Valsartan augments the beneficial effect of rosuvastatin with respect to lipid profile, oxidative stress, and the nitric oxide pathway in high-fat diet-induced hypercholesterolemic rats

Omnia E. Baheg[a], Yousreya A. Maklad[a], Amina Gamal El Din[b], Manal A. Badawy[b], Sanaa A. Kenawy[c]

[a]Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Division, [b]Pathology Department, Medical Research Division, National Research Center, [c]Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Correspondence to Yousreya A. Maklad, MD, Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Division, National Research Centre, 12311 Dokki, Giza, Egypt
Tel: +20 100 112 0565; fax: +20 333 709 31; e-mail: yousreya_maklad@yahoo.com

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Background
The renin–angiotensin system contributes considerably to a variety of cardiovascular diseases and is the target of angiotensin receptor blockers (ARBs). Recent studies have reported that in experimental models, as well as some human studies, ARBs had shown the ability to affect lipid metabolism in a modest but significant way. In addition to their primary mode of action, statins and ARBs have common additional properties such as restoration of endothelial activity and antioxidant properties. These properties may potentially aid the improvement treatment of cardiovascular disease.

Objective
The present study was designed to evaluate the possible beneficial effects of both therapies valsartan (ARB) and rosuvastatin (3-hydroxy-methylglutaryl coenzyme reductase inhibitor) beyond their blood pressure-lowering and cholesterol-lowering effects, and the possibility that valsartan may enhance the beneficial effects of rosuvastatin in high-fat diet-induced hypercholesterolemic (HC) rats with respect to lipid profile, oxidative stress, and the nitric oxide pathway.

Materials and methods
HC was induced in male albino Wistar rats by a daily gavage of a cocktail containing 1 l peanut oil, 100 g cholesterol, and 100 g cholic acid over a period of 21 days. These animals were assigned randomly to the following groups: HC, HC/rosuvastatin, HC/valsartan, and HC/rosuvastatin+valsartan.

Results
Daily gavage of the cocktail for 3 weeks induced a significant increase in plasma total cholesterol (TC), triglyceride (TG), and low-density lipoprotein and a significant reduction in high-density lipoprotein (HDL), but did not induce any significant changes in arterial blood pressure and heart rate. Meanwhile, the plasma nitric oxide level was reduced to 17.49% of its normal level and the plasma malondialdehyde level was significantly increased by 32.53%. Coadministration of valsartan with rosuvastatin normalized plasma HDL, significantly decreased plasma TC and low-density lipoprotein to a greater extent than monotherapy with each drug as well as ameliorated the effect of HC diet on the plasma TG level in HC rats. Moreover, the combined treatment induced a significant increase in the plasma nitrate+nitrite level compared with the corresponding HC value and normalized the plasma malondialdehyde level with respect to the effect of rosuvastatin or valsartan alone. In addition, histopathological and morphometric studies of the aorta and liver showed marked improvement after combined treatment with rosuvastatin and valsartan when compared with the HC group.

Conclusion
Conclusively, coadministration of rosuvastatin and valsartan in high-fat diet-induced HC rats conferred a greater degree of protection as it ameliorated the increase in the plasma TC and TG and restored HDL to its normal value, improved the endothelial function, and reduced oxidative stress, together with improvement in the histopathological features in rats that had previously received a high-fat diet.

Keywords:
hypercholesterolemia, hypertension, lipid profile, oxidative stress

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Introduction

Hypercholesterolemia (HC) is a lipoprotein metabolic disorder characterized by high serum low-density lipoprotein (LDL) and cholesterol. It is a major health problem and is a challenge for health professionals because of the close correlation between cardiovascular diseases (CVDs) and lipid abnormalities [1,2]. HC and increased plasma concentrations of LDL-cholesterol are one of the most important risk factors involved in the pathogenesis of atherosclerosis that lead to CVDs [3–5].

Considerable evidence points to the interplay between HC and hypertension, acting through the renin–angiotensin system (RAS), to increase cardiovascular risk [6–8]. Angiotensin