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# Hypocholesterolemic Effect of Quercetin-Rich Onion Peel Extract in C57BL/6J Mice Fed with High Cholesterol Diet

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**Abstract** Onion peel (OP) extract is known as a rich source of flavonoids, mainly quercetin. We hypothesized that OP has hypocholesterolemic effects. To investigate the effect of OP, C57BL/6J mice were divided into 4 dietary groups ($n$=10); normal diet (ND); high cholesterol diet (HC); and high cholesterol diet with 100 or 200 mg OP extract (OP-100 or OP-200, respectively) per kg of body weight. After 12 weeks, lower values of liver weight, serum total cholesterol levels, LDL cholesterol, atherogenic index, cardiac risk factor, hepatic triacylglycerol, and total cholesterol, and higher fecal cholesterol levels were observed in the OP-200 than in the HC group. The hepatic mRNA expression levels of low-density lipoprotein receptors (LDL-R) and cholesterol 7-alpha-monooxygenase (CYP7A1) were high in the OP-200 compared to the HC group. These observations suggest that OP promoted lowering of serum and hepatic cholesterol in mice primarily via fecal excretion.

**Keywords:** onion peel, atherosclerosis, cholesterol, low-density lipoprotein receptor, cholesterol 7-alpha-monooxygenase

## Introduction

Hypercholesterolemia or excessively high plasma cholesterol levels, is a strong predictor and contributory risk factor in the development of atherosclerosis-related diseases such as ischemic heart diseases, carotid artery diseases, and hypertension. According to WHO, by 2030 there will be 23.6 million people globally affected by cardiovascular diseases (CVD), making it the leading cause of mortality worldwide (1). Excessive consumption of diets rich in saturated fats and cholesterol is the leading cause for increase in plasma cholesterol levels (2). With globalization consumption of high-calorie foods, a poor dietary fatty acid profile along with high sodium and cholesterol levels has increased the burden of hypercholesterolemia (3).

Currently, drugs like statins are used for lowering cholesterol; however, numerous side effects arise with its use, including muscle damage, liver dysfunction, and increased blood glucose levels (4). Similarly, dietary modification plays a vital role in preventing and treating high blood cholesterol by ameliorating the LDL to HDL cholesterol ratio (5). Studies on humans and animals have shown that diet supplemented with fruits and vegetables have beneficial effects on CVD (6). Based on a meta-analysis study, up to 3% risk for coronary negative events can be lowered by 1% decrease in plasma cholesterol levels, which can be only achieved through appropriate food modification (7). Polyphenols and flavonoids present in functional foods (vegetables and fruits) combat CVD and other risk factors (8).

Onion (*Allium cepa* L.) is a universally consumed functional food rich in flavonoids, mainly quercetin and saponins (9), which suppress oxidative stress and inflammation as well as induce lipolysis (10). More than 500,000 tons of onion peel waste is produced annually in the European Union and other parts of the world. Moreover, because of the odor associated with onion peels, this organic waste is also unsuitable for use as fodder or organic fertilizer and therefore poses a major problem of disposal (11). Onion peels (OP) have been used as coloring agents and additives in foods in some parts of the world (12). Earlier reports indicated the presence of high concentration of quercetin in the outer layer of onions and it has anti-oxidative and antidiabetic properties (10,12). However, the hypocholesterolemic property of OP extract has not been yet reported. Therefore, the present study was performed to evaluate the effectiveness of OP extract on modulating the expression of genes regulating hepatic cholesterol metabolism with changes in body weight (BW), feed intake, serum and liver lipid profile on high cholesterol diet induced hyper-cholesterolemic mice.

## Materials and Methods

**Animals and diets** Male C57BL/6J mice were obtained at 4 weeks of age from Charles River Laboratory (Tokyo, Japan) and were allowed to acclimate to their surroundings for 1 week. The animals were then randomly divided into four groups ($n$=10): normal diet (ND); high cholesterol (HC) control; high cholesterol diet with OP extract, 100 mg/kg BW (OP-100); and high cholesterol diet with OP extract, 200 mg/kg BW (OP-200). The OP extract was orally administered using oral gavage; the ingredient compositions of the experimental diets (Research Diets, New Brunswick, NJ, USA) are shown in Table 1. The animals were housed in a controlled environment at 23±1°C with alternating 12 h light-dark cycles and a relative humidity of 50±5%. The animals were given free access to food and water during the entire experimental period of 12 weeks. The feed intake was measured every other day and BW was measured on a weekly basis. The experimental protocol was approved by the Animal Care and Use Committee of Chonbuk National University.

