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Effect of *Quercus infectoria* and *Rosa damascena* on Lipid Profile and Atherosclerotic Plaque Formation in Rabbit Model of Hyperlipidemia

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Abstract: Hyperlipidemia is the cause of many complications in the human societies. In this study, the effect of methanol extracts of *Quercus infectoria* (QI) galls and *Rosa damascena* (RD) Mill flower were studied on lipid profile and atherosclerotic plaques formation in hyperlipidemic rabbits. Thirty-six New Zeland white rabbits randomly divided into 6 groups as control (I), hyperlipidemic (II), hyperlipidemic+QI (III), hyperlipidemic+RD (IV), +Atorvastolin (V) and hyperlipidemic+Orlistat (VI) and were fed with high fat diet (0.5% cholesterol and 16% hydrogenated vegetable oil) for 45 days. At the end of the study period, lipid profile and plaque formation were assessed. Total Cholesterol (TC), Low Density Lipoprotein (LDL) and Triglyceride (TG) levels were significantly increased in hyperlipidemic group compared with control group ($p<0.001$). Methanol extract consumption of *Quercus infectoria* significantly decreased plasma levels of TC, TG and LDL ($p<0.001$). It also decreased plaques formation in semi lunar valve and thoracic aorta. *Rosa damascena* mill flower methanol extract moderately decreased the levels of TC, TG, LDL and plaques formation but it was not significant. HDL levels and weight of animals did not show significant difference among groups. Based on the doses used in this study, our finding indicated that QI but no RD methanol extract has anti atherogenic and hypolipidemic activities.

Key words: *Quercus infectoria, Rosa damascena*, hyperlipidemic rabbit, HMG CoA reductase and pancreatic lipase

INTRODUCTION

Atherosclerosis is the main cause of death in developed and most developing countries and its incidence is related to lipid disorders (Mohammadi *et al*., 2009). Obesity, high blood pressure, smoking, diabetes, hypercholesterolemia and inflammation are considered as risk factors of atherosclerosis (Huang *et al*., 2007). The popularity of medicinal plants is increasing due to their lesser side effects and better compatibility (Karim *et al*., 2011). Traditional medicines have been used for the treatment of various diseases by mankind for centuries. Since plants are rich resources of biological active compounds, they must be examined for the discovery of new anti hyperlipidemic agents (Ozcelika *et al*., 2005). *Quercus infectoria* (QI) Olivier (Fagaceae) is a small tree found in Greece, Asia Minor and Iran. The galls of QI have also been pharmacologically documented to possess anti alpha-glucosidase, antidiabetic, analgesic, antiviral, antibacterial, anti alpha-mannosidase and anti-inflammatory activities (Umachigi *et al*., 2008; Gholamhoseinian *et al*., 2008a, b). *Rosa damascena* (RD) (Rosaceae) is also used in various conditions including menstrual bleeding, digestive disorders and headache (Rakhshandeh *et al*., 2004). Pancreatic Lipase (PL) is involved in triglyceride absorption from small intestine and HMG CoA reductase is involved in endogenous cholesterol biosynthesis (Jung *et al*., 2005; Sharma *et al*., 2005). If somehow initial movement of triglycerides and cholesterol from the lumen is blocked or cholesterol endogenous synthesis be inhibited, hyperlipidemia can be prevented (Sharma *et al*., 2005). A wide variety of plant compounds exhibited anti PL (Sharma *et al*., 2005; Won *et al*., 2007) and anti HMG CoA reductase activities (Jung *et al*., 2005; Xie *et al*., 2007). We found that methanol extract of QI galls and RD Mill flower

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are potent inhibitors of PL and HMG CoA reductase *in vitro* and then we convinced to examine their effects *in vivo*. Therefore, the present study was designed to assess the therapeutic effects of two mentioned extracts on lipid profile and atherosclerotic plaques formation in rabbits with long-term high fat diet.

