

Full Length Research Paper

Regression of hypercholesterolemic atherosclerosis in rabbits by hydroalcoholic extracts of *Hypericum perforatum*

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Regression and suppression of atherosclerotic lesion may be a realistic goal in some patients. Antioxidants and hypolipidemic agents suppress the development of hypercholesterolemic atherosclerosis and induce regression of atherosclerosis. The objectives of this study were to determine effects of *Hypericum perforatum* L. (HPL) on regression of atherosclerosis in hypercholesterolemic rabbits. Rabbits were assigned to four groups: Group I control diet (75 days); Group II cholesterol diet (75 days); Group III cholesterol diet (45 days) followed by regular diet (30 days); Group IV cholesterol diet (45 days) followed by regular diet and HPL (30 days). Blood samples were collected from rabbits before and after 45 days and 75 days respective for measurement of biochemical factors. At the end, aorta was removed for assessment of atherosclerotic plaques. It increases in serum total cholesterol, triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoproteinB (apoB), AI, high-sensitive C-reactive protein (hs-CRP), Malondialdehyde (MDA), oxidized LDL (OX-LDL), apolipoproteinA (apoA), white blood cell (WBC), fibrinogen and platelet in Group II, III, IV on the 45th days, comparing with Group I and the beginning of the study. At the end, biochemical factors serum decreased in Groups III and IV. There was more Biochemical factors serum and atherosclerotic lesions decreased in Groups IV compared to Group III in regression period. The reduction in atherosclerotic lesions was associated with a reduction in oxidative stress. These results suggest that regular diet following a high cholesterol diet accelerates atherosclerosis; HPL treatment prevents the progression of atherosclerosis on Group IV; prevention of progression is associated with a reduction of inflammatory factors and antioxidant mechanism may induces regression of atherosclerosis lesion.

Key words: Atherosclerosis, regression, *Hypericum perforatum*, rabbits, oxidized low density lipoprotein (OX-LDL), C-reactive protein, white blood cell (WBC), fibrinogen, platelet.

INTRODUCTION

Atherosclerosis is a complex process; lipid oxidation and inflammation play a central role in the development of atherosclerosis (Güldiken et al., 2005). Lipid peroxidation products generated during low density lipoprotein oxidation are chemotactic for both monocytes and T cells, and alterations cause macrophages migration into the subendothelial (Güldiken et al., 2005) and subsequent uptake of oxidized low density lipoprotein (OX-LDL) by

scavenger receptors would result in foam cell formation and later develops to fatty streaks (Libby and Aikawa, 2002).

Recent reports indicate that the reversal or regression of lesions can be achieved by aggressive lipid lowering or drug treatment (Oka and Chan, 2005; Frisinghelli and Mafri, 2007). As lipids, such as cholesterol and low density lipoprotein cholesterol (LDL-C), are the major component of these early lesions, one of the approaches to reduce the development of lesions in early stages is lipid lowering through dietary interventions (Boban et al., 2008).

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Other parameters also associated with elevated cardiovascular risk, are apolipoproteins. Apolipoproteins are important components of lipoprotein particles, and there is accumulating evidence that measurement of various forms of apolipoproteins may improve the prediction of the risk of cardiovascular disease (Genest et al., 1992; Talmud et al., 2002).

Although the etiology of chronic subclinical inflammation is unknown, elevated atherogenic apolipoprotein B100 (apoB)- lipoproteins, such as VLDL, LDL, and lipoprotein(a) (Lp(a)) have well described roles in the stimulation of inflammatory cascade (for review of in vitro and animal studies (Faraj et al., 2010). On the other hand, in clinical studies, plasma triglycerides, total cholesterol (TC) and LDL cholesterol (LDL-C) have little, if any, association with inflammatory markers, in particular hsCRP, the most studied inflammatory marker (Ridker et al., 2002; Bermudez et al., 2002). Addition reduced the frequency of progression of coronary lesions, increased the frequency of regression. Moreover, changes in plasma apo B levels correlated well with the degree of coronary artery disease (CAD) regression (Walldius and Jungner, 2004). Antioxidants and hypolipidemic agents suppress the development of hypercholesterolemic atherosclerosis and induce regression of atherosclerosis (Prasad, 2008).

Hypericum perforatum L. (HPL), known as St John's Wort, is a perennial herbaceous plant of the Hypericaceae family, is well known antidepressant herbal remedy contains hypericin, pseudohypericin, hyperforin, quercitine and quercitrin as one of the major active constituents antihypertensive (Kumar et al., 2010; Carlo et al., 2001), and mild anti-inflammatory drugs (Barnes et al., 2001).

Phytochemical analysis of HPL shows that it is a rich source of flavonoids, and much of its antioxidant activities are attributed to these compounds (Zou et al., 2004). This study assessed the ability of hydroalcoholic extracts of HPL to reduce atherosclerotic plaque formation and also its ability to regression atherosclerosis lesion in hypercholesterolemic rabbits by analyzing biochemical markers, such as serum lipids, inflammatory markers and malondialdehyde (MDA), an index of levels of oxygen radicals and white blood cell (WBC), platelet, Fibrinogen are risk factors for atherosclerotic vascular disease.

MATERIALS AND METHODS

Plant material

H. perforatum L. was provided from Isfahan Natural Resource Institute and authenticated by Dr. Lili Ghaemmaghami at the Biology Department, School of Science, Isfahan University. The voucher specimen was deposited in Isfahan University Herbarium under the number 13648.

