

Full Length Research Paper

Inhibition of Early Atherogenesis by Co-Administration of *Amaranthus caudatus* and *Hypericum perforatum* Extracts

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Accepted 16 September, 2025

Hypercholesterolemia, a high cholesterol diet and oxidative stress increase serum low density lipoprotein (LDL) levels resulting in increased risk for development of atherosclerosis. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against contagious and degenerative diseases. The aim of this study was to assess the anti-fatty streak effects of concurrent hydroalcoholic extracts of *Amaranthus caudatus* L. (*A. caudatus*) and *Hypericum perforatum* L. (*H. perforatum*) as this were investigated in hypercholesterolemic rabbits and was compared with lovastatin. Rabbits were randomly divided into four groups which were fed with normal diet (control), high-cholesterol diet (hypercholesterolemic control group), high-cholesterol + concurrent hydroalcoholic extracts of *A. caudatus* (75 mg/kg daily) and *H. perforatum* (75 mg/kg daily) (treatment group), and high-cholesterol + lovastatin (10 mg/kg daily) (treatment group) for 60th days and then blood samples were obtained to measure plasma cholesterol, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoproteinA (apoA), apolipoproteinB (apoB), malondialdehyde (MDA), C-reactive protein (CRP), Ox-LDL and AI aorta was also obtained for histological evaluation. The observation showed decrease in plasma cholesterol, TG, HDL, LDL, apoB, CRP, MDA, OX-LDL, AI and increased apoA and HDL between treatment groups and hypercholesterolemic control groups ($P>0.05$). The mean size of produced fatty streak also showed significant reduction in the treatment groupIII compared to the hypercholesterolemic group ($P<0.05$). This results showed that combination of hydroalcoholic extracts of *A. caudatus* and *H. perforatum* has anti-fatty streak effects in hypercholesterolemic rabbits.

Key words: Atherosclerosis, *Amaranthus*, *Hypericum*.

INTRODUCTION

Atherosclerosis is a disease with a multifaceted aetiology, and diet is one of the most important environmental factors influencing the development of cardiovascular disease (CVD) (Covas et al., 2009; de Lorgeril et al., 1999; Buckland et al., 2009; Trichopoulou et al., 2003). A lipid profile characterized by reduced high density

lipoprotein-cholesterol (HDL-c) concentrations and increased low density lipoprotein-cholesterol (LDL-c) and triglycerides concentrations as well as increased total cholesterol-to-HDL-c ratio constitutes a high risk for cardiovascular diseases (Ingelsson et al., 2007; Hadaegh et al., 2010). Oxidative stress impairment or altered antioxidant type have been suggested as critical keys in the start of certain chronic diseases (Pérez-Matute et al., 2009; Valdecantos et al., 2009). It is now known that oxidized LDL-cholesterol is a much greater

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risk factor for the development of atherosclerosis than its non-oxidized precursor (Berliner and Heinecke., 1996). In this sense, oxidized low-density lipoproteins (ox-LDL), a recognized oxidative stress marker, has been positively associated with central obesity (Weinbrenner et al., 2006) and atherosclerosis (Santiago et al., 2010). One of the most examined free radical damages is an oxidative damage to lipids in the process of lipoperoxidation. Increased lipid peroxidation has been recognized as a key mechanism for the development of atherosclerosis (Harrison et al., 2003). Oxidatively modified LDL play a crucial role in pathogenesis of atherosclerosis (Nagyova et al., 2004, Sumegová et al., 2007). After reduction of their ability, the speed of oxidative procedure markedly increases leading to the formation of lipoperoxides (Esterbauer et al., 1992). Lipoperoxides can be further decomposed and give rise to various secondary products of lipoperoxidation, several biologically active compounds. Some of them have been used as biological markers of lipoperoxidation, like malondialdehyde (MDA) (Viigimaa et al., 2010). Recent studies point out that long-term supplementation of vitamin E markedly reduces LDL oxidability in several diseases associated with oxidative stress. Vitamin E in *in vitro* conditions can protect LDL not only against oxidation but also it prevents accumulation of oxidised LDL in macrophages in addition to esters of cholesterol. According to Kwon et al. (2009), vitamin E increases activity of antioxidant enzymes and has a positive effect on prevention against hypercholesterolemia and atherogenesis (Kwon et al., 2009; Clark, 2008). Epidemiological studies have exposed an association between increased consumption of antioxidant-rich vegetables and fruits and a decreased risk of coronary heart disease (CHD) (Ness and Powles, 1997). Flavonoids from a variety of sources have been reported to prevent LDL oxidation *in vitro* and show markedly hypolipidemic activity *in vivo*, suggesting the effectiveness of flavonoids for the prevention and treatment of atherosclerosis (Dufull et al., 2003; Yokozawa et al., 2002; Koshy et al., 2001). Role of inflammation in the pathogenesis of atherosclerosis particularly the associations between C-reactive protein (CRP), a plasma protein synthesized in liver, with cardiovascular risk factors and disease risk has gained much attention in the recent past (Ridker, 2009). CRP is a sensitive inflammatory marker and modulated by mediators of the inflammatory cascade (e.g., interleukin 6). High levels of CRP have been shown to be an independent predictor of cardiovascular risk for all degrees of severity of the metabolic syndrome (Ridker et al., 2003). Flavonoids and anthocyanins have been found to acquire anti-inflammatory (Sautebin et al., 2004), exhibits free radical scavenging activity (Noda et al., 2002), protects against endothelial dysfunction (Serraino et al., 2003), decreases myocardium damage (Amorini et al., 2003; Pas kon et al., 2009) and potent antioxidant