MATERIALS AND METHODS

Plant materials: The plants were collected during spring 2009 in Kerman province, Iran and authenticated by Dr. Mirtajaddini, Department of Botany, Bahonar University, Kerman, Iran. A voucher specimen of each plant (*Rosa damascena*: KF 1135, *Quercus infectoria*) was deposited in the herbarium center, faculty of pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Extraction method: The air-dried flowers of RD and galls of QI (300 g) were milled to fine powder for extraction by maceration method in 1000 mL methanol at room temperature for 72 h. The extract was evaporated in vacuum to yield a waxy mass from RD and a powder mass from QI and then was kept in dark vials at -20°C until the time of the experiments (Gholamhoseinian *et al*., 2009).

Animal study design: Thirty-six New Zeland white rabbits weighing 2-3 kg were used in this study. They were kept individually in similar conditions and 12 h/12 h light dark cycle with light at day time and at room temperature (22-25°C). Animals had free access to food and water *ad libitum*. The rabbits were randomly divided into the six groups as control (I), hyperlipidemic (II), hyperlipidemic+QI methanol extract (III), hyperlipidemic+RD methanol extract (IV), hyperlipidemic+Atorvastatin (V) and hyperlipidemic+Orlistat (VI) (n=6). Group I was fed with normal rabbit chow and group II with normal rabbit chow supplemented with cholesterol and oil (cholesterol 0.5% and hydrogenated vegetable oil 16%) for 45 days. Groups III to VI, in addition to the diet of group II, received 1.5 g kg^{-1} of the diet of QI methanol extract (group III), 1.5 g kg^{-1} of the diet of RD methanol extract (group IV), 50 mg kg^{-1} of the diet of Atorvastatin (group V) and 50 mg kg^{-1} diet of Orlistat (group VI), respectively (Yu *et al*., 2005; Huang *et al*., 2007). All experiments were approved by the ethics committee of the Kerman University of Medical Sciences.

Biochemical analysis: The animals were anesthetized by intraperitoneal injection of sodium thiopental (50 mg kg^{-1}) and then were sacrificed and blood samples were collected. At the end of study period, blood samples were taken and plasma levels of Total Cholesterol (TC), triglyceride (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) levels were measured by enzymatic assays.

Histological analysis: Finally, animal's hearts were removed, cleaned and fixed in 10% buffered formalin. A cross-shaved incision was made on the upper part of heart to obtain the semi lunar valve and thoracic aorta was opened longitudinally and horizontally sectioned. Two sequential 4 μm thick sections were cut from thoracic aorta and semi lunar valve and then embedding in paraffin were stained with hematoxylin and eosin (H and E) (Reilly *et al*., 2008). Then, prepared slides were evaluated and scored independently by two pathologists blinded to animals, groups.

The presence of atherosclerotic plaques was scored between zero to six as stated below (King *et al*., 2009; Reilly *et al*., 2008):

Class zero: Normal valve, Class one: Focal lipophage collections (×40)
Class two: Lipophage collections (×10)
Class three: Diffuse (Intiman lipophage collection×4)
Class four: Fatty plaques
Class five: Fibro fatty plaques
Class six: Fibro sclerotic plaques (Reilly *et al*., 2008)

Statistical analysis: All data are presented as Mean±SD. Statistical analysis was performed by analysis of variance (ANOVA) and Post-Hoc Tukey test; p-values of less than 0.05 were considered to be significant (we also performed non-parametric test Kruskal-Wallis for plaques analysis that was significant, then we analyzed based on ANOVA).

RESULTS

Biochemical analysis: Table 1 is showing the biochemical results of this study. In the hyperlipidemic group, TC, LDL and TG levels significantly increased compared with the control group (I) ($p<0.001$). In comparison with the hyperlipidemic group, QI extract and Atorvastatin but not RD extract and Orlistat significantly decreased TC and LDL levels ($p<0.05$, respectively). On the other hand, TG levels significantly reduced only in III and VI groups when compared with the hyperlipidemic group (II). HDL levels and animals weight did not show significant difference among groups.