Preparation of hydroalcoholic extract

Above-ground parts of the plant were dried for 10 days at room

temperature. The dried plants were ground by an electric blender. One hundred g of HPL plant's powder was soaked in 96% ethanol for 72 h and then filtered, and concentrated by a distiller in a vacuum. The concentrated solution was decanted in three consecutive steps (once with 100 ml and twice with 50 ml of chloroform). The resulting solution was vaporized and desiccated in 50°C under sterile conditions (Eseyin et al., 2007). The dried powder obtained from the last step kept in dark glass bottle at 4°C until use.

Flavonoid and anthocyanines measurement

The flavonoid content was measured by spectrophotometric in 424 nm wave length (Petry et al., 2001) and anthocyanines were measured by the same methods at 535 nm wave length (Schutz et al., 2006).

Animals and treatment

Twenty mature male New Zealand rabbits with average body weight of 2 kg were used for the study. The animals were provided from Razi Institute, Karaj, Iran. The animals were housed in an air-conditioned animal room at 23±2°C, relative humidity 40 to 70%, with 12 h/12 h light/dark photoperiod for 2 weeks, Standard Rabbit Chow, purchased from Pasteur Institute of Iran. The rabbits were randomly divided into four groups of five rabbits each; animals in group 1 received a normal diet (ND) throughout the experiment (75 days). Animals in group 2 received ND supplemented with 1% cholesterol which is considered to be a high cholesterol diet (HCD) throughout the experiment (75 days). Animals in group 3 and 4 received HCD for 45 days and then respectively ND and ND + HPL (150 mg kg daily) for 30 days.

Isfahan Cardiovascular Research Center Ethics Committee which is a member of office for human research protections, US department of health and human services, approved the present study, and the animals were handled according to guidelines of Isfahan University of Medical Sciences for Laboratory Animal Sciences for the care and use of laboratory animals.

Measuring the biochemical factors

Blood samples (from a central ear artery) after 12 h of fasting were collected before time 0, 45th and 75th days. The plasma was obtained by centrifuging the blood samples at 2000 rpm for 15 min. Total cholesterol, TG, LDL-C and HDL-C were measured using special kits (DiaSys, Germany) which utilized the colorimetric method, in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo). Concentrations of apoA and apoB were also measured using special kits (DiaSys, Germany) in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo) according to the turbidimetric method; CRP was also measured by rabbit CRP ELISA (Rapidbio, USA). We measured Ox-LDL by rabbit Ox-LDL ELISA (Rapidbio, USA). MDA was estimated by the double heating method of Draper and Hadley (Draper and Hadley, 1990). The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. Atherosclerosis index was calculated according to the following formula: AI = LDL - C/HDL-C (Zou et al., 2005). Fibrinogen was measured by the Clauss method with the use of reagents supplied by Diagnostica Stago (France) for the WBC and platelet count (Coulter Counter T890).

Assessment of atherosclerotic changes in aorta

On the 75th day, all the groups of the animals were sacrificed by

rapid intracardiac pentobarbital (40 mg/kg intravenously) at the end of the protocol, and aortas were removed for assessment of atherosclerotic change. The aorta rinsed with normal saline solution and put in 10% formalin to be preserved for the next step. Cuts of aortic tissue were stained by Hematoxyline-Eosin method. Chekanov scale was used for grading of atherosclerotic plaques and the results were determined on a scale of 1 to 4 in relation to the thickness of media layer as follows:

Grade 1: Plaque less than half as thick as the media with some form of endothelial dysfunction.

Grade 2: Plaque at least half as thick as media with accumulation of intracellular lipid, macrophages, and smooth muscle cells.

Grade 3: Plaque as thick as the media with an abundance of macrophages, smooth muscle cells, and connective tissue.

Grade 4: Plaque thicker than the media with a large intracellular intimal lipid core and inflammatory cell infiltration (Chekanov, 2003).

All histopathological evaluations were done by biochemical analysis blinded to the experimental design.

Statistical analysis

Results are expressed as the mean \pm SD. All analyses were performed using SPSS 13 statistical software. Data were analysed by univariate ANOVA. If a resultant fraction was found to be significant, that is, established at $p < 0.05$, a post-ANOVA Duncan's test was used to specify pair-wise differences.

RESULTS

Amount of flavonoids and anthocyanins

The results showed that each 100 g powder of HPL plant results in 8.33 ± 0.033 g hydroalcoholic extract powder of HPL. It was also demonstrated that the amount of flavonoids and anthocyanins in 100 g of HPL extract is 0.435 ± 0.0031 and 2.299 ± 0.99 mg, respectively.

Biochemical factors

1. The basal values were not significantly different among the study groups. The changes in the serum TG of the four experimental groups are summarized in Table 1.

2. In the groups fed with high cholesterol diet (Groups II, III, IV), on the 45th days, increase significantly in concentrations of total cholesterol, TG, LDL-C, HDL-C, apoB, AI, CRP, MDA, OX-LDL, apoA, WBC, fibrinogen and platelet in comparison with Group I and to zero time (Table 1) were observed.

3. In the group fed with high cholesterol diet (Group II), on the 75th days, increase significantly in concentrations of total cholesterol, TG, LDL-C, HDL-C, apoB, AI, CRP, MDA, OX-LDL, apoA, WBC, fibrinogen and platelet in comparison with Group I and to zero time (Table 1).

4. The levels significantly decreased in concentrations of total cholesterol, TG, LDL-C, HDL-C, apoB, AI, CRP, MDA, OX-LDL, WBC, fibrinogen and platelet whereas significantly increased level apoA Groups III and IV in

comparison with Group II at the end time (Table 1).

