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Hypocholesterolemic Property of *Yucca schidigera* and *Quillaja saponaria* Extracts in Human Body

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This study was undertaken to observe the effects of the blend of partially purified *Yucca schidigera* and *Quillaja saponaria* extracts on cholesterol levels in the human's blood and gastrointestinal functions, and to determine if a new cholesterol-lowering drug can be developed by the further purification of the extracts. Ultrafiltration and sequential diafiltration increased the amounts of steroidal saponin in aqueous *yucca* extract and terpenoid saponin in aqueous quillaja extract from 9.3% and 21.4% to 17.2% and 61.8%, respectively. Taking 0.9 mg of the blend (6:4, v:v) of the resulting filtrates a day for 4 weeks resulted in the decreases in total and LDL cholesterol levels in blood plasma of hyper-cholestrolemic patients with enhancement in gastrointestinal symptoms of patients.

Key words: *Yucca schidigera*, *Quillaja saponaria*, Hypercholesterolemia, Saponin

INTRODUCTION

Cholesterol, a representative sterol and a component of all euaryotic plasma membranes, is necessary for the growth and viability of higher organisms. However, several diseases, such as arteriosclerosis, cardiac insufficiency and hyperpiesia, are caused by the excessive intake of cholesterol. Especially, coronary heart disease is the major cause of death in many western industrialized countries, and recently has become a more signitiitiant health risk in Korea. Therefore, the excessive amount of cholesterol in diet needs to be removed, because the minimum amount of cholesterol necessary for living is produced in the body. Numerous efforts have been made to reduce the cholesterol level in foods and living organisms by utilizing saponins (Griminger and Fisher, 1958; Malinow *et al.*, 1977; Nakaue *et al.*, 1980; Newman *et al.*, 1957; Sim *et al.*, 1984). Saponins are natural detergents found in many plants, which consist of a fat soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water soluble carbohydrates. The worldwide two major commercial sources of saponins are *Yucca schidigera*, which grows in the arid Mexican dessert, and *Quillaja saponaria*, a tree that grows in the arid areas of Chile. The saponins water-extracted from the trunk of *yucca* and the bark of *quillaja* contain steroid and triterpenoid nucleuses, respectively. It has been known for many years that saponins form insoluble complexes with cholesterol (Lindahl *et al.*, 1957). Saponins form micelles with sterols such as cholesterol and bile acids. Sapogenin, the hydrophobic portion of saponin associates with the hydrophobic sterol nucleus, in a stacked micellar aggregation (Oakenfull and Sidhu, 1989). It was demonstrated that dietary saponin reduced blood cholesterol levels in chickens (Griminger and Fisher, 1958; Newman *et al.*, 1957) and this effect was considered as a result of saponins binding to cholesterol in the bile in the intestine, and preventing its reabsorption. Efforts to reduce egg cholesterol levels by feeding saponin sources to laying hens had generally not been successful (Nakaue *et al.*, 1980; Sim *et al.*, 1984). The main source

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of egg cholesterol was endogenous synthesis in the ovary, so reductions in blood cholesterol in laying hens did not result in lowered egg cholesterol. Dietary saponins also reduce blood cholesterol levels in mammals (Oakenfull and Sidhu, 1989). Malinow *et al.* (1977) showed that alfalfa saponins have hypocholesterolemic activity in non-human primates. Cholesterol-lowering properties of saponins in human are of obvious interest. However, there is little clinical trial information. Chapman *et al.* (1997) observed that the Masai people of East Africa have low serum cholesterol levels in spite of a diet rich in animal fats, and contributed the low cholesterol levels to the Masai diet, in which saponin-rich herbs were added to milk and meat-base soups. This appeared to be the only study reported in which a saponin product had been given directly to human subjects.

Therefore, this study was undertaken to observe the effects of *Yucca schidigera* and *Quillaja saponaria* extracts on cholesterol levels in the human's blood and gastrointestinal functions, and to produce a nutraceutical ingredient economically as a preliminary study for the further purpose of development of a new cholesterol-lowering drug without severe side effects.

