*) skin extract (OSE) on pancreatic lipase (PL), the key enzyme of the digestion and absorption of dietary fat in the small intestine, and to evaluate its potential for the inhibition of body-weight gain. OSE inhibited PL with an IC_{50} of 53.70 mg/mL, which means as potent as 0.07635% of the activity of orlistat. At 3 and 4 hr after administration of OSE, the plasma triacylglycerol concentration was significantly lower in the OSE-treated rats than control. Body-weight gain and parametrial adipose tissue weights were significantly lower in mice fed the high-fat diet (fat comprises 31% of total calories) with 5%(w/w) OSE than in control. The results suggest that OSE may be an effective nutraceutical for the inhibition of body-weight gain.

Keywords: onion skin, quercetin, pancreatic lipase, body weight

Introduction

Obesity is caused by the results of an imbalance between energy intake and expenditure. Excess energy is stored in fat cells that enlarge or increase in number. Moreover, obesity is a strong risk factor for various diseases, such as hypertention, hyperlipidemia, atherosclerosis, and diabetes (1). Therefore, the effective ways to prevent obesity include inhibition of fat absorption from intestine and increase of metabolic rate and fat oxidation. Dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase. Therefore, interference with fat absorption by pancreatic lipase inhibition should cause suppression of weight gain. Orlistat (Xenical^{R}), a potent inhibitor of pancreatic lipase (2), has been proved useful in the treatment of obesity, but side effects such as fecal incontinence have also arisen due to its potency (3, 4). Therefore, lipase-inhibitory materials derived from natural products without side effects would be useful, although the activities are weaker than that of orlistat. The existence of lipase inhibitors in natural products has been demonstrated in products including chitosan (5), chondroitin sulfate (5), oolong tea extract (6), Platycodi radix extract (7), Nomame Herba extract (8), green tea extract (9), the polyphenolic constituents of *Salacia reticulata* (10), grape seed extract (11), 3-methylethergalangin from *Alpinia officinarum* (12), the polyphenols of oolong tea (13), peanut shell extract (14), and saponins from *Acanthopanax sessiliflorus* leaves (15).

In the course of a search for a lipase inhibitor from various foodstuffs and natural products, we found that onion skin extract (OSE) inhibited porcine pancreatic lipase activity. The present study was designed to clarify whether OSE could inhibit pancreatic lipase, whether OSE could reduce the elevation of plasma triacylglycerol level after oral administration of a lipid emulsion, and whether OSE could prevent the body weight gain induced by feeding a high-fat diet. Because quercetin is a main flavonoid in OSE, it was expected that quercetin would be one of active components responsible for the observed inhibitory effect of OSE.

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Materials and Methods

Preparation of the onion skin extract Fresh onions were purchased from the Hanaro Mart (Seoul, Korea). The skin was removed from the inner part. The dusted or contaminated skin was discarded. The skin was air-dried in a sunless place and powdered with a grinder (Hanil Electronics, Seoul, Korea). The powder was extracted with 60%(v/v) fermented alcohol (manufactured by fermentation from starch of rice, barley, or yam; Korea Alcohol Sales Co., Ltd., Seoul, Korea) at a ratio of 25 mL/g for 4-5 hr at 65°C, followed by filtration with cartridge filters (pore size 25 μm). The combined filtrate was concentrated in a rotary vacuum evaporator (Buchi Labortechnik AG, Flawil, Switzerland) below 40°C until 90-94% of extracting solvent was removed and 98.5-99% of extracting solvent was removed. The residue was spray-dried at inlet temperature 188°C and outlet temperature 85°C and 10,000×g. The spray-dried residue was dissolved in 50% dimethyl sulfoxide (DMSO) and used for lipase-inhibitory bioassay. It was also used for animal study.

Quantitative analysis of the extract Quercetin in the extract was determined by high-performance liquid chromatography (HPLC) using a Jasco PU-980 liquid chromatograph (Jasco Corporation, Tokyo, Japan) fitted with a Waters Xterra^{TM}, reversed-phase C_{18} column (150× 4.5 mm, i.d., 5 μm, Waters Corporation, Milford, MA, USA). The mobile phase consisted of water adjusted to pH 2.5 with trifluoroacetic acid (solvent A) and acetonitrile (solvent B). The elution was used as follows: 0-30 min, 80% A/20% B at 0 min, 50% A/50% B at 30 min. The flow rate was 1 mL/min, and the peaks were detected at 365 nm using a UV-975 UV/VIS detector (Jasco

Corporation)(16).