and can protect LDL against oxidation by macrophages (Sautebin et al., 2004). Flavonoid-rich extract of *Hypericum perforatum* was prepared and its antioxidant activity was determined by a series of models *in vitro* (Zou et al., 2004). Therefore, in the study, the hypocholesterolemic effects of this flavonoid-rich extract was investigated by observing its effects on serum lipid levels and antioxidant enzyme activity in rats fed a cholesterol-rich diet (Zou et al., 2005). *H. perforatum* L. (Hypericaceae), commonly known as St. John's wort, (Sánchez-Mateo et al., 2009] is a traditional medicinal plant used in the West for the treatment of respiratory diseases, skin wounds, and dermatitis long before it was used as a conventional therapy in the clinical treatment of mild-to-moderate depression (Chatterjee et al., 1998). In addition, studies also have shown that *H. perforatum* L. has antiviral (Singer et al., 1999), antimicrobial (Moure et al., 2001), wound healing (Hollman et al., 1996) and anticancer activities (Meyer et al., 1998; Hunt et al., 2001) and hypocholesterolemic effects (Zou et al., 2005). The extracts of *H. perforatum* L. contain a variety of pharmacological active constituents including phloroglucinols, naphthodianthrone, and a broad range of flavonoids. *H. perforatum* L. contains a number of compounds such as quercetin, rutin, hypericin, hyperforin, and myricetin, etc. (Barnes et al., 2001). In *H. perforatum*, anthocyanins were found in dried crude extract (Barnes et al., 2001; Jurgentliemk and Nahrstedt, 2002).

A. caudatus (Amaranthaceae) (*Amaranthus caudatus* L.) is commonly known as "Peddathotakura" in Telugu (Rastrelli et al., 1995). *A. caudatus* is traditionally used in jaundice, amoebiasis, and kidney diseases (Rinderle et al., 1989; Yineger et al., 2008), as blood purifier, diuretic, abortifacient, vermifuge, astringent and of liver diseases (Sripathi and Sankari, 2010). *A. caudatus* seeds showed cholesterol-lowering, *in vitro* antioxidant and α -amylase inhibition activities (Ashok et al., 2010; Plate and Arêas, 2002). *A. caudatus* contains anthocyanin (Pas kon et al., 2009), agglutinin (Broekaert et al., 1992), triterpenoid saponins and ionol derived glycoside (Transue et al., 1997), as well as vitamin E isomers (Rastrelli et al., 1998) and amaranthin (Bruni et al., 2002). *A. caudatus* contains amino acids namely, lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan (Council of Scientific and Industrial Research, 1988).

As there are been no reports available on the hypocholesterolemic and antiinflammatory effect of concurrent hydroalcoholic extracts of *A. caudatus* and *H. perforatum*, the present study was undertaken to evaluate its ability to reduce its total cholesterol, LDL-cholesterol (LDL-C), triglyceride (TG), low-density lipoprotein (LDL), apolipoprotein B (apoB), malondialdehyde (MDA), C-reactive protein (CRP), Ox-LDL, AI increased apoA, HDL and reduced of fatty streak

formation in hypercholesterolemic rabbits and was compared with lovastatin.