MATERIALS AND METHODS

Materials

Yucca schidigera and *Quillaja saponaria* used in this study were harvested in Mexico and Chile, respectively, and processed to produce the extracts by Celltechs Corporation in Korea. The trunk of the *yucca* were mechanically ground, squeezed in a press to produce *yucca* juice, and then, partially concentrated by evaporation to produce *yucca* extracts. The *quillaja* extracts were produced by the aqueous extraction of the bark of the *quillaja* and partial evapo-concentration.

Sample preparation

The extracts of *yucca* and *quillaja* were partially purified and further concentrated by ultrafiltration processes using 100 kDa molecular weight cut-off membrane (Romicon Type PM 10), in order to increase the saponin fraction in the extracts by removing large molecular weight materials. After ultrafiltration, the amounts of salt, sugar and phenols in the extracts were then reduced by a diafiltration process using 20 kDa molecular weight cut-off membrane (Romicon Type PM 10). The concentrated *quillaja* and *yucca* extracts were freeze-dried and blended in 6:4 proportion (w:w basis) which was determined in our preliminary study as the optimum proportion of *quillaja* and *yucca* extracts for masking bitter taste and unpleasant odor, and named YQ2.

Quantifications of saponins

The amounts of steroidal saponins in the ultrafiltrated and dried *yucca* extract were determined by butanol extraction method (Bernardo *et al.*, 1996; Miamki *et al.*, 1995). The dried extract was defatted with ether 3 times and extracted with butanol 5 times. The butanol fraction was evaporated and the residue was determined as steroidal saponins in *the yucca* extract.

RP-HPLC was carried out based on the report of Martin and Briones (2000), in order to quantify the *quillaja* saponins, mostly triterpenoidal saponins. A Waters model 501 chromatograph equipped with a Waters model 441 detector and a column (WATO 27324, 3.9×300 mm) was used. The sample loop was 20 μL. Solvent A was ultrapure water 1.5 g/kg TFA, and solvent B was ultrapure acetonitrile with 1.5 g/kg TFA. The gradient was started with 30% of B in A, increasing to 45% in 35 min, and maintained for 5 min at 45%. The flow rate was 1 mL/min and detection was performed at 220 nm. Both *quillaja* extract and ultrafiltrated qullaja extracts prepared as mentioned previously were diluted 10 times with distilled water and then injected. In all chromatograms, saponins were considered to elute after 6 min. This is based on the fact that hydrophilic tannins and polyphenolics elute in the early part of the organic solvent gradient (1996). The area of saponins in the sample was compared to that of the standard, and the concentration of saponins was estimated as;

$$13.5 \times (A_{sample}/A_{standard})$$

Characterization of saponins

Infrared (Perkin-Elmer 2000 series IR) spectrometer was used to verify the structures of two extracts. Wavelength (cm^{-1}) was in the range from 4,000 to 400. IR spectra were measured in the form of KBr (Aldrich) pellet. The molecular weights of the polymers were measured by static light scattering (Varian CARY 1E UV/VIS spectrometer, He/Ne laser source at 488 nm). The incident beam was unpolarized and the thermostated sample cell is placed on a motor-driven precision goniometer that enables the photomultiplier detector to be moved accurately from 20° to 150° at scattering angle. The intensity was monitored by photomultiplier. The temperature was kept constant during the measurement using a water circulator at 25°C. Samples were prepared at 4 different concentrations (10, 5, 2.5 and 1.25 g/L) in DMSO and filtrated through a membrane filter (0.1mm pore size). Particle size was also obtained by dynamic light scattering at 25°C after the samples were filtrated by syringe filter of 0.1 mm pore size. The concentration of samples was fixed at 1 g/L in distilled water.