**Preparation of onion peel extract** OP was prepared using onion peel powder supplied by the Research Center for Industrial Development of Biofood Materials (Chonbuk National University, Jeonju, Korea). The peels were washed three times in tap water, extracted with 60% aqueous ethanol solution for 1 h at 80°C using an extractor (Kyungseo Machines Co., Ltd., Incheon, Korea) and then concentrated. The final concentration of soluble solids was 50°Bx, measured using a refractometer (Atago Co., Tokyo, Japan). The concentrate was then lyophilized, pulverized, and used. We measured quercetin levels (Table 2) in both the onion inner flesh and the onion peel. The total quercetin amount in the onion peel was 80% higher than in the inner flesh, indicating a high level of quercetin accumulation in the peel rather than the onion flesh. Therefore, we chose the peel to investigate its hypocholesterolemic effect.

**Collection of serum and tissue samples** After 12 h of overnight fasting, each mouse was anesthetized with diethyl ether inhalation and blood was collected by orbital vein puncture. Serum was isolated from clotted blood by centrifugation at 1,100×$g$ for 15 min at 4°C (Micro 17R; Hanil Science Co. Ltd., Gangneung, Korea). Tissues were carefully excised, rinsed, and weighed. Both tissue and serum samples were stored at −80°C until analyses.

**Analyses of tissue lipids** Hepatic triacylglycerol (TAG) and total cholesterol (TC) were extracted from the liver tissue (13). Briefly, chloroform/methanol solution (2:1, v/v) was added to the homogenized liver tissues, vortexed, and centrifuged at 6,296×$g$; the lower phase was collected and evaporated at room temperature under a fume hood (Daihan Labtech Co. Ltd., Namyangju, Korea). The remaining semi-dried pellet was dissolved in 1% Triton X-100 (Yakuri Pure Chemicals Co. Ltd., Kyoto, Japan). The resulting solution was used to estimate hepatic TAG and TC. Serum sample, fecal sample, and hepatic lipid profiles were measured using a commercially available kit (Asan Pharmaceutical Co., Seoul, Korea) and a spectrophotometer (Shimadzu, Kyoto, Japan).

**Quantitative real-time polymerase chain reaction (PCR) analyses** Total RNA was extracted from the liver tissue using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and its concentration was measured spectrophotometrically. The extracted RNA was reverse transcribed into complementary DNA using a high-capacity

**Table 1.** Ingredient composition of the experimental diets fed to mice

| Ingredient (g) | Normal diet[1)] | High cholesterol diet[2)] | | |
|---|---|---|---|---|
| | | HC | OP100 | OP200 |
| Casein, 30 Mesh | 200 | 75 | 75 | 75 |
| Soy protein | - | 130 | 130 | 130 |
| DL-Methionine | 3 | 2 | 2 | 2 |
| Corn starch | 150 | 275 | 275 | 275 |
| Maltodextrin10 | - | 150 | 150 | 150 |
| Sucrose | 500 | 30 | 30 | 30 |
| Cellulose, BW200 | 50 | 90 | 90 | 90 |
| Soy bean oil (AIN-76A: Corn oil) | 50 | 50 | 50 | 50 |
| Cocoa butter | - | 75 | 75 | 75 |
| Coconut oil,76 | - | 35 | 35 | 35 |
| Mineral MixS10001 | 35 | 35 | 35 | 35 |
| Calcium carbonate | - | 5.5 | 5.5 | 5.5 |
| Sodium chloride | - | 8 | 8 | 8 |
| Potassium citrate | - | 10 | 10 | 10 |
| Vitamin Mix V10001 | 10 | 10 | 10 | 10 |
| Choline bitartrate | 2 | 2 | 2 | 2 |
| Cholesterol, USP | - | 12.5 | 12.5 | 12.5 |
| Sodium cholic acid | - | 5 | 5 | 5 |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| kcal | 3902 | 4128 | 4128 | 4128 |
| kcal% | 3.9% | 4.1% | 4.1% | 4.1% |
| Oral administration | DW[3)] | DW | OP100 | OP200 |