5. On 75th days, in the group fed with to Standard diet (Group III) the levels of significantly decreased in concentrations of total cholesterol, LDL-C, HDL-C, apoB, WBC, fibrinogen and platelet in comparison with Group II (Table 1).

6. The levels of lipid parameters during treatment with HPL in addition to Standard diet (Group IV) are shown in Table 1. Group the levels of apoB, TG, cholesterol, LDL-C, Ox-LDL, MDA, hs-CRP, WBC, fibrinogen and platelet and AI, were found to be significantly decreased compared to the rabbits fed normal diet (Group III) during treatment. On the other hand, the levels of apoA and HDL-C significantly increased in Group IV (Table 1).

Atherosclerotic changes in aorta

Representative photographs of the atherosclerotic changes in the aortas from the four groups stained with Hematoxyline-Eosin method shown in Figure 1, and the extent of atherosclerosis in the groups is summarized in Table 2. There were no atherosclerotic plaques in the aortas of rabbits in Group I on regular diet. The grade of fatty streak formation in aorta of Group II on 1% cholesterol diet for 45th days and 75th days was significantly increased.

The extent of atherosclerosis in the Group IV of rabbits on HPL and regular diet for 30th days following 45th days on 1% cholesterol diet was significantly decreased as compared with Group II. The extent of atherosclerosis in the group of rabbits (Group III) on regular diet for 30th days following 1% cholesterol diet for 45th days was significantly greater than in the rabbits on Group IV. This shows that the atherosclerotic process continued even on the regular diet. These results suggest that HPL treatment for 30th days induce regression of atherosclerosis, however, it did prevent the progression of atherosclerosis and regular diet following high cholesterol diet was unable to regress atherosclerosis (Figure 1 and Table 2).

DISCUSSION

The present study has shown that high cholesterol 1% diet increased the serum levels of cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, apoB, apoA, MDA, CRP, AI, OX-LDL, WBC, fibrinogen and platelet in rabbits. Similar changes have been reported earlier that a high cholesterol diet increased the serum levels of TG, TC, LDL-C, HDL-C and the risk ratio of TC/HDL-C in rabbits with 0.5% cholesterol for 2 months (Prasad, 2008).

The results using the rabbit's model showed that *H. perforatum* has significant antihyperlipidemic, anti-inflammatory and antiatherogenic effects and promoted the regression of atherosclerosis lesion. This was evidenced by a decreased total cholesterol, triglyceride

Table 1. Pooled data for change in biochemical parameters in all four groups on the start day 45th day and 75th day.

Biochemical parameters		Group I	Group II	Group III	Group IV
Cholesterol(mg/dl)	0	46.2±5.263079	48.333±4.72581	48±3.91578	46.2±5.263
	45	48.4±15.1096	958.3444±41.01626	1042.667±20.52641	1029±23.881
	75	48.44433±4.725816	1146.667±55.07571	994.2233±12.5033 ^b	764.8±24.5070 ^{bc}
TG(mg/dl)	0	66.2±11.18928	70.4334±13.3166	67±12.75408	69±4.24
	45	66.2±11.18928	169±38.10512	227.5557±24.16039	202.8±20.147
	75	67.445±13.3166	224±34.60467	180.7778±75.03555 ^b	148.8±14.815 ^{bc}
LDL-C(mg/dl)	0	15.28±3.366304	15.46667±3.9372	16±3.413691	15.28±3.363
	45	14.28±8.493056	841.6±36.31914	878.557±78.2116	840.6±45.56
	75	15.46667±9.97156	969.0667±120.4128	818.53333±102.8258 ^b	445.28±187.076 ^{bc}
HDL-C(mg/dl)	0	18±3.937004	18±3.937004	18±3.937004	18.2±3.937
	45	18±3.937004	88±6667	132.33±12.05543	130.8±13.45
	75	19.333±4.50925	108±6.45644	89.6667±9.50438 ^b	108.4±12.7 ^{bc}
ApoB(mg/dl)	0	8.4±2.073644	7.6±2.08166	7.75±1.70782	8.4±2.07
	45	8.4±2.073644	56.33±13.05118	55±2.64575	47.2±8.44
	75	7.9±2.081666	59.333±11.50362	40±5.03322 ^b	29±8.81 ^{bc}
ApoA(mg/dl)	0	39.2±10.75639	36.33±14.15392	38.25±12.1758	38.25±12.17
	45	39.2±10.75639	42±5.56776	51.666±5.85946	60.5±5.87
	75	36.333±14.15392	28.6566±1.154701	41.666±5.85946 ^b	49.6±4.4 ^{bc}
MDA(mol/l)	0	0.5±0.12247	0.466±0.20816	0.475±0.170783	0.45±0.125
	45	0.46±0.15165	07±0.18488	0.733±0.20277	0.68±0.211
	75	0.46666±0.20816	0.8±0.16055	0.7±0.1 ^b	0.5±0.21 ^{bc}
hs-CRP(mg/l)	0	7.8±3.910882	7.8±3.910882	8.3±4.327432	7.8±3.9
	45	7.8±3.910882	14.6±2.281082	21.733±5.37368	24.48±5.47
	75	7.8±3.910882	17.833±4.64582	19.6±7.23394	6.14±4.56 ^{bc}
OX-LDL(ng/ml)	0	20.26±5.49299	21.15±5.911853	20.26±5.49299	21.15±4.91
	45	20.26±5.49299	32.166±2.40069	41.13±6.59114	33.94±8.648
	75	20.36±6.916	38.033±5.80075	38.233±7.7588	19.28±5.009691 ^{bc}
AI	0	0.85±0.341565	0.833±0.41633	0.85±0.341565	.83±0.34
	45	0.85±0.341565	9.6±0.1.442221	8.3433±1.46374	6.4±1.1
	75	0.85±0.341565	9.2333±1.101514	7.7±1.053565 ^b	5.1±1.4 ^{bc}
WBC(μl)	0	4760±832.4662	4966.667±550.7571	5150±580.2298	5240±541.294
	45	4760±832.4662	36333.33±14153.92	35500±7053.368	35380.234±7929.5
	75	4966±550.7571	42366.67±17048.39	39734±7245.502 ^b	20820±6692.352 ^{bc}
Platelet(μl)	0	426000±55879.3	439250±54707.56	426000±55879.3	437567±55232
	45	426000±55879.3	563000±159056.6	589666.7±34961.88	550600±78340.92
	75	439333±67002.49	638000±59774.58	577333.3±104078.5 ^b	492600±34623.21 ^{bc}
Fibrinogen(μl)	0	156.6±24.47039	153.663±30.66486	156.6±24.47039	148.33±33.22
	45	156.6±24.47039	267.333±35.21837	240.333±34.21837	240.45±37.961
	75	153.6667±30.66486	299.3333±46.5224	258.4±47.96176 ^b	200±18.77 ^{bc}