MATERIALS AND METHODS

A. caudatus L. and *H. perforatum* L. were collected from Isfahan Natural Resource Institute. They were identified by Lili Ghaemmaghami and a voucher specimen was deposited at the Herbarium of the Department of Biology, Faculty of Science and Isfahan University (voucher no. 13649 and 13648). Aerial parts (stems, leaves and flowers) were dried for 10 days at room temperature; it was extracted with 96% ethanol for 72 h and then filtered, and concentrated by vacuum distillation. Solvent was evaporated under vacuum and crude extract was obtained as a dark reddish colour and kept in dark glass bottles at 4 °C until use (Eseyin et al., 2007).

Twenty male New Zealand white rabbits weighing 1.5 to 2 kg were obtained from the central animal house of Tehran Razi Institute. The animals were housed at the temperature of 21 to 23 °C in a light-controlled room with a 12 h light-dark cycle and ambient humidity (50 to 60%). All the rabbits were initially fed a normal diet (Pars Dam, Tehran, Iran) for 2 weeks and then randomly divided into four groups of five rabbits each; rabbits fed a normal diet were used as normal controls, hypercholesterolemic control group (n = 5, fed 1% high-cholesterol diet), treatment group (n = 5, fed 1% high-cholesterol diet supplemented with combination hydroalcoholic extracts of *A. caudatus* (75 mg/kg daily) and *H. perforatum* (75 mg/kg daily), treatment group (n = 5, fed 1% high-cholesterol diet with lovastatin (10 mg/kg daily). Animals were fed for 60th days and each diet was set at 100 g/rabbit per day with water available *ad libitum*. The rabbits were weighed weekly.

Blood samples from a superficial ear vein were taken at 0, 30th and 60th days after 12 h of fasting to analyze plasma total cholesterol (Ch), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) and triglyceride (TG) were measured using special kits (DiaSys, Germany) which utilized the colorimetric method, in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo). Concentrations of apolipoprotein A (apoA) and apolipoprotein B (apoB) were also measured using special kits (DiaSys, Germany) in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo) according to the turbidimetric method; C-reactive protein (CRP) was also measured by rabbit CRP ELISA (Rapidbio, USA). We measured Ox-LDL by rabbit Ox-LDL ELISA (Rapidbio, USA). Malondialdehyde (MDA) was estimated by the double heating method of Draper and Hadley. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid with MDA. Atherosclerosis index was calculated according to the following formula: $AI = LDL-C/HDL-C$ (Zou et al., 2005). At the end of experiment, the animals were killed, the aorta were removed and tissue sections (5 µm) aorta fixed by immersion at room temperature in 10% formalin for pathological examination. The histological sections were stained with hematoxylin/eosin (H&E). 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 a pathologist

blinded to the experimental design.

Statistical analysis

All values were expressed as mean ± SD. Significant differences among the groups were determined by one-way ANOVA using the SPSS 13.0 software package program. Values of $p < 0.05$ were taken as statistically significant.

RESULTS

At the onset, no significance was found between the mean values among the study groups. In rabbits fed on high cholesterol diet (Groups II), there was significantly increase in Cho, TG, LDL-C, HDL-C, apoB, AI, CRP, MDA, OX-LDL and apoA on the 30th days and after 60th days, as compared to the beginning of period. A non significant decrease of Cho, TG, LDL-C, apoB, AI, CRP, MDA, OX-LDL and apoA and HDL-C, were observed in rabbits fed with high-cholesterol diet + combination of *Amaranthus* + *Hypericum* (Group III) and of high-cholesterol diet + lovastatin (group IV) on the 30th days and after 60th days, as compared to Group II. There was decrease in Cho, TG, LDL-C, apoB, AI, CRP, MDA, OX-LDL and increased in apoA and HDL-C between the high-cholesterol diet + combination of *Amaranthus* + *Hypericum* (Group III) and high-cholesterol diet + lovastatin (Group iv) on the 30th days and after the 60th days (Table 1). Pathological assessment of aorta showed that rabbits which had received a normal diet did not develop any fatty streaks (Table 2 and Figure 1a) and those which had received a high-cholesterol diet showed fatty streak formation (Table 2 and Figure 1b), The rabbits which had received a high-cholesterol diet with combination of hydroalcoholic extracts of *A. caudatus* and *H. perforatum* did not develop any fatty streaks was significantly smaller ($p < 0.05$) than that the Group II (Table 2 and Figure 1c) whereas there was a nonsignificant reduction in the aorta atherosclerotic rabbits fed rabbits which had received a high-cholesterol diet with lovastatin for 60th days compared with rabbits fed on high-cholesterol diet (Group II) (Table 2, and Figure 1d). There was reduction in the aorta atherosclerotic rabbits fed on a high-cholesterol diet with a combination of hydroalcoholic extracts of *A. caudatus* and *H. perforatum* for the 60th days (Group V) compared with rabbits fed on high-cholesterol diet with lovastatin (Group IV) (Table 2 and Figure 1c and d).