[1)]Normal diet-AIN-76A diet
[2)]High cholesterol diet-Paigen's atherogenic rodent Diet
[3)]DW, distilled water 1 mL/kg BW; OP100, ethanol extract of onion peel extract 100 mg/kg BW; OP200 ethanol extract of onion peel extract 200 mg/kg BW

**Table 2.** Composition of quercetin in onion peel extract[1)]

| | Quercetin aglycone | Q-3-glycone | Q-4-glycone | Q-3,4-diglycone | Total quercetin |
|---|---|---|---|---|---|
| Onion inner flesh | 1.413 | ND[2)] | 1.099 | 0.043 | 2.464 |
| Onion skin peel | 7.402 | ND | 4.177 | ND | 11.580 |

[1)]mg/g of dry weight
[2)]ND, Not detected

**Table 3.** Oligonucleotide primer sequences used in the study

| | Gene[1)] | Forward sequence | Reverse sequence |
|---|---|---|---|
| 1 | CYP7A1 | 5´-CGCATGTTTCTCAACGACACA-3´ | 5´-ATGCCCAGAGGATCACAAGGT-3´ |
| 2 | LDL-R | 5´-GATGGACCAGGCCCCTAACT-3´ | 5´-GGTGTCAGCCACAGATACGCT-3´ |
| 3 | β-actin | 5´-ATGGATGACGATATCGCT-3´ | 5´-ATGAGGTAGTCTGTCAGGT-3´ |

[1)]Relative quantification of gene expression with real-time PCR data was calculated relative to ß-actin; CYP7A1, Cholesterol 7 alpha monooxygenase; LDLR, Low-density lipoprotein receptor

complementary DNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Then, the RNA expression level was quantified by quantitative real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems) and the 7500 Real Time PCR system (Applied Biosystems) according to the manufacturer's protocol. The gene-specific primers used are given in Table 3. Relative quantification of gene expression with real-time PCR data was calculated relative to â-actin.

**Statistical analyses** The data were analyzed by one-way ANOVA using SPSS version 17.0 program (SPSS Inc., Chicago, IL, USA). Values are expressed as means±standard deviation. The differences among groups were assessed using Duncan's multiple range test. Statistical significance was considered at $p<0.05$.

## Results and Discussion

**Feed intake, body weight, and liver weight** Feed intake, BW, and liver weight are shown in Table 4. The feed intake of HC control, OP100, and OP200 groups was lower than that of the ND group. Our result is congruent with Kim *et al*. (10); they reported that the feed intake in animals fed with high-fat diet was less compared with that in animals fed with normal diet. Similarly, in our study, the feed intake in animals fed with high cholesterol diet was less compared with that in animals fed with normal diet. The lower intake of diet reflected in the BW of the animals and the high cholesterol diet-fed animals had a lower BW than the ND group. Interestingly, the feed intake of the OP200 group was similar to that of the ND group; however, the BW of the OP200 group was lower than that of the ND group. In a study (14) by Teratani *et al*. (14), it was noted that cholesterol-rich diet did not induce weight gain. Similarly, in our study, the HC group had a lower BW than the ND group.

Liver weight gain was observed in animals fed with high cholesterol diet. Similarly, in our study, we found that liver weight was the highest in the HC control group. It was noteworthy to observe that supplementation of onion peel extract suppressed the liver weight gain by 6.6% in the OP100 group and 7.8% in the OP200 group compared to the HC group.