Histological results: Plaques formation showed some differences in the study groups (Table 2). There was no obvious change in the control group. Severities of lesions in semi lunar valve were more than thoracic aorta in groups II to VI (Fig. 1a-i). Plaque formation in semi lunar valve for groups II to VI showed significant increase

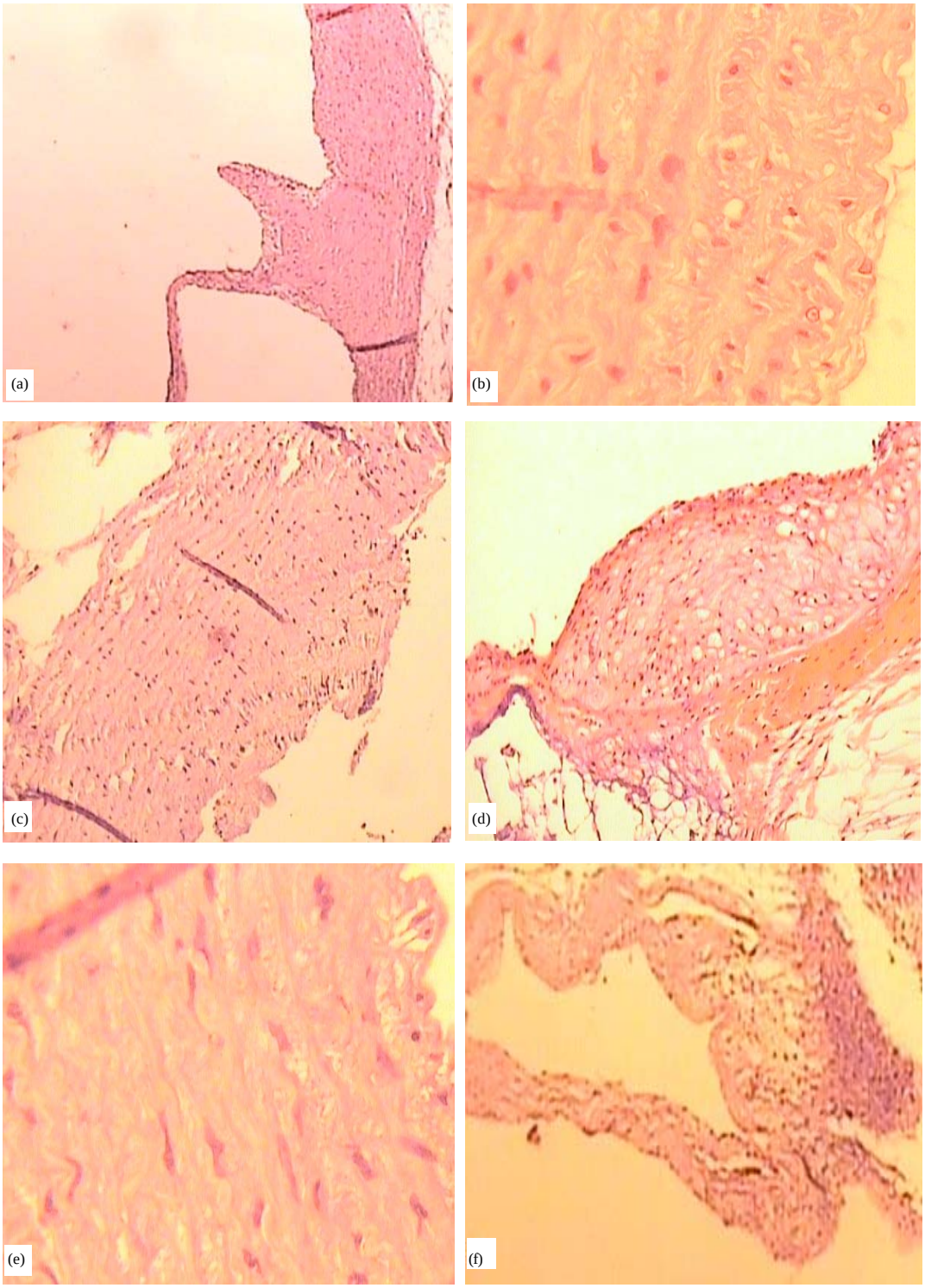

Fig. 1: Continued