***In vitro* assay for the measurement of inhibitory effect of OSE on pancreatic lipase** The method was followed as the previous paper (17), in which a pH-Stat is used to add standard NaOH automatically when fatty acids, liberated by the action of lipase, decrease the pH of the reaction. It was used a pH-Stat (DK-2400; Radiometer A/S, Copenhagen NV, Denmark) consisting of a PHM290 digital pH meter with XC161 combined pH electrode, SAM7 sample stand, PHM290 pH-Stat controller, and T201 temperature sensor. Fifty mmol/L NaOH with an autoburette (Model ABU901; Radiometer) was added. Lipase (Type II; from Porcine pancreas) and tributyrin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Orlistat was purchased from Roche Korea Co. (Seoul, Korea) in the commercial form, Xenical. The concentration of orlistat was estimated from the manufacturer's manual (orlistat 120.0 mg per Xenical 317.24 mg). Parameters were optimized and set as follows; temperature, 30°C; titration, upscale; and endpoint, pH 7.00.

Substrate emulsion was prepared from blending 15 mL tributyrin, 50 mL emulsification reagent (17.9 g NaCl, 0.41 g KH_2PO_4, 540 mL glycerol, 6.0 g gum arabic in 1,000 mL deionized water) and 235 mL deionized water. The assay system contained 9.0 mL substrate emulsion, 0.50 mL plant extract in 50% DMSO, and 0.50 mL lipase preparation (1.0 LU/mL) in a total volume of 10 mL. The final concentrations of the reactants were: tributyrin, 50.0 mL/L; lipase 0.05 LU/mL; onion skin extract, 5, 50, 500 μg/mL. The reaction was linear during the first 10 min, and run for 10 min. The concentration of DMSO in the reaction (1%) did not affect the enzyme activity.
Calculations:

$$\text{Specific lipase activity, LU/mL} = (T/t_T - B/t_B) \times 0.05 \times 1/0.5$$

where, the specific lipase activity was defined in Lipase Units (LU) per mL i.e., μmoles released butyric acid per mL enzyme preparation (pH 7.0; 30°C) per min; T and B represent the volume of a 50 mmol/L solution of NaOH in μL, used to titrate the test and blank, respectively; t_T and t_B are the reaction times, in minutes, for the test and blank, respectively; and 1/0.5 converts enzyme preparation volume to μL. The concentration of each test sample giving 50% inhibition of the enzyme activity (IC_{50}) compared to the reaction of control was calculated from the least squares regression line of the semi-logarithmic plot against the remaining activity. Orlistat was assayed as a positive control. The assay was run in triplicate for each sample.

Measurements of plasma triacylglycerol level after oral administration of lipid emulsion in rats After male 10 week-old Sprague Dawley rats (Jungang Lab Animal Co., Seoul, Korea) had been deprived of food overnight, 3 mL of lipid emulsion (6 mL soybean oil, 80 mg cholic acid, 2 g chloestryloleate plus 6 mL saline) or the lipid emulsion (3 mL) plus the onion skin extract (final concentration, 50 mg/kg body weight) were administered orally to the rats. Blood samples were taken from the ophthalmic venous plexus 0, 1, 2, 3, and 4 hr after administration of the lipid emulsion with or without the extract using a capillary tube (heparinized), and centrifuged at 2,000×g for 5 min in a centrifuge to obtain the plasma (7). The plasma triacylglycerol concentration was determined using a triacylglycerol test kit (Sigma Chemical Co.).

Animal studies: feeding effect of OSE on female ICR mice Female 4 week-old ICR mice (Jungang Lab Animal Co.) were divided into 2 groups, with each group matched for mean body weight, after 1 week of feeding. The content of fat in the diet was 15%(w/w), and fat comprised 31% of total calories. OSE was provided at 5%(w/w) in the diet instead of cornstarch. The control diet represented a modified AIN-76 semipurified diet for laboratory rodents (Table 1)(18). Diets were provided three times a week. Body weights were measured once a week. Eight weeks after consuming the experimental diets, blood was taken from each mouse by venous puncture under anesthesia with diethyl ether, the mice were then killed with an overdose of diethyl ether. The plasma was prepared and frozen at -70°C until analysis. The liver and parametrial adipose tissues were quickly removed and weighted, and the liver tissue was stored at -70°C until analysis. The liver triacylglycerol and total cholesterol

Table 1. Composition of experimental diets (g/1,000 g diet)