**Effect of OP extract on serum and hepatic lipid profiles** Serum and hepatic lipid profiles are shown in Table 5. The high cholesterol diet-fed groups (HC, OP100, and OP200) showed lower levels of serum and hepatic TAG compared to the ND group. Among the high cholesterol diet-fed groups, OP100 and OP200 showed a declining tendency in TAG compared to the HC group. Consumption of sucrose-rich diets promotes increase in serum TAG (15), while high cholesterol diet intake increases accumulation of cholesterol mainly in the serum and liver. The ND diet contained 50% sucrose and 0% cholesterol compared to the HC diet with 3% sucrose and 1.25% cholesterol; therefore, a high amount of TAG was seen in the ND group while increased cholesterol levels were observed in the HC group. The high cholesterol diet did not induce a rise in serum and hepatic TAG levels in the HC, OP100, and OP200 groups, in contrast to what was observed in the ND group. However, among the HC groups, quercetin-rich onion peel extract was effective in lowering hepatic TG by 24.5% and 22.5% in the OP100 and OP200 groups, respectively, compared to the HC control group. Our result is in

**Table 4.** Feed intake, body weight, liver weight, atherogenic index, and cardiac risk factor in mice fed the experimental diets

| | Normal diet | High Ccholesterol diet | | |
|---|---|---|---|---|
| | ND[1)] | HC | HC-OP 100 | HC-OP 200 |
| Feed intake (g) | 2.41±0.34[a2)] | 2.25±0.24[b] | 2.23±0.27[b] | 2.35±0.32[ab] |
| Body weight (g) | 29.13±1.87[a] | 24.55±1.20[b] | 23.90±1.96[b] | 24.75±1.56[b] |
| Liver weight (g) | 1.00±0.07[c] | 1.66±0.10[a] | 1.55±0.12[b] | 1.53±0.16[b] |
| Cardiac risk factor[3)] | 1.29±0.10[c] | 3.61±1.09[a] | 2.53±0.79[b] | 1.98±0.52[b] |
| Atherogenic index[4)] | 0.29±0.10[c] | 2.73±1.11[a] | 2.53±0.79[a] | 1.98±0.52[b] |

[1)]ND, AIN-76A diet; HC, 1.25% high cholesterol diet (Paigen's atherogenic rodent diet); OP-50, 1.25% high cholesterol diet+onion peel 50 mg/kg; OP-100, 1.25% high cholesterol diet+onion peel 100 mg/kg; OP-200, 1.25% high cholesterol diet+onion peel 200 mg/kg.

[2)]All values are mean±SD. Values with different superscripts within a row are significantly different among the high cholesterol diet groups by ANOVA with Duncan's multiple range test at $p<0.05$.

[3)]Cardiac risk factor=TC/HDL-C

[4)]Atherogenic index=(TC − HDL-C)/HDL-C

**Table 5.** Serum and hepatic lipid parameters and fecal cholesterol levels in mice fed with the experimental diets

| | | Normal diet | High cholesterol diet | | |
|---|---|---|---|---|---|
| | | ND[1)] | HC | HC-OP 100 | HC-OP 200 |
| Serum (mg $dL^{-1}$) | TAG | 168.94±24.67[a2)] | 115.49±20.59[b] | 109.55±18.21[b] | 111.51±31.62[b] |
| | TC | 175.90±25.42[c] | 263.59±38.91[a] | 261.94±32.85[a] | 222.79±37.28[b] |
| | HDL-cholesterol | 137.28±23.69[a] | 74.45±11.60[b] | 81.13±11.35[b] | 81.63±12.91[b] |
| | LDL-cholesterol | 10.09±5.56[c] | 168.46±46.94[a] | 167.09±42.05[a] | 123.11±31.12[b] |
| Liver (mg $dL^{-1}$) | TAG | 32.50±6.48[a] | 25.33±2.14[b] | 19.10±2.59[c] | 19.63±2.59[c] |
| | TC | 144.68±29.30[b] | 303.84±38.07[a] | 270.00±19.96[b] | 272.40±21.59[b] |
| Fecal (mg $dL^{-1}$) | TC | 144.26±42.49[c] | 259.56±36.42[b] | 282.21±33.53[a] | 324.71±94.67[a] |

[1)]ND, AIN-76A diet; HC, 1.25% high cholesterol diet (Paigen's atherogenic rodent diet); OP-50, 1.25% high cholesterol diet+onion peel 50 mg/kg; OP-100, 1.25% high cholesterol diet+onion peel 100 mg/kg; OP-200, 1.25% high cholesterol diet+onion peel 200 mg/kg

[2)]All values are mean±SD ($n$=10). Values with different superscripts within a row are significantly different among the groups by ANOVA with Duncan's multiple range test at $p$<0.05.

agreement with our earlier report showing the hepatic TAG-lowering effect of quercetin-rich extract of sea buckthorn (13).