Group I: Fed with standard diet (control group) (75th days). Group II: Standard diet + cholesterol (75th days). Group III: Standard diet + cholesterol for 45th days and Standard diet for 30th days. Group IV: Standard diet + cholesterol for 45th days and Standard diet + *Hypericum perforatum* for 30th days. ^bSignificant difference between Group II with Groups III Group IV (p<0.05). ^cSignificant difference between Group III with Group IV (p<0.05).

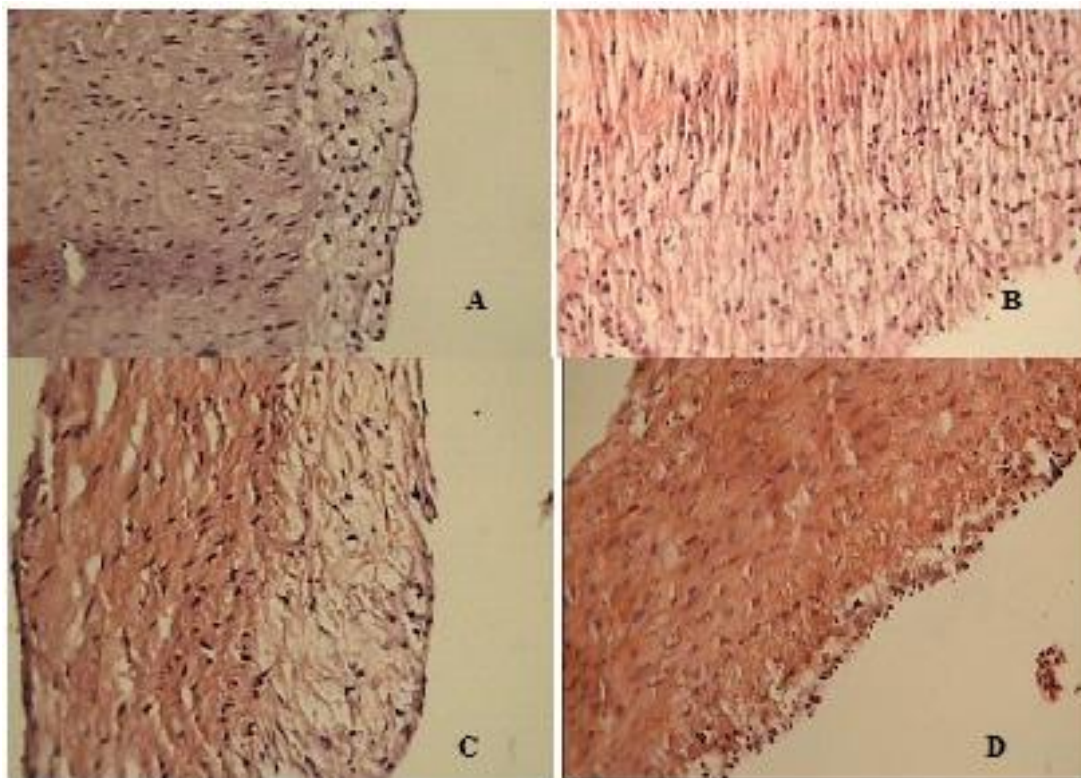


Figure 1. Histology of aorta of atherosclerotic plaque in studied group. A: Standard diet + cholesterol (45th day). B: Standard diet + cholesterol (75th days). C: Standard diet + cholesterol for 45th days and Standard diet + cholesterol for 30th day. D: Standard diet + cholesterol for 45th day and Standard diet + *A. caudatus* for 30th day. ^aSignificant difference between Group IV and Group III with Group II. ($p < 0.05$) ^bSignificant difference between Group III with Group IV ($p < 0.05$).

Table 2. Comparison of atheroma plaque in aortic cuts of three groups of rabbits fed with high cholesterol diets.