Ingredient[1)]	Control group	OSE group
Casein[2)]	200	200
Sucrose[3)]	200	200
Cornstarch[4)]	350	300
Corn oil[5)]	50	50
Lard	100	100
Cellulose[6)]	50	50
Vitamin mixture[7)]	10	10
Mineral mixture[8)]	35	35
Choline bitartrate[9)]	2	2
Methionine[10)]	3	3
BHT[11)]	0.003	0.003
Onion skin extract	-	50
Total (g)	1000.003	1000.003

[1)]Modified AIN-76 diet composition. [2)]Casein, high nitrogen, 87% protein (Dyets, Bethelehem, PA, USA). [3)]From CJ (Korea). [4,5,6)]From Dyets. [7)]AIN-93VX Vitamin Mixture (Dyets), niacin 3.00, calcium pantothenate 1.60, pyridoxine HCl 0.70, thiamine HCl 0.60, riboflavin 0.60, folic acid 0.20, biotin 0.02, vitamina E acetate (500 IU/g) 15.00, vitamin B12 (0.1%) 2.50, vitamin A palmitate (500,000 IU/g) 0.80, vitamin D3 (400,000 IU/g) 0.25, vitamin K1/dextrose mix (10 mg/g) 7.50, sucrose 967.23 g/kg. [8)]AIN-93G mineral mixture (Dyets), calcium carbonate 357, potassium phosphate monobasic 196, potassium citrate·H_2O 70.78, sodium chloride 74.0, potassium sulfate 46.6, magnesium oxide 24.0, ferric citrate USP 6.06, zinc carbonate 1.65, manganous carbonate 0.63, cupric carbonate 0.3, potassium iodate 0.01, sodium selenate 0.01025, ammonium paramolybdate·$4H_2O$ 0.00795, sodium metasilicate·$9H_2O$ 1.45, chromium potassium sulfate·$129H_2O$ 0.275, lithium chloride 0.0174, boric acid 0.0815, sodium fluoride 0.0635, nickel carbonate 0.0318, ammonium vanadate 0.0066, sucrose finely powdered 221.026 g/kg. [9-11)]From Harlan (IA, USA).

concentrations were measured as follows: a portion (0.5 g) of the liver was homogenized in Krebs Ringer phosphate buffer (pH 7.4, 4.5 mL), the homogenate (0.2 mL) was extracted with chloroform/methanol (2:1, v/v, 4 mL) and the extract was concentrated under a nitrogen stream. The residue was analyzed using the triacylglycerol and total cholesterol test kits (Sigma Chemical Co.). Feces were collected for 3 days before the feeding experiments ended. Feces were stored at -20°C until analyzed. Feces was freeze-dried and mechanically homogenized. The pharmaco-dynamic effect of OSE was assessed by measuring fecal fat content. Total fecal fat excretion of the mice was expressed as grams of fat per day, and percentage total fat absorbed was calculated from the daily fat intake and the daily fecal fat excretion and expressed as a percentage of the daily fat intake.

Statistical analysis The results are expressed as means ± SD or SEM, and statistical significance between means was determined by use of student's *t*-test and ANOVA in SAS program.

Results and Discussion

Pancreatic lipase-inhibitory activity *in vitro* The OSE inhibited the pancreatic lipase activity in dose-dependent manner in the assay system using tributyrin emulsified with gum arabic. Quercetin present in OSE also inhibited pancreatic lipase activity (Table 2). OSE inhibited PL with an IC_{50} of 53.70 μg/mL, which means as potent as 0.07635% of the activity of orlistat at the weight basis. In OSE at the concentration of 53.70 μg/mL (the IC_{50} value of OSE; Table 2), quercetin was present at 3.222 μg/mL. The calculation was based on the content of quercetin in OSE, i.e., 60.0 mg/g OSE analyzed by HPLC. The IC_{50} value of quercetin was 527.08 μg/mL. It is important to note that the concentration of quercetin required to inhibit 50% of the control was equivalent to 527.08 μg/mL, whereas 50% inhibition was seen in the OSE only with a concentration of 3.222 μg/mL, i.e., 0.61% of the IC_{50} value of quercetin. The OSE showed advantages over a single compound in the inhibition of PL.