DISCUSSION

Biochemical assessments demonstrate that concurrent hydroalcoholic extracts of *A. caudatus* and *H. perforatum* attenuates development of atherosclerosis through

Table 1. Effect of combination of *Amaranthus* + *Hypericum* and lovastatin on plasma lipids and biochemical factors in cholesterol-fed rabbits at 60th days.

Group (days)	Duration	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Apolipoprotein A (mg/dl)	Apolipoprotein B (mg/dl)	Malondialdehyde (mol/L)	OX-LDL (ng/ml)	C-reactive protein(mg/L)	AI
I	0	41.8±4.711688	61.8±16.54388	31.42±10.41769	20±5.567764	46.8±4.32435	16±5.33854	0.46±0.151658	20.26±5.492996	8±3.852921	1.52±0.40866
	30	44.4±5.176872	61±4.472136	22.46±5.748739	17±2.345208	45.2±4.32435	9.2±2.58844	0.5±0.158114	20.26±5.492996	8±3.852921	1.316±0.4458
	60	44.4±6.024948	59.6±2.607681	22.36±5.76437	18.4±3.646917	41.4±12.30041	10.2±2.58844	0.42±0.130384	21.64±4.217582	8±3.852921	1.138±0.1848
II	0	44.4±111.6817	61±4.472136	21.5±5.10294	18.4±1.516575	45±4.41588	8.4±2.0736441	0.44±0.151658	20.26±5.492996	8±3.852921	9.72±1.05211
	30	812.6±111.6817	154.8±33.70757	808.8±66.186857	83.2±9.984989	21.2±4.32435	46.2±9.093954	1.66±1.00896	33.34±2.3755	14.42±3.568193	9.76±1.43635
	60	1086±91.81503	355.4±157.9519	987.4±51.354649	101.6±10.62073	25.8±4.024922	66.2±6.90652	3.16±1.851486	38.16±6.505229	30.7±13.72771	9.76±1.43635
III	0	41.8±6.140033	66.2±11.18928	25.62±10.569863	18.4±3.507136	45.2±5.357238	10±1.8708287	0.42±0.164317	20.26±0.645848	8±.852921	1.568±0.6454
	30	590±121.2209	101±13.36039	665.72±146.3963	109.6±18.68957	53±9.327379	49.4±6.465292	0.6±0.273861	25.64±6.300635	7.56±2.33088	6.088±1.1536
	60	765±111.6132	133.6±42.76447	809.4±61.610064	129.4±14.74788	58±14.74788	41±9.823441	0.6±0.158114	27.92±4.139082	10.72±3.977059	6.262±0.5714
IV	0	44.4±6.348228	66.2±11.18928	20.3±5.4990908	17.2±2.863564	46.4±4.505552	9.6±4.393177	0.5±0.158114	20.26±5.492996	8±.852921	1.08±0.16437
	30	922.4±99.95149	142.6±46.77392	853.4±60.793092	97.8±26.66833	47.2±10.54514	55.4±5.22494	0.86±0.642651	34±8.915997	14.28±5.042519	9.22±2.68088
	60	1019.25±27.572	196±77.85242	932.8±65.705403	114.6±12.42176	39.2±11.47606	58±11.97915	0.8±0.173205	30.98±4.605106	13.18±2.970185	8.22±1.35904

Group I: Fed with Normal diet (control group) (60th days); Group II: Hypercholesterolemic diet (60th days); Group III: Hypercholesterolemic diet + combination of *Amaranthus* + *Hypericum* (treatment group) (60th days); Group IV: hypercholesterolemic diet + lovastatin (treatment group) (60th days); 0: Blood sampling Phase I; 30: Blood sampling Phase II; 60: Blood sampling Phase III.