Group	Plaque thickness	Plaque stage	Plaque thickness to media thickness
Ila	1.01±0.1222	1	More than half of media thickness
Ilb	2.76±0.35355	2	Plaque at least half as thick as media
III	1.266±0.873689 ^a	1	More than half of media thickness
IV	0.65±0.494 ^{ab}	1	More than half of media thickness

Group Ila: Standard diet + cholesterol (45th days). Group Ilb: Standard diet + cholesterol (75th days) Group III: Standard diet + cholesterol for 45th days and Standard diet for 30th days. Group IV: Standard diet + cholesterol for 45th days and Standard diet + *A. caudatus* for 30th days. ^aSignificant difference between Group IV and Group III with Group II. ($p < 0.05$). ^bSignificant difference between Group III with Group IV. ($p < 0.05$).

and LDL-cholesterol, apoB, MDA, CRP, AI, OX-LDL, WBC, fibrinogen and platelet.

Therefore, inflammatory factors are considered to play an important role in the development and progression of atherosclerotic lesions. C-reactive protein (CRP), a classic acute-phase reactant, is an important sensitive marker of low grade inflammation; increased concentrations of high sensitive-CRP (hs-CRP) have been shown in several studies to be associated atherosclerosis and coronary artery disease (Yan et al., 2004; Guldiken

et al., 2005). Since a previous study reported that CRP is stimulated by ROS, the decrease in these inflammatory factors may be due largely to the improvement in plasma antioxidant status by anthocaynins contained in black rice pigment fraction (Lyon et al., 2003; Wang et al., 2007).

Anthocyanins have recently been considered as important phytochemicals with potential activities, such as anti-oxidation, anti-inflammation, etc. whereas also documented that plant anthocyanins are beneficial to cardiovascular health (Wang et al., 2007). *H. perforatum*

found to have anthocyanins which are water-soluble flavonoid pigments that appear red to blue, according to pH (Harborne and Williams, 2001). Anthocyanins might trap reactive oxygen in plasma and interstitial fluid of the arterial wall, hence preventing oxidation of LDL-C and acting as an antiatherogenic particle in the plants (Yamakoshi et al., 1999).

In recent years, catechin in green tea including the prevention of cancer and cardiovascular diseases and anti-inflammatory are under investigation (Chacko et al., 2010).

Tedeschi et al. (2003) suggest that extracts from *H. perforatum*, may be a promising anti-inflammatory principle in chronic inflammatory diseases. Oxidative modification of LDL is thought to play an important role in atherogenesis. Therefore, it has been hypothesized that increased formation of OxLDL in the vascular intima is responsible for monocyte recruitment (Seo et al., 2010). There is also evidence that oxidized LDL has a pathogenic role in the development of atherosclerosis. Uptake of oxidized LDL by macrophages and smooth muscle cells leads to the formation of fatty streaks, a key event in early atherosclerosis.

These findings suggest that improvement in LDL and HDL cholesterol and inhibition of oxidized LDL concentrations may result in the prevention of atherosclerotic lesions. Studies in both rats and humans also reported that intake of these polyphenols suppressed LDL cholesterol concentrations and susceptibility of LDL to oxidation and increased HDL cholesterol concentrations (Baba et al., 2007) flavonoids, quercetin and apigenin, belonging to the large family of polyphenols. We recently reported that quercetin, a plant-derived flavonoid, can protect erythrocytes against non-oxidative, enzymatic damage and anti-inflammatory activity by quercetin (Christopher et al., 2010).

In vitro and animal studies indicate that quercetin supplementation has the potential to exhibit multiple immunomodulatory effects including augmentation of neutrophil chemotaxis and respiratory burst activity, macrophage function and natural killer (NK) cell lytic activity (Heinz et al., 2010).

Quercetin was shown to significantly inhibit TNF α and nitric oxide synthesis in Lipopolysaccharide (LPS) activated macrophages and Kupffer cells as well as suppress induced expression of Interleukin-8 (IL-8) and Monocyte chemoattractant protein-1 (MCP-1) in human synovial cells. Apigenin inhibited tumor necrosis factor- α (TNF α) induced synthesis of Interleukin-6 (IL-6) and IL-8 in human endothelial cells. Whereas both quercetin and apigenin decreased induced expression of cell adhesion molecules in endothelial cells (Strzelecka et al., 2005; Reiterer et al., 2004).

TNF α , a pleiotropic cytokine produced by activated macrophages, stimulates synthesis of cell adhesion molecules and induces iNOS expression leading to production of biologically active nitric oxide (NO)

(Aggarwal et al., 2001). An elevated total white blood cell (WBC) count, fibrinogen and platelet are risk factor for atherosclerotic vascular disease (Okopien et al., 2004; Bradran and Nasri, 2006; Pignatelli et al., 2006).

WBC-derived macrophages and other phagocytes are believed to contribute to vascular injury and atherosclerotic progression (Lee et al., 2001). In addition to its role as an acute phase protein, increased fibrinogen is known to enhance platelet aggregation, increase smooth muscle cell proliferation and migration and affect the structure and function of cross-linked fibrin. Fibrinogen may therefore have a more direct role in the development of atherothrombotic coronary artery disease (Mills et al., 2002).

Epidemiological evidence indicates that populations with high intake of green tea catechins benefit in terms of body weight and body fat, glucose homeostasis, and cardiovascular health effects via several mechanisms including inhibition of adipocyte differentiation and proliferation, reduction of fat absorption, leading finally to reduction in fat mass, triacylglycerides in hyperlipidemia models, and free fatty acids and total cholesterol (Thielecke and Boschmann, 2009).