Table 2. Effect of onion skin extract (OSE) on pancreatic lipase activity *in vitro*[1,2]

Group	Conc. (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
OSE	4.55	6.01±0.01^c	
	45.46	47.15±5.34^b	53.70
	454.55	87.78±0.92^a	
Quercetin	4.55	6.66±0.09^c	
	45.46	12.47±4.10^b	527.08
	454.55	44.03±2.14^a	
Orlistat	0.01	22.82±5.87^c	
	0.09	69.29±22.82^b	0.041
	0.91	95.02±0.00^a	

[1]Results are the mean±SD, n=5.
[2]Superscripts mean significantly different within groups ($p<0.05$).

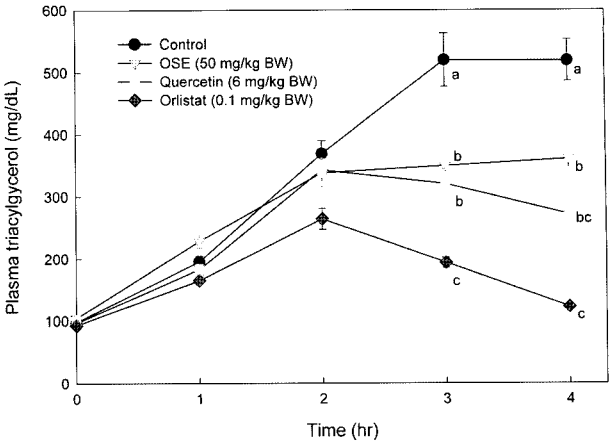

Fig. 1. Effect of onion skin extract on rat plasma triacylglycerol level after oral administration of a lipid emulsion. Each point represents the mean±SEM, n=6. Means with different letters (a, b, c) within a same time are significantly different from each other at $\alpha=0.05$ as determined by Duncan's multiple range test.

Rat plasma triacylglycerol level At 3 and 4 hr after the administration of OSE (50 mg/kg BW) or quercetin (6 mg/kg BW) with oil emulsion, the plasma triacylglycerol concentrations were significantly lower in treated rats than in the control group (Fig. 1). Fifty mg of OSE reduced plasma triacylglycerol as potent as that of 6 mg of quercetin. In OSE at the concentration of 50 mg/kg, quercetin was present at 3 mg/kg. The calculation was based on the content of quercetin in OSE, i.e., 60.0 mg/g OSE analyzed by HPLC.

The observed effects are likely induced by more than one bioactive component of OSE.

Food consumption and body, parametrial adipose tissue, and liver weights This study was undertaken to test whether OSE inhibits the increase in body weight of normal mice receiving a high-fat diet, a diet-induced obesity condition in mice mimics what was observed in humans (7). Consumption of a high-fat diet containing 10% lard for 8 weeks increased body weight (Table 3). The OSE fed 5%(w/w) in diet inhibited the increase in the weight gain in mice fed high-fat diet for 8 weeks. No abnormalities were observed in the necropsy performed at the end of the experiment, after feeding the mice for 8 weeks, and body weight gain in the OSE-treated mice (0.53 g/week/mouse) was 23.3% lower than that of the control (0.69 g/week/mouse) ($p<0.01$). Mean energy consumption for 8 weeks was not significantly different between the high fat-diet and the high-fat diet plus OSE (5%) groups as calculated from food intake in Table 3: 14.7 kcal/(day·mouse) in the high-fat diet group; 15.7 kcal/(day·mouse) in the high fat diet plus 5% OSE group.

The increases in parametrial adipose tissue were significantly inhibited by 33.7% with consumption of the high fat-diet containing 5% OSE compared with feeding the high-fat diet alone (Table 3).

Plasma and hepatic triacylglycerol and total cholesterol The concentrations in plasma triacylglycerol and total

Table 3. Effect of onion skin extract on the body weight change and food intake in ICR mice fed the high-fat diet with OSE[1-3)]

Groups	Initial body weight (g)	Final body weight (g)	Body weight change (g/week)	Food intake (g/day)	Parametrial adipose tissue (g/100 g BW)
Control	22.73±1.28	27.90±2.62	0.69±0.18	3.35±0.45	1.99±0.85
OSE(5%, w/w diet)	22.68±1.30	26.94±1.93	0.53±0.15**	3.79±0.56	1.32±0.61*

[1)]Five week-old female ICR mice were kept on the experimental diet for 8 weeks.
[2)]Each value represents mean±SEM of 10 mice.
[3)]Significantly different from the group treated with lipid emulsion alone at the same time, *p<0.05, **p<0.01.