Clinical analysis

Eighty-six patients, having more than 220 mg/dL

triglyceride level in blood, were given either the YQ2 or placebo in a random selection double-blind study at Miz Medi Hospital in Seoul, Korea. Before the test, the patients who had diseases or medications affecting to lipid metabolism or gastrointestinal function within recent 3 months, were excluded in this study. The amounts of cholesterol and triglyceride in the blood were analyzed on their first visit to compare the amounts after treatments. Each patient was given 0.3 g of the YQ2 or placebo three times a day after dilution with 180 mL of water for 4 weeks, and the amounts of cholesterol and triglyceride in the blood were analyzed. Gastrointestinal symptoms were also scored by questionnaire at baseline and 4 weeks after initiation of intaking YQ2. The symptoms in the questionnaire were composed of abdominal bloating, gas distension, constipation, and diarrhea (symptom score: very severe-4, severe-3, moderate-2, mild-1, and none-0).

Statistical analysis

Three replications were performed in a completely randomized design. Data were analyzed using the general linear model procedures of SAS (1992) to determine differences between treatment means. Pair-wise comparison of all treatment means was performed using the Least Significant Difference (LSD) procedure with significance defined at $P<0.05$.

RESULTS AND DISCUSSION

Ultrafiltration and diafiltration increased the amounts of butanol-extractable fraction (presumably steroidal saponin) in resulting aqueous *yucca* extract and terpenoid saponin in aqueous qullaja extract from 9.3% and 21.4% to 17.2% and 61.8% (dry base). The increment of saponin content in *yucca* extract by the filtrations was relatively small due to the low molecular weight of *yucca* saponins and their passing through the 20 kDa molecular weight cut-off membrane (although they exist in the form of micelles). Moreover, diafiltration of *yucca* extract was not feasible due to increasing viscosity as the diafiltration process went on, and consequently, it was concluded that diafiltration process was not efficient for the partial concentration of *yucca* saponins. On the other hand, *quillaja* saponins did not pass through the 20 kDa molecular weight cut-off membrane because they had relatively high molecular weight of 1.8 kDa and formed aggregates of 50 or more molecules (Kensil *et al.*, 1991; Oakenfull and Sidhu, 1989). Nevertheless, in these processes there were some losses of very large aggregates (>100 kDa) and monomer molecules which always co-existed in equilibrium with aggregates. The increasing viscosity of the *quillaja* extract with diafiltration process was not so severe as the *yucca* extract, and thus, the *quillaja* saponin was concentrated efficiently. The partially purified and concentrated *quillaja* and *yucca* extracts were, then, freeze-dried and blended in 6:4 proportion (w:w basis) to produce YQ2 as mentioned previously.

The FT-IR spectra of two extracts were shown in Fig. 1. The FT-IR spectra of two extracts showed the broad peak of hydroxyl group at 3,300cm^{-1}. Peaks from ring structure was also observed in 3,000~3,100 cm^{-1} and 1,100~1,200 cm^{-1}. IR results indicated that two extracts had the typical shape of saponin which had both hydroxyl group and ring structure.

Molecular weights of *yucca* and *quillaja* saponins were 12,000 and 63,500 g/mol, respectively. These data was close to the expected molecular weight. Particle size of saponins was connected with micelle formation. *Quillaja* saponins could form the micelle because of the amphiphilic structure. Its particle size by intensity was 670 ± 92 nm. In contrast, *yucca* saponin did not form micelles in spite of the existence of aggregates.

Eighty-six patients were included in the clinical tests at Miz Medi Hospital in Seoul, Korea. These were divided into two groups. The first group of 51 patients received YQ2 for 4 weeks while the other group of 36 patients received placebo composed of lactose, whey and protein. The clinical characteristics of the patients are described in Table I, and the clinical results after the intake of either YQ2 or placebo for 4 weeks were described in Fig. 2. High density lipoprotein (HDL) and low density lipoprotein (LDL) were the major carriers for cholesterol transportation in blood, where the former transported 25% cholesterol in serum and the latter transported 75% (Choi *et al.*, 2001). YQ2 significantly reduced total cholesterol and LDL levels without significant changes in HDL level, indicating that