Several studies showed that flavonoids such as quercetin and catechin inhibit platelet aggregation, could modulate platelet activity. The results of most studies show that flavonoids interact with arachidonic acid metabolism, thus inhibiting platelet thromboxane A₂ production, a potent aggregating and vasoconstricting agent (Pignatelli et al., 2000).

quercetin and catechin alone or in combination significantly inhibited calcium mobilization and IP₃ formation because of their ability to quench hydrogen peroxide. Therefore, these findings indicate that quercetin and catechin inhibit platelet function by virtue of antioxidant activity and improved endothelial function, increased antioxidant activities and an improved pressure control (Pignatelli et al., 1999; Thielecke and Boschmann, 2009).

Studies suggest that reactive oxygen species (ROS) generated during hypercholesterolemia via cytokines and increases the levels of platelet activating factor (PAF) may in part contribute to the development of hypercholesterolemic atherosclerosis and that suppression of production and activity of cytokines and PAF may reduce the development of hypercholesterolemic atherosclerosis (Prasad and Lee, 2007).

Regular diet following a high cholesterol diet did not lower the high cholesterol diet-induced rise in MDA. However, *H. perforatum* did lower MDA in the present study. Increased levels of MDA in spite of an increase in the antioxidant reserve in aorta with a high cholesterol diet could be due to amounts of reactive oxygen species greater than can be handled by the increased levels of antioxidant reserve.

Atherogenesis is associated with an increase in lipid hypercholesterolemic peroxidation product MDA an indirect index of levels of ROS, and a decrease in

antioxidant reserve of aorta where antioxidant with decrease in the oxidative stress was associated with a decrease in MDA (Lee and Prasad., 2003). Antioxidants and suppressants of sources of ROS are effective in the suppression of development of atherosclerosis.

The concentration of low density lipoprotein cholesterol (LDL-C) is one of the most important predictors of atherosclerosis and coronary heart disease (CHD). There is abundant evidence that a reduction in LDL-C lowers morbidity and mortality in patients with CHD (Stojakovic et al., 2007). Recently, it was shown that high concentrations of LDL-triglycerides (LDL-TG) are correlated with the accumulation of remnants and dense LDL as well as low-grade systemic inflammation and vascular damage (Marz et al., 2004).

Low HDL cholesterol is the most frequent lipid disorder in patients with coronary artery disease. Apolipoprotein A-I (apoA-I) is a major protein component of HDL and plays a pivotal role in its formation. ApoA-I level seems to be a better indicator of coronary risk than HDL cholesterol level (Belalcazar et al., 2003). Recent evidence suggests that apolipoproteins, especially apolipoprotein B (apoB), may be more strongly associated with CHD incidence than LDL (Sacks, 2006; Walldius and Jungner, 2006).

Apolipoprotein plays a role in transporting lipid particles and is considered a direct measurement of proatherogenic particles (Chien et al., 2007). In an earlier study, we also observed a positive correlation between urinary catechin excretion and plasma HDL cholesterol. Showed that the increase and regulation of apolipoprotein A1 expression induced by genistein was mediated by the nitrogen-activated protein kinase signaling pathway (Baba et al., 2007).

It has been reported that flavonoids from *H. perforatum* showed antidepressant activity, which was considered to be associated with their antioxidant actions (Butterweck et al., 2000; Luo et al., 2004). Zou et al. (2005) reported the radical scavenging activity and antioxidant activity of a flavonoid-rich extract of *H. perforatum in vitro* (Zou et al., 2004). While some studies have investigated *H. perforatum* and *Hypericum lysimachioides* for their hypocholesterolemic effects (Zou et al., 2005; Hakimoglu et al., 2007).

Recently reported that the citrus flavonoids, naringenin and hesperetin, markedly reduce basal apoB secretion in hepatoma (HepG2) cells. These flavonoids, found predominantly in grapefruit and oranges, reduce plasma lipids and atherosclerosis. The hypocholesterolemic effects observed in vivo were associated with reduced hepatic HMG-CoA reductase and ACAT activities. In HepG2 cells that the flavonoid-induced reduction in basal apoB secretion was associated with reduced ACAT and microsomal triglyceride transfer protein (MTP) activities, as well as reduced expression of ACAT2 and MTP (Borradaile et al., 2002; Pignatelli et al., 2006).

Regular diet following a high cholesterol diet, although reduces serum lipid levels, in comparison with Group II

but further increases atherosclerosis lesions in the aorta.

Prasad (2008) show despite a decrease in serum cholesterol in the rabbits on regular diet following high cholesterol diet, the levels of aortic MDA did not decrease. One possible explanation could be that a regular diet following high cholesterol diet does not lower the cholesterol concentration of aortic tissue in spite of a decrease in serum cholesterol. The possibility is that tissue cholesterol-induced production of MDA counteracted the increase in the antioxidant reserve of the aorta. The increases in cholesterol content of the aorta could increase the levels of ROS resulting in atherosclerotic changes (Prasad, 2008).

In the regression phase of the present study demonstrated that ethanol extract HPL following the high cholesterol diet can decrease atherosclerosis lesions in the aorta after consumption HPL in regression period Group IV in compared to the rabbits on regular diet following high cholesterol diet (Group III). Slowing of progression and regression of atherosclerosis could not be due to lowering of serum lipids because decreases in serum lipids were similar in both the groups on regular diet without HPL (Group III) following high cholesterol diet.