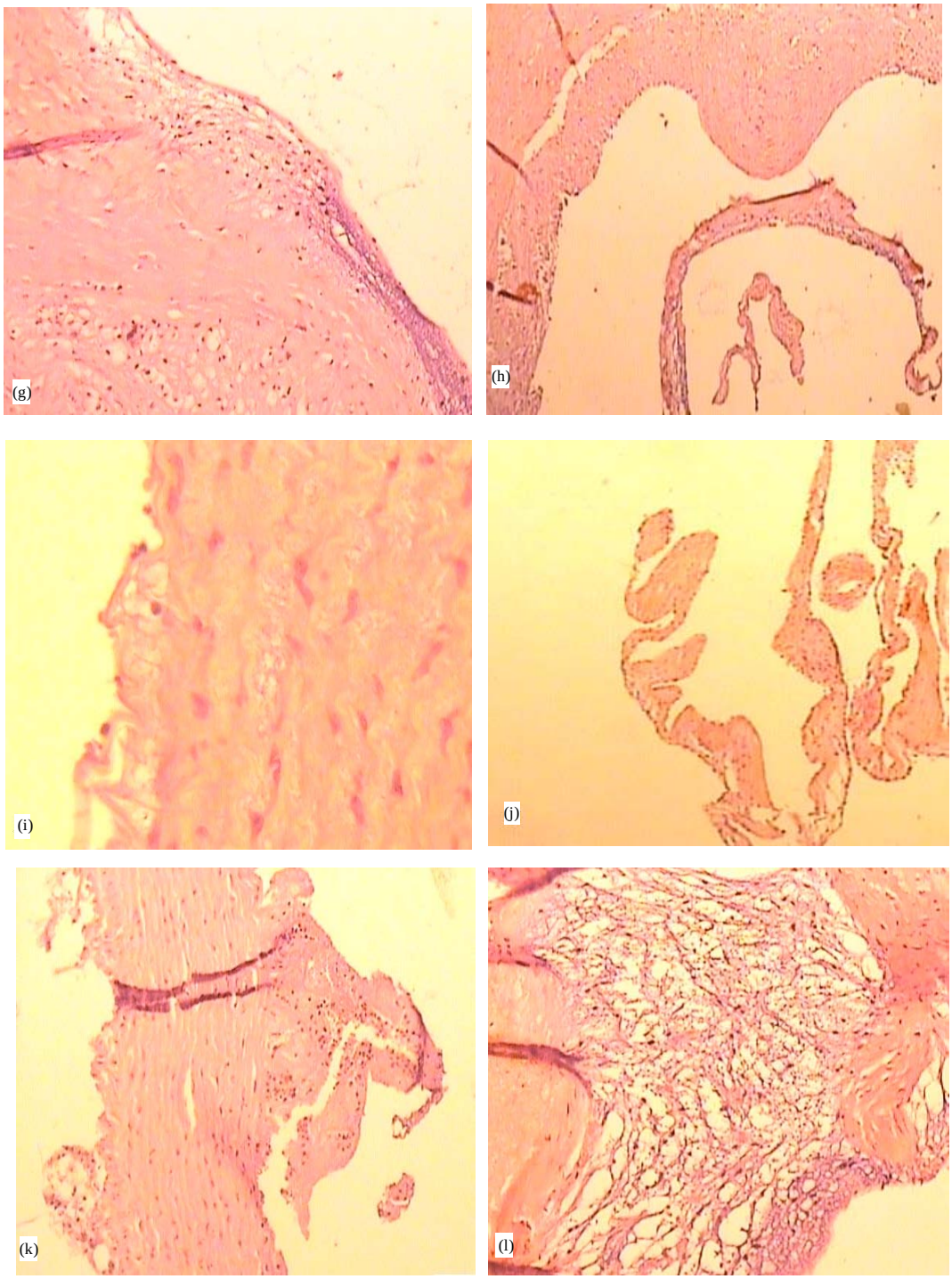

Fig. 1(a-l): Sections of thoracic aorta and semi lunar valve stained by H and E in studied groups. (a and b) Standard group (I), (c and d) Hyperlipidemic group (II), (e and f) Hyperlipidemic+QI methanol extract group (III), (g and h) Hyperlipidemic+RD methanol extract group (IV), (i and j) hyperlipidemic+Atorvastatin group (V) and (k and l) hyperlipidemic+Orlistat group (VI)