However, a steep rise in serum and hepatic total cholesterol and serum LDL-cholesterol levels was noted in all high cholesterol diet groups compared to the ND group. Interestingly, supplementation of onion peel extract averted the rise of TC and LDL cholesterol by 0.6% and 0.8%, respectively, in the OP100 group and ($p$<0.05) 15.5% and 26.9%, respectively, in the OP200 group compared to the HC group.

Oral supplementation of quercetin alone at 0.01 to 1.0 g/kg BW in rodents did not produce any significant difference in the serum and hepatic lipid profile (16). Similarly, in human subjects, oral supplementation of quercetin at 50 to 150 mg/day (17) or even 500 mg/day (18) failed to produce a significant difference in the serum lipid profile. However, quercetin-rich plant-based supplements have shown to lower total cholesterol in animals (13) and humans (19). In our study, animals receiving 0.06 mg/day quercetin obtained from the onion peel extract showed effective lipid-ameliorating activity. We believe that bioactive compounds in the extract apart from quercetin produce a combinational effect resulting in the hypocholesterolemic effect.

Low HDL cholesterol is a well-established risk factor for coronary heart disease (CHD) (20). In this study, HDL-cholesterol was lower in the high cholesterol diet-fed groups compared to the ND group. Although not significant, the OP extract-supplemented group showed an inclining tendency of HDL-cholesterol by 8.9% and 9.6% in the OP100 and OP200 groups, respectively, compared to the HC control group. Our study is also in agreement with the Wu *et al*. (21) study; in their study, they did not find any significant difference in the HDL level between the supplemented and control groups fed with a high cholesterol diet.

**Fecal excretion of total cholesterol** Fecal cholesterol levels are shown in Table 5. The fecal cholesterol level was higher in the high cholesterol-fed animals than in the animals fed with ND. It was observed that among the high cholesterol diet-fed groups, the fecal cholesterol level was significantly high ($p$<0.05) in the OP200 and OP100 groups than the HC group. The major pathway of cholesterol elimination occurs through fecal excretion of bile acids and cholesterol. A higher amount of fecal cholesterol implies efficient removal of stored cholesterol (22). Many studies showed that quercetin and quercetin-rich plant supplements (23) increase fecal cholesterol excretion (24). In congruence with the aforementioned report, in our study, quercetin-rich OP facilitated the fecal excretion of cholesterol, thereby decreasing serum and hepatic cholesterol levels.

**Effect of OP extract on cardiac risk factor and atherogenic index** Cardiac risk factor and atherogenic index increases with increasing Cardiovascular risk, can be easily calculated from standard lipid profile (25). High HDL-cholesterol and low TC levels imply a low atherogenic index. In this study, we analyzed both cardiac risk factor and atherogenic index (Table 4). We observed that the intake of high cholesterol diet resulted in higher values of cardiac risk factor and atherogenic index in all HC groups compared to the ND group. Interestingly, supplementation of onion peel extract resulted ($p$<0.05) in 30% and 45% reduction of cardiac risk in the OP100 and OP200 groups, respectively. Similarly, the atherogenic index decreased significantly by 7.3 and 27.5% in OP100 and OP200 groups, respectively, indicating a dose-dependent effect. Supplementation of plant-based products has been found to correlate with increased excretion of fecal cholesterol and lower levels of cardiac risk factor and atherogenic index (26,27). In this study, increased fecal excretion of cholesterol in the supplemented groups led to the reduction of total cholesterol, thereby lowering the atherogenic index and cardiac risk factor.

**Effect of OP extract on hepatic cholesterol metabolism-regulating genes** The relative mRNA expression levels of genes, namely low density lipoprotein receptor (LDL-R) and cholesterol 7-alpha-monooxygenase (CYP7A1) involved in hepatic cholesterol metabolism, are shown in Fig. 1A and 1B. The highest level of LDL-R mRNA was seen in the OP200 group, while the CYP7A1 mRNA level was not significantly different among the high cholesterol diet groups.