The main mechanism of slowing of progression and regression of atherosclerosis may possibly be a reduction in oxidative stress. Several epidemiological studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease. Food rich in antioxidants plays an essential role in the prevention of cardiovascular diseases (Gerber et al, 2002; Di Matteo and Esposito, 2003). It was also reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen et al, 1993).

In the present study, we found that HPL could be useful in reduces atherosclerotic lesions and the rate of lesions regression may be hypolipidemic effect, anti-inflammatory and antioxidant mechanism also contribute to antiatherogenic effect hydroalcoholic extracts of *H. perforatum* .

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REFERENCES

- Aggarwal BB, Samanta A, Feldmann M (2001). TNF α In: Durum SK, Hirano T, Vilcek J, Nicola NA, editors. Cytokine Reference. London: Academic Press, pp. 413-434.

- Bradran A, Nasri H (2006). Association between white blood cell count and levels of serum homocysteine in end-stage renal failure patients treating with hemodialysis. *J. Ayub Med. Coll. Abbottabad*, 18(1): 22-26
- Belalcazar LM, Merched A, Carr B, Oka K, Chen K, Pastore L, Beaudet A, Chan L (2003). Long-Term Stable Expression of Human Apolipoprotein A-I Mediated by Helper-Dependent Adenovirus Gene Transfer Inhibits Atherosclerosis Progression and Remodels Atherosclerotic Plaques in a Mouse Model of Familial Hypercholesterolemia. *Circulation*, 107: 2726-2732.
- Boban PT, Nambisan B, Sudhakaran PR (2008). Dietary mucilage promotes regression of atheromatous lesions in hypercholesterolemic rabbits. *Phytother. Res.*, 23(5): 725-730
- Butterweck V, Jurgenliemk G, Nahrstedt A, Winterhoff H (2000). Flavonoids from *Hypericum perforatum* show antidepressant activity in the forced swimming test. *Planta Med.*, 66: 3-6.
- Barnes J, Anderson LA, Phillipson JD (2001). St John's Wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *J. Pharm. Pharmacol.*, 53: 383-600.
- Borradaile NM, Dreu LE, Barrett PHR, Huff MW (2002). Inhibition of hepatocyte apoB secretion by naringenin: enhanced rapid intracellular degradation independent of reduced microsomal cholesteryl esters. 43: 1144-1154
- Chien KL, Hsu HC, Su TC, Chen MF, Lee YT, Hu FB (2007). Apolipoprotein B and non-high density lipoprotein cholesterol and the risk of coronary heart disease in Chinese. *J. Lipid Res.*, 48: 2499-2505.
- Chekanov VS (2003). Low frequency electrical impulses reduce atherosclerosis in cholesterol fed rabbits. *Med. Sci. Monitor.*, 9(8): BR302-9.
- Di MV, Esposito E (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. *Curr. Drug Targets: C.N.S. Neurol. Disord.*, 2: 95-107.
- Di Carlo DG, Borrelli F, Ernst E, Izzo AA (2001). St John's Wort: Prozac from the plant kingdom. *Trends Pharmacol. Sciences*, 22: 292-297.
- Draper HH, Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.*, 186: 421-31.
- Eseyin O, Ebong P, Ekpo A, Igboasoiki A, Oforah E (2007). Hypoglycemic Effect of the Seed Extract of *Telfairia occidentalis* in Rat. *Pak. J. Biol. Sci.*, 10(3): 498-501.
- Frisinghelli A, Mafriaci A (2007). Regression or reduction in progression of atherosclerosis, and avoidance of coronary events, with lovastatin in patients with or at high risk of cardiovascular disease: A review. *Clin. Drug Investig.*, 27(9): 591-604.
- Gerber M, Boutron-Rault MC, Hereberg S, Riboli E, Scalbert A, Siess MH (2002). Food and cancer: state of the art about the protective effect of fruits and vegetables. *Bull. Cancer*, 89: 293-312.
- Güldiken S, Demir M, Arıkan E, Tuğrul A (2005). The Level of Serum High Sensitive C-Reactive Protein in Women with Hyperthyroidism. *Turk. J. Endocrinol. Metab.*, 3: 85-88.
- Genest J Jr, McNamara JR, Ordovas JM (1992). Lipoprotein cholesterol, apolipoprotein A-I and B and lipoprotein (a) abnormalities in men with premature coronary artery disease. *J. Am. Coll. Cardiol.*, 19: 792-802.
- Harborne JB, Williams CA (2001). Anthocyanins and other flavonoids. *Nat. Prod. Rep.*, 18(3): 310-333.
- Hakimoğlu F, Kizil G, Kanay Z, Kizil M, Isi H (2007). The effect of ethanol extract of *Hypericum lysimachioides* on lipid profile in hypercholesterolemic rabbits and its *in vitro* antioxidant activity. *Atherosclerosis*, 192(1): 113-122.
- Lee P, Prasad K (2003). Suppression of oxidative stress as a mechanism of reduction of hypercholesterolemic atherosclerosis by cyclooxygenase inhibitor in rabbits. *Int. J. Angiol.*, 12: 13-23.
- Lyon CJ, Law RE, Hsueh WA (2003). Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology*, 144: 2195-2200.
- Libby P, Aikawa M (2002). Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nat. Med.*, 8: 1257-1262.
- Luo L, Sun Q, Mao YY, Lu YH, Tan RX (2004). Inhibitory effects of flavonoids from *Hypericum perforatum* on nitric oxide synthase. *J. Ethnopharmacol.*, 93: 221-225.
- Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA (2001). White Blood Cell Count and Incidence of Coronary Heart Disease and Ischemic Stroke and Mortality from Cardiovascular Disease in African- American and White Men and Women. *Am. J. Epidemiol.*, 154: 758-764.
- Marz W, Scharnag IH, Winkler K (2004). Low-density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: the Ludwigshafen Risk and Cardiovascular Health study. *Circulation*, 110: 3068-3074.
- Mills JD, Mansfield MW, Grant PJ (2002). Elevated fibrinogen in the healthy male relatives of patients with severe, premature coronary artery disease. *Eur. Heart J.*, 23, 1276-1281.
- Oka K, Chan L (2005). Inhibition and regression of atherosclerotic lesions. *Acta Biochim. Pol.*, 52: 311-319.
- Okopien B, Krysiak R, Madej A, Belowski D, Zielinski M, Kowalski J, Herman ZS (2004). Effect of simvastatin and fluvastatin on plasma fibrinogen levels in patients with primary hypercholesterolemia. *Pol. J. Pharmacol.*, 56: 781-787.
- Prasad K (2008). Regression of hypercholesterolemic atherosclerosis in rabbits by secoisolaricresinol diglucoside isolated from flaxseed. *Atherosclerosis*, 197: 34342.
- Prasad K, Lee P (2007). A Study on Regression of Hypercholesterolemic Atherosclerosis in Rabbits by Flax Lignan Complex. *J. Cardiovascular Pharmacol. Therap.*, 12(4): 304-313.
- Petry RD, Ortega GG, Silva WB (2001). Flavonoid content assay: influence of the reagent concentration and reaction time on the spectrophotometric behavior of the aluminium chloride flavonoid complex. *Pharmazie*, 56(6): 465-470.
- Pignatelli P, Pulcinelli FM, Lenti L, Ghiselli A, Gazzaniga PP, Violi F (1999). Vitamin E inhibits collagen-induced platelet activation by blunting hydrogen peroxide. *Arterioscler. Thromb. Vasc. Biol.*, 19: 2542-2547.
- Pignatelli P, Pulcinelli FM, Celestini A, Lenti L, Ghiselli A, Gazzaniga PP (2000). The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. *J. Clin. Nutr.*, 72: 1150-1155.
- Pignatelli P, Santo SD, Buchetti B, Sanguigni V, Brunelli A, Violi F (2006). Polyphenols enhance platelet nitric oxide by inhibiting protein kinase C-dependent NADPH oxidase activation: effect on platelet recruitment. *FASEB J.*, 20: 1082-1089.
- Stojakovic T, Scharnagl H, März W, Winkelmann BR, Boehm BO, Otto SO (2007). Low density lipoprotein triglycerides and lipoprotein(a) are risk factors for retinal vascular occlusion. *Clin. Chim. Acta*, 382: 77-81.
- Sacks FM (2006). The apolipoprotein story. *Atheroscler. Suppl.*, 7: 23-27.
- Strzelecka M, Bzowska M, Koziel J, Szuba B, Dubiel O, Nunez DR, Heinrich M, Bereta J (2005). Anti-inflammatory effects of extracts from some traditional Mediterranean diet plants. *J. Physiol. Pharmacol.*, 56: 139-156
- Schutz K, Persike M, Carle R, Scriber A (2006). Characterization and quantification of anthocyanins in selected artichoke (*Cynara scolymus* L.) cultivars by HPLC-DAD-ESI/MS. *Anal. Bioanal. Chem.*, 384(7-8): 1511-1517.
- Tedeschi E, Menegazzi M, Margotto D, Suzuki H, Forstermann U, Kleinert H (2003). Anti-inflammatory actions of St John's wort: inhibition of human iNOS expression by down regulating STAT-1 activation. *J. Pharmacol. Exp. Therap.*, 307: 254-261.
- Talmud PJ, Hawe E, Miller GJ, Humphries SE (2002). Nonfasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease risk in middle-aged UK men. *Arterioscler. Thromb. Vasc. Biol.*, 22: 1918-1923.
- Reiterer G, Toborek M, Hennig B (2004). Quercetin protects against linoleic acid-induced porcine endothelial cell dysfunction. *J. Nutr.*, 134: 771-775.
- Walldius G, Jungner I (2006). The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy—a review of the evidence. *J. Int. Med.*, 259: 493-519.
- Walldius G, Jungner I (2004). Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-

- modifying therapy. *J. Intern. Med.*, 255: 188–205
- Wang Q, Han P, Zhang M, Xia M, Huilian ZH, Ma J, Hou M, Tang Z, Ling W (2007). Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pac. J. Clin. Nutr.*, 16: 295-301.
- Yen GC, Duh PD, Tsai CL (1993). The relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem.*, 41: 67–70.
- Yan JC, Zhu J, Gao L, Wu ZG, Kong XT, Zong RQ, Zhan LZ (2004). The effect of elevated serum soluble CD40 ligand on the prognostic value in patients with acute coronary syndromes. *Clin. Chim. Acta*, 343: 155-159.
- Yamakoshi J, Kawaka S, Koga T, Arial T (1999). Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis*, 142(1): 139-149.
- Zou Y, Lu Y, Wei D (2005). Hypocholesterolemic effects of a flavonoid-rich extract of *Hypericum perforatum* L. in rats fed a cholesterol-rich diet. *J. Agric. Food Chem.*, 53(7): 2462-2466.
- Zou Y, Lu Y, Wei D (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum Perforatum* L. *in vitro*. *J. Agric. Food Chem.*, 52: 5032-5039.