Table 1: Lipid profile at baseline and 45 days in 6 studied groups

Parameters (n = 6)	Baseline	45 days
Total cholesterol (mg dL^{-1})		
Standard	53.00±11.9	56.00±12.8[c]
Hypercholesterolemic	48.00±8.60	1327.60±139.0[a]
Hypercholesterolemic+QI extract	52.00±12.2	642.10±224[bd]
Hypercholesterolemic+RD extract	59.83±16.3	1088.00±412.0[a]
Hypercholesterolemic+atorvastatin	68.83±19.7	630.80±182[bd]
Hypercholesterolemic+orlistat	60.33±10.9	1050.00±341.0[a]
Triglyceride (mg dL^{-1})		
Standard	86.10±11.28	90.60±12.8[c]
Hypercholesterolemic	7.22±16.45	211.00±514.0[a]
Hypercholesterolemic+QI extract	73.00±27.50	115.80±50.8[d]
Hypercholesterolemic+RD extract	82.10±23.11	142.60±43.2
Hypercholesterolemic+atorvastatin	101.00±23.92	144.60±51.4
Hypercholesterolemic+orlistat	90.10±32.44	110.30±16.7[d]
LDL-C (mg mL^{-1})		
Standard	21.50±8.31	20.50±7.91[c]
Hypercholesterolemic	16.20±13.2	536.20±42.5[a]
Hypercholesterolemic+QI extract	15.00±10.2	324.80±71.1[ad]
Hypercholesterolemic+RD extract	15.67±10.9	395.10±72.7[a]
Hypercholesterolemic+atorvastatin	17.00±8.74	351.10±105[ad]
Hypercholesterolemic+orlistat	23.00±13.1	445.30±130[a]
HDL-C (mg mL^{-1})		
Standard	135.60±16.47	141.30±17.80[d]
Hypercholesterolemic	158.00±30.30	319.00±51.45[b]
Hypercholesterolemic+QI extract	154.80±31.03	417.60±145.2[a]
Hypercholesterolemic+RD extract	197.50±83.72	326.10±130.2[b]
Hypercholesterolemic+atorvastatin	206.30±144.7	247.10±55.26
Hypercholesterolemic+orlistat	176.10±19.39	228.80±42.64

Data are the Mean±SD. [a]p<0.001, [b]p<0.05 compared to standard group (I). [c]p<0.001, [d]p<0.05 compared to Hyperlipidemic group (II)

Table 2: Mean of the atherosclerotic plaques formation in studied groups in semilunar valve and thoracic aorta

Parameters (n = 6)	Mean±SD
Atherosclerotic plaques in heart lats	
Standard	0.0±0.0
Hypercholesterolemic	5.0±0.0
Hypercholesterolemic+QI extract	3.5±0.8[a]
Hypercholesterolemic+RD extract	5.1±0.4
Hypercholesterolemic+atorvastatin	3.7±0.5[a]
Hypercholesterolemic+orlistat	5.1±0.4
Atherosclerotic plaques in thoracic aorta	
Standard	0.0±0.0
Hypercholesterolemic	4.0±0.7
Hypercholesterolemic+QI extract	1.6±1.5[a]
Hypercholesterolemic+RD extract	3.7±0.5
Hypercholesterolemic+atorvastatin	3.3±0.5
Hypercholesterolemic+orlistat	3.1±0.4

Data are Mean±SD. Plaques formation in heart lats, [a]p<0.001 compared to Hyperlipidemic group. Plaques formation in thoracic aorta, [a]p<0.001 compared to Hyperlipidemic group

compared to the control group (p<0.001). However, the severity of lesions significantly reduced in Groups III and V compared with the hyperlipidemic group. The patterns of plaque formation in thoracic aorta were similar to those in aortic valve in tests groups but, compared to the hypercholestrolemic group just group III showed significant decrease of plaque formation (p<0.001) (Table 2, Fig. 1).