Table 4. Effect of the extract of onion skin on triacylglycerol and total cholesterol levels in plasma of ICR mice fed the high-fat diet with OSE[1,2,3)]

Group	Triacylglycerol (mg/dL)	Cholesterol (mg/dL)
Control	85.32±9.02	153.43±29.61
OSE (5%, w/w diet)	67.76±5.43*	124.42±17.48*

[1)]Five week-old female ICR mice were kept on the experimental diet for 8 weeks.
[2)]Each value represents mean±SEM of 10 mice.
[3)]Significantly different from the group treated with lipid emulsion alone at the same time, *p<0.05.

cholesterol were significantly decreased by 20.6% with feeding the high-fat diets containing a 5% OSE compared with feeding the high-fat diet alone (Table 4). The concentrations in plasma total cholesterol were significantly decreased by 18.9% with feeding the high-fat diets containing a 5% OSE compared with feeding the control (Table 4). Furthermore, the consumption of the high-fat diet plus OSE significantly inhibited the increase in hepatic triacylglycerol concentrations by 7.4% (Table 5). However, the consumption of OSE did not affect hepatic total cholesterol concentrations (Table 5).

Fecal fat absorption Feeding high-fat diet supplemented with OSE significantly increased fecal dry weight by 34.6% and consequently caused a significant increase in fecal fat output by 21.8% (Table 6).

The OSE inhibited the hydrolysis of tributyrin emulsified with gum arabic in the pancreatic lipase activity assay. On the basis of this result, it was performed experiments that examined the reducing effect of the OSE on plasma triacylglycerols elevated in rats after oral administration of a lipid emulsion containing corn oil and clarified its effect. The results suggest that the OSE might exert antiobesity and antihyperlipidemic actions, which were mediated through delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity. In the long-term (8 weeks) experiments, 50 g/kg diet OSE significantly reduced body and parametrial adipose tissue weights, although no significant difference in energy consumption was found.

OSE increased the fecal dry weight and fecal fat output, and decreased fat absorption, possibly by inhibiting PL, decreasing the digestibility of dietary fat. Daily observations did not reveal any other visible behavioral, physiological, or anti-nutritional effects of OSE in the experimental animals. Diarrhea is a common side effect of PL inhibitor orlistat (Xenical^{R}) in humans (3, 4). However, rats treated with OSE did not have diarrhea for the duration of the experiment. The accumulation of triacylglycerol in the liver (Table 3), induced by a high-fat diet was reduced by the consumption of OSE, possibly due to the inhibition of pancreatic lipase and the subsequent reduction of the intestinal fat absorption. Such effects should, in turn, result in the suppression of hydrolysis and absorption of triacylglycerols.

Table 5. Effect of the extract of onion skin on triacylglycerol and cholesterol levels in liver of ICR mice fed the high-fat diet with OSE[1-3)]

Group	Liver weight (g/100 g B.W.)	Triacylglycerol (mg/g liver)	Cholesterol (mg/g liver)
Control	3.56±0.24	23.06±3.72	4.31±0.78
OSE (5%, w/w diet)	3.54±0.27	21.36±7.89*	4.23±0.82

[1)]Five week-old female ICR mice were kept on the experimental diet for 8 weeks.
[2)]Each value represents mean±SEM of 10 mice.
[3)]Significantly different from the group treated with lipid emulsion alone at the same time, *p<0.05.

Table 6. Effect of the extract of onion skin on fat absorption in ICR mice fed the high-fat diet with OSE[1-3)]

Group	Fecal dry weight (g/day)	Fat intake (g/day)	Fecal fat (g/day)	% of amount ingested	Calculated fat absorption[4)] (g/day)
Control	0.263±0.062	0.557±0.152	0.011±0.005	97.44±1.39	0.546±0.152
OSE (5%, w/w diet)	0.354±0.108*	0.445±0.181*	0.018±0.006*	94.04±2.47**	0.427±0.179*

[1)]Five week-old female ICR mice were kept on the experimental diet for 8 weeks.
[2)]Each value represents mean±SEM of 10 mice.
[3)]Significantly different from the group treated with lipid emulsion alone at the same time, *p<0.05 and **p<0.01.
[4)]Deduced fecal fat from fat intake.

The OSE actions may, at least in part, be attributed to the inhibition of fat absorption in the digestive tract, and the activation of lipid metabolism in the liver. The observed effects are likely induced by more than one bioactive component of OSE.

In conclusion, there were shown that oral administration of OSE reduced postprandial increase of triacylglycerol induced by an oil emulsion, and that OSE reduced body weight and pararmetrial adipose tissue in female high-fat diet-treated mice. OSE may become a promising candidate for a novel nutraceutical for weight control.

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