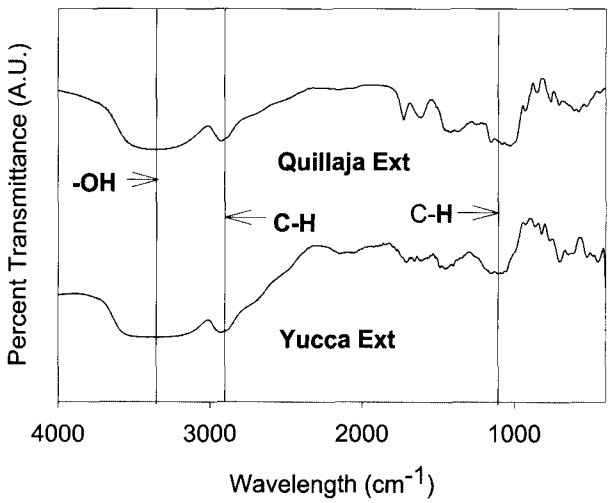

Fig. 1. IR spectra of *Yucca schidigera* and *Quillaja saponaria* extracts. IR spectra were measured in the form of KBr pellet. Wavelength (cm^{-1}) was in the range from 4,000 to 400.

Table I. Clinical characteristics of subjects

	YQ2 group	Placebo group
Number	51	35
Age (year)	60.1± 1.3	55.8± 7.8
Total cholesterol (mg/dL)	246.5±20.2	239.8±16.4
LDL[a] (mg/dl)	161.1±25.8	150.9±25.5
HDL[b] (mg/dl)	53.4±13.2	58.1±17.2
Triglyceride (mg/dL)	167.9±68.2	137.9±61.1

[a]LDL : low density lipoprotein
[b]HDL : high density lipoprotein
Values represent mean±S.D.

YQ2 effectively decreased total cholesterol level through lowering LDL level, the most harmful lipid. The mechanism of YQ2 was not completely known in lipid metabolism. However, recent researches (Harwood *et al.*, 1993; Jenkins and Atwal, 1994; Oakenfull and Sidhu, 1989) suggested that the saponins in YQ2 had the similar molecular structure to cholesterol and the unique characteristics having both hydrophilicity and lipophilicity. YQ2 tightly binded to the digested lipid, bile acid, mixed-micelle and intestinal cells absorbing lipid in the small intestine. The materials bound with YQ2 became the insoluble unabsorbed form, and then, excreted as stool. Although another possible mode of action via increased intestinal cell turn-over rate (Gee *et al.*, 1997; Milgate and Roberts, 1995) has been suggested, it is generally accepted that the principal action of saponins on blood cholesterol is by sequestration of cholesterol and bile acids in the intestine. Because the mechanisms of YQ2 is similar to orlistat (Xenical, Roche co.) known as an anti-obesity medicine in lipid metabolism, it may be possible for YQ2 to be treated for the obesity. Although YQ2 was not more effective against hypercholesterolemia than HMG-CoA reductase inhibitors (statins; lowering 20-40% LDL level), more effective than the other medicines such as bile acid sequestrants (lowering 10-20% LDL level), nicotinic acid, and fibric acids in lipid metabolism. Most of hypercholesterolemic patients knew the efficacy of the lipid-lowering medicines, however, they want becoming treated by diet and exercise therapy rather than by medication due to the anxiety about becoming a real patient as well as the negative gastrointestinal side-effects of the medicines, such as gas distension, belching, constipation, and diarrhea. YQ2 contributed not only to the excellent tolerability but also to the improvements in gastrointestinal motility (Fig. 3). The saponins in YQ2 prevented the oxidation of secondary bile acid, known as a carcinogen of colon cancer (Koratkar and Rao, 1997; Rao and Sung, 1995), and the growth of pathogens, such as protozoa and gram-negative bacteria (Bingham, 1976; Olson *et al.*, 1996), in the large intestine, and thus, could enhance the gastrointestinal functions.