DISCUSSION

The present study was conducted to evaluate the effects of QI and RD methanol extracts on lipid profile and atherosclerotic plaque formation of hyperlipidemic rabbits. The results showed that QI but not RD extract significantly decreased TC, LDL, TG and atherosclerotic plaque in comparison with the hyperlipidemic group. None of the extracts had any obvious effect on HDL when compared with the hyperlipidemic group. Decremental effects of QI extract on plasma levels of TC and TG were similar to Atorvastatin and Orlistat, respectively.

Digestion of dietary triglycerides representing 90-95% of the total ingested fat is driven in the intestine by PL. The most common anti-obesity drug, Orlistat, is a potent irreversible inhibitor of pancreatic lipase and it has been proved to be effective in the treatment of human obesity by causing 35% reduction in fat absorption (Slanc *et al*., 2009).

Statins inhibit the HMG CoA reductase competitively and have beneficial effects on hypercholesterolemia and cardiovascular events and are among the most widely prescribed drugs in the world (Carbonell and Freire, 2005).

An *In vitro* experiment demonstrated that although methanol extracts of QI and RD have anti PL and HMG CoA reductase activities, the suppressive effect of QI on these enzymes is more prominent than RD. In line with our previous study, the present study indicated the diminishing effect of QI extract on plaque formation and lipids profile obviously. These findings suggest that part of anti hyperlipidemic effect of QI can be through PL and HMG Co A reductase inhibition (Gholamhoseinian *et al*., 2010a, b).

Many plant compounds such as polyphenols have an affinity for protein through hydrophobic and hydrogen bonds and they showed ability to reduce activity of some enzymes like PL (Birari and Bhutani, 2007). The constituents of QI are including 50-70% tannin and small amount of free gallic acid, methyl gallate and ellagic acid (Umachigi *et al*., 2008; Basri and Fan, 2005; Pithayanukul *et al*., 2009). It is well established that tannin is a phenolic compound that is soluble in water, alcohol and acetone and can precipitate proteins (Basri and Fan, 2005). Tannin as the main constituent of QI may be responsible for many of its effects (Basri and Fan, 2005).

Pancreatic Cholesterol Esterase (pCE) and intestinal (ACAT) are involved in the cholesterol movement from small intestine through the brush border (Park *et al*., 2002). Sterols have been found in some parts of plants. Plant sterols have been investigated as good and safe alternatives to lower plasma cholesterol levels. Phytosterols structurally are similar to cholesterol but remarkably are different in intestinal absorption and metabolic fate. Phytosterols intestinal absorption is poor and they can disrupt cholesterol intestinal absorption without any serious side effects (Moghadasian, 2000; Ros, 2000). Therefore, plants with high content of sterols and other hypolipidemic components have a great value to reduce absorption and plasma levels of cholesterol. Inhibition of pCE and intestinal ACAT can be another mechanism applied by QI to lower cholesterol levels.

It had been reported that synthetic PL and HMG CoA reductase inhibitors have some unpleasant side effects. Myopathy, liver damages and potential drug-drug interaction are some adverse effects of Statins. Consumption of synthetic drugs against PL leads to hyperuricemia, gastric irritation, flushing, dry skin, oily spotting, fecal incontinence and abnormal liver function (Gholamhoseinian *et al*., 2010a; Deng, 2009).

Overall, methanol extract of QI galls showed hypotriglyceridemic, intensive hypocholestrolemic and anti-atherogenic activities in high fat diet fed rabbits. Regarding the less side effects of botanical drugs, we suggest that QI gall potentially can be a suitable alternative for cholesterol and TG lowering agents in hyperlipidemic patients. Despite the inhibitory effect of RD on lipid digestion enzymes as reported in *in vitro* study, the decremental effect of 1.5 g kg^{-1} diet of RD on plasma lipids in our study was not supported. Hence, the use of higher doses of RD in next similar studies is the other suggestion of authors.

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