decreasing CHO, TG, HDL, LDL-C, apoB, CRP, MDA, OX-LDL and AI and as well as increasing apoA and HDL-C. Histological evaluation of cardiac vasculature regarding fatty streak formation indicates that concurrent hydroalcoholic extracts of *A. caudatus* and *H. perforatum* L may prevent progression of atherosclerotic lesions more than lovastatin. Hypercholesterolemia and the consequent atherosclerosis is one of the most important risk factors for ischemic heart disease (Freedman et al., 2003; Khoo et al., 2003). Atherosclerosis is characterized by accumulation of intra- and extracellular lipids, monocyte/macrophage infiltration, and foam cell formation of connective tissue components (Prasad and Kalra, 1989; Keaney et al., 1993). Hypercholesterolemia damages the vascular endothelium and results in impaired endothelium

dependent vasomotor function, which is improved by lipid-lowering therapy (Boak and Chin-Dusting, 2004). Oxidative stress induced by reactive oxygen species (ROS) also plays an important role in several diseases including atherosclerosis and coronary heart disease (Kwiterovich, 1997; Tribble, 1999). Malondialdehyde, a secondary product of lipid peroxidation is widely used as marker of lipid peroxidation. Lipid peroxide levels in tissue were found to be significantly elevated in hypercholesterolemic rats (Hung et al., 2006), higher levels can lead to peroxidation of biological membranes (Tiwari, 1999). The antioxidant enzymes, mainly superoxide dismutase and catalase are first-line defensive enzymes against free radicals (Parathasarathy et al., 1999). Antioxidants reduce the oxidation of LDL and

decrease the concentration of free radicals, which inactivate nitric oxide and may be effective in reversing endothelial function associated with hypercholesterolemia (Bok et al., 1999). It is generally assumed that some antioxidants can prevent atherosclerosis by protecting LDL from oxidation and are also associated with an antihypercholesterolemic effect (Chen et al., 1999; Freyschuss et al., 2001). There are however reports on the effect of *Hypericum* on hypercholesterolemia (Zou et al., 2005; Hakimo et al., 2007). Result demonstrated that the consumption of extruded flavonoid-rich extract of *H. perforatum* (FEHP) reduces TC, TG, LDL-C, AI and MDA and increased the concentration of serum HDL-C. The ability of FEHP to suppress the lipid peroxidation may partially be attributed to the antiradical activities of the flavonoid

Table 2. Pathological evaluation of aorta in cholesterol fed rabbits.

Group	Diet	(μ)	Plaque stage
I	Normal diet	0 $\pm$ 0	-
II	High-cholesterol diet	3.166667 $\pm$ 0.288675	3
III	High-cholesterol diet + <i>Amaranthus</i> + <i>Hypericum</i>	0.866667 $\pm$ 0.305505*	1
IV	High-cholesterol diet + <i>lovastatin</i>	1.166667 $\pm$ 0.737111	1

Group I: Fed with Normal diet (control group) (60th days); Group II: Hypercholesterolemic diet (60th days); Group III: Hypercholesterolemic diet + combination of *Amaranthus* + *Hypericum* (treatment group) (60th days); Group IV: hypercholesterolemic diet + lovastatin (treatment group) (60th days). * Significant difference between Group III and Group II ($p < 0.05$).

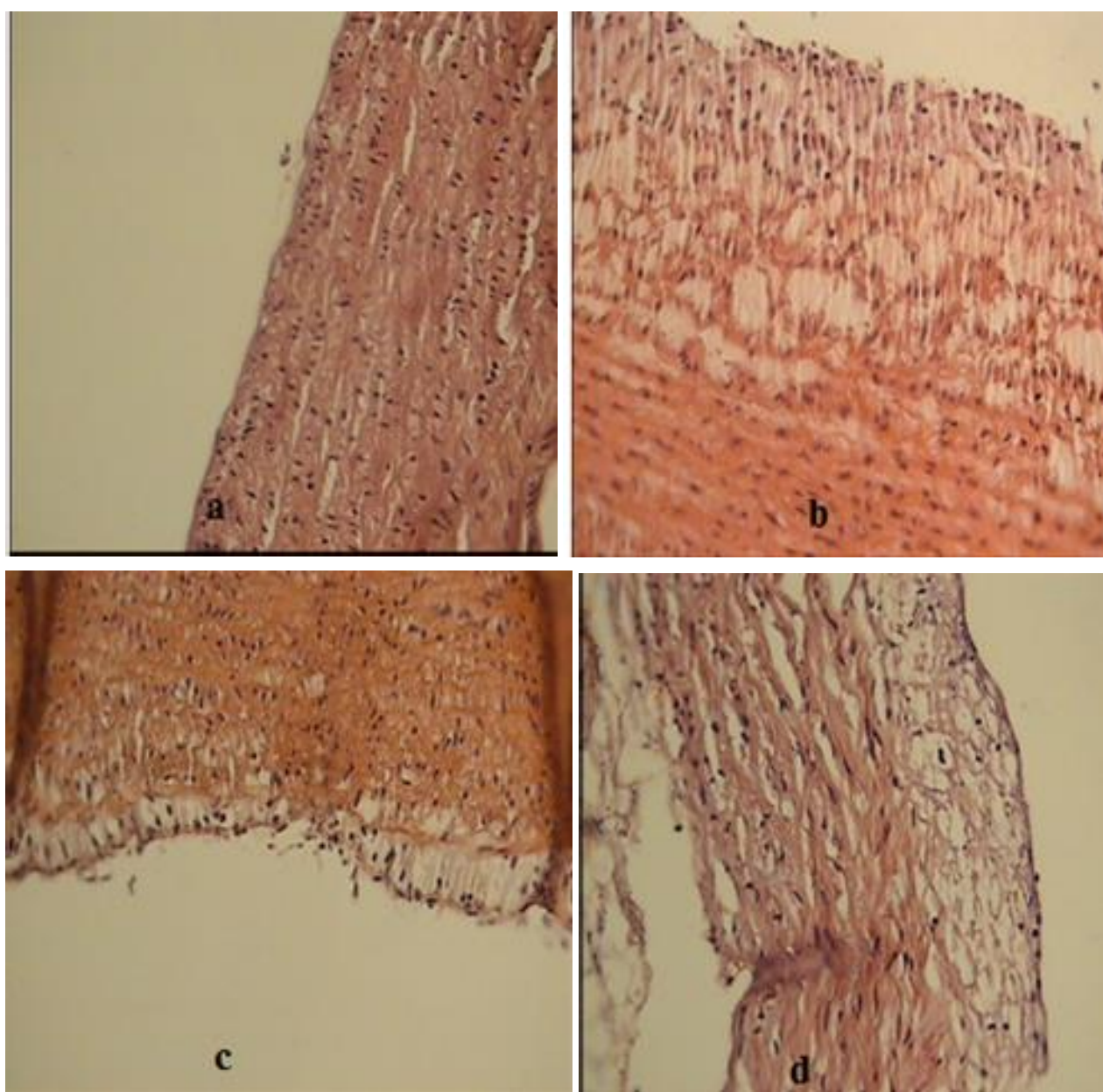


Figure 1. Pathological evaluation of aorta in all group. a: Aorta of normal diet (control group); b: Aorta of high-cholesterol diet (control group); c: Aorta of high-cholesterol diet + concurrent of *Amaranthus* + *Hypericum* (treatment group); d: Aorta of high-cholesterol diet + lovastatin (treatment group).

components known to act by free radical scavenging or chain-breaking mechanisms (Zou et al., 2004; Conforti et al., 2002; El-Sherbiny et al., 2003). Recent studies have demonstrated that different species of *Hypericum* contains compounds such as flavonoids, xan-thones and phenolic and can be used as antioxidants (Mulinaccia et al., 2008). Ethanol extract of *Hypericum lysimachioides* was investigated for hypolipidemic and its *in vitro* antioxidant activity. Histological study demonstrated that ethanol extract of *H. lysimachioides* can reduce atherosclerosis lesions in the aorta and some fatty changes in liver (Hakimo glu et al., 2007). Therefore, *Hypericum* extract, with a potential antioxidant activity in animal models is useful in the treatment of pathological situations in which ROS play an important role such as acute inflammation (Tedeschi et al., 2003; Menegazzi et al., 2006). Studies have suggested that primary component extract of *H. perforatum* L. was quercetin strong antioxidant activity. It prevented the peroxidation of lipid membranes in liposome and inhibited linoleic acid peroxidation. FEHP was also an effective superoxide anion radical scavenger. FEHP inhibited deoxyribose degradation mainly via the chelating iron ions rather than scavenging hydroxyl radical directly. The results of the study which demonstrated the radical scavenging activity and antioxidant activity of FEHP indicated that FEHP might be proposed as a dietary supplement or drug for the treatment of various coronary heart diseases (Zou et al., 2004). Quercetin has been reported to exert free radical scavenging, TNF- α inhibition effects (Horvathova et al., 2003; Park et al., 2000). Studies have demonstrated that the consumption of extruded amaranth (*A. caudatus*) reduces LDL and total cholesterol in hypercholesterolemic rabbits (Plate and Arêas, 2002). Our previous studies showed antiatherosclerotic and antiinflammatory effects of hydroalcoholic extracts of *A. caudatus* (Kabiri et al., 2010). Andrea et al. (2002) shows positive effect of *A. caudatus* extract on decrease of cholesterol level, LDL-C, VLDL-C and TG (Andrea et al., 2002). There are numerous reports about the hypocholesterolemic effect of plant proteins, particularly those obtained from soybean as well as wheat gluten and potato and oat proteins (Horigome and Cho 1992; Schrijver, 1990). The results obtained suggest that the protein concentrate from *A. cruentus* as supplement in the diet contributes to the prevention of cardiovascular disease (Escudero et al., 2006). Amaranth grains contain about 15 protein and 60% starch (Buckland et al., 2009; Trichopoulou et al., 2003). Tocopherols in amaranth seeds include c-and d-tocotrienols, the unsaturated forms of vitamin E. All of them have antioxidant activity, and they are under analysis as hypocholesterolemic agents (Lehmann et al., 1994). Amaranth also contains squalene, a feedback inhibitor of HMG-CoA reductase activity (Goldstein and Brown, 1990). Therefore, the contents of dietary fibers, trace elements, proteins, antioxidant compounds (Newman et al., 1992; Tosi et al.,

2001) suggested that *Amaranth* and its products could be a valuable substitute for cereals in allergic hypercholesterolemic patients (Czerwin et al., 2004). Anthocyanins have anti-inflammatory and free radical scavenging activity (Pergola et al., 2006). A great number of studies have shown that anthocyanins prevents endothelial damages and act as an inhibitor of endothelial cell death (Pergola et al., 2006; Borradaile et al., 2002). *Amaranth* indicates that herbal supplementation of *Amaranthus* may exert an antiradiation and antioxidative influence in the body organs. *Amaranthus* is one of the carotene rich foods available round the year. Bhatia et al. (2002a) also showed that another variety, *Amaranthus blitum* renders protection oxidative stress suggesting that it may either scavenge or reduce the free radicals generation (Bhatia and Jain, 2002).

In this study, we have used the drug, lovastatin for comparing with effects of combination of hydroalcoholic *A. caudatus* and *H. perforatum*. The most important lipid-modifying drugs are statins, specifically 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. They have been shown to reduce serum cholesterol levels and significantly reduce cardiovascular events and mortality in patients with or without coronary artery disease. The clinically beneficial effects of statins are usually assumed to result from their ability to reduce cholesterol production. Over the past few years, it has become clear that statins have many effects further than lipid lowering capabilities that make them of prospective benefit in patients. Statins help nitric oxide synthesis and improve endothelial function (Drexler and Horning, 1999) and (Koh et al., 2000), and inhibit the production of inflammatory cytokines and chemokines, improve autonomic function, and quash myocardial remodeling (Hayashidani et al., 2002; Lefer, 2002). In addition, statins can reduce vascular inflammation decrease platelet aggregability and thrombus deposition (Lacoste et al., 1995), and increase endothelium consequent nitric oxide production (Laufs and Liao, 1998).

Our results demonstrated that combination of hydroalcoholic extracts of *A. caudatus* and *H. perforatum* L significantly inhibited fatty streak formation in arteries compared with high-cholesterol diet group without significant effect on plasma concentrations cholesterol, TG, HDL, LDL, apoB, CRP, MDA, OX-LDL, AI, apoA and HDL and was effective than lovastatin, possibly through increasing antioxidant capacity and reduction of lipid peroxidation. Further studies are needed to clarify the exact mechanism underlying the antiatherogenic effect of combination of hydroalcoholic extracts of *A. caudatus* and *H. perforatum* in hypercholesterolemic rabbits.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge research funding from Isfahan Cardiovascular Research Center, Isfahan

University of Medical Sciences, a WHO Collaborating Center (Grant Number 84143). Grateful thanks to authorities of Graduate Studies at Isfahan University for supporting this project as part of a thesis in partial fulfillment of the requirements for the degree of Master of Sciences.

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