Recently, although many investigators have researched on the natural lipid-lowering products, most of the studies on the natural products were not well designed or had been failed to prove the lipid-lowering effect in the human body. However, this clinical study improved that YQ2 could be available to treat the hypercholesterolemia by

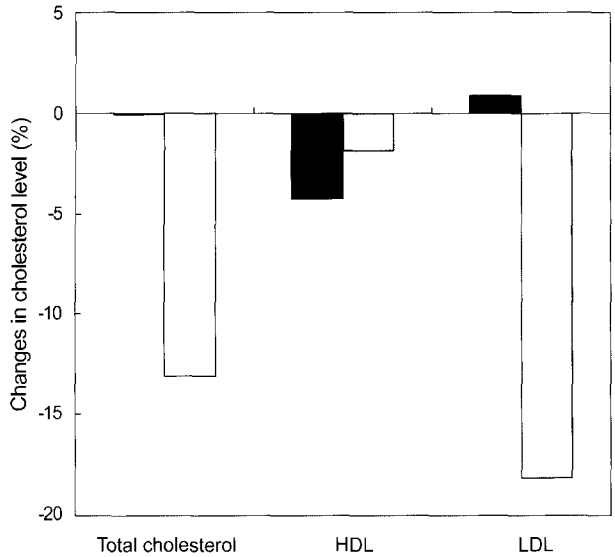

Fig. 2. Percent changes of chorlesterol level in blood plasma. Eighty-six patients were given either the YQ2 (□; n=51) or placebo (■; n=35) in a random selection double-blind study at Miz Medi Hospital. Each patient was given 0.3 g of the YQ2 or placebo three times a day after dilution with 180 mL of water for 4 weeks, and then, the amounts of cholesterol and triglyceride in the blood were analyzed.

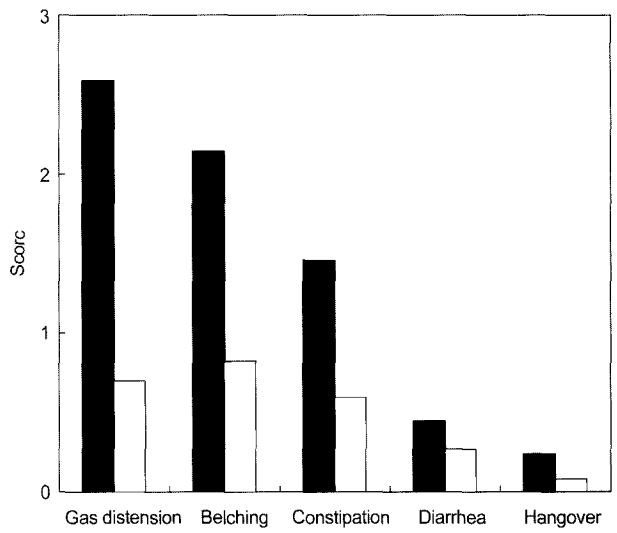

Fig. 3. Changes in symptoms of gastrointestinal motility (■: Before taking YQ2; □: After taking YQ2 for 4 weeks). Gastrointestinal symptoms were scored by questionnaire at baseline and 4 weeks after initiation of therapy. The symptoms in the questionnaire were composed of abdominal bloating, gas distension, constipation and diarrhea (symptom score: very severe-4, severe-3, moderate-2, mild-1 and none-0).

taking directly as a tablet or adding to beverages or foods, especially the foods rich in fat.

In conclusion, even the treatment of the partially purified *yucca* and *quillaja* saponins resulted in decreased cholesterol levels in the blood of hypercholesterolemic patients, implying that a new cholesterol-lowering drug could be developed by the further purification of YQ2 saponins. Additionally, the intaking of 0.9 mg of YQ2 a day, which might be too a small amount to give the beverages and foods unfavorable tastes, was enough to reduce the cholesterol level. Moreover, it was feasible to apply YQ2 in beverage or food production due to its good emulsifying and foaming properties (Martin and Briones, 2000).

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