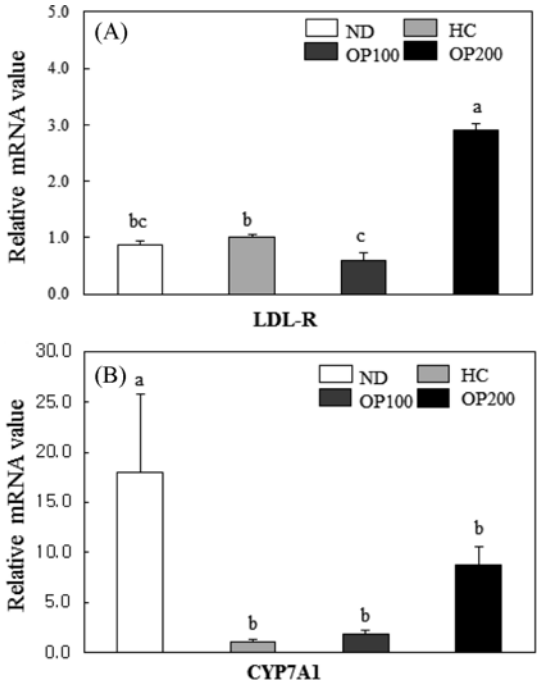


**Fig. 1.** Effects of onion peel extract on mRNA expression of hepatic cholesterol metabolism-related genes in mice fed on experimental diets. (A) LDL-R (low-density lipoprotein receptor) and (B) CYP7A1 (cholesterol 7 alpha-monooxygenase) as measured by qRT-PCR. Data are expressed as means±SD ($n$=3), with different alphabets indicating a significant difference among the treated groups with onion peel extract, according to ANOVA with Duncan's multiple range test ($p$<0.05).

However, the supplementation of onion peel extract promoted an inclining tendency of CYP7A1 mRNA than the high cholsterol diet control group. Risk of atherosclerosis is directly proportional to an increase in the total cholesterol level and inversely proportional to the LDL-R activity (28). Hepatic LDL-R plays a crucial role in the removal of LDL cholesterol from plasma and other peripheral cells (29) and avoiding the formation of atherosclerotic lesions (30). However, high cholesterol-fed mice show down-regulation of LDL-R, thereby increasing the possibility of elevated blood cholesterol levels. In contrast, onion peel extract (OP200) supplementation reverts the down-regulation of hepatic LDL-R mRNA levels in high cholesterol-fed mice. One study demonstrated that green tea elicits a hypocholesterolemic effect via LDL-R up-regulation (31). *In vitro* supplementation of quercetin has been found to up-regulate the expression of LDL-R (32). A similar result was also observed in our study-the onion peel extract rich in quercetin up-regulated LDL-R. Altogether we surmise that the reason underlying hypocholesterolemic effects of the onion peel extract could be due to increase in the LDL-R activity leading to increase in the removal of plasma LDL cholesterol especially in the OP 200 group.

The cholesterol 7-alpha- monooxygenase gene acts as a catalyst to convert cholesterol to bile acid, which is the major pathway for disposal of cholesterol in mammals (33). In another study, plasma cholesterol decreased through up-regulation of CYP7A1 (34). The CYP7A1-increasing tendency in the OP200 group shows that cholesterol removal was increased in OP extract-treated mice resulting in fecal excretion of cholesterol.

Our study has certain limitations. The duration of our study is short (12 weeks) and hence we cannot anticipate the sustained effects of OP extract. A long-term study is required to be conducted in future to know the side effects of OP extract when used over a long period.

In summary, several lines of evidence support our hypothesis that OP extract aids in the lowering of serum and hepatic cholesterol via up-regulation of LDL-R and CYP7A1 gene expression, thereby facilitating the removal of cholesterol via fecal excretion. Apart from the cholesterol-lowering effect, the extract has proven to be beneficial in averting lipogenesis in the liver (35). Our findings suggest that OP may act as a phytochemical to regulate hypocholesterolemia; however, relevant studies in humans are required to explore its use as a hypocholesterolemic food supplement.

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**Disclosure** The authors declare no conflict of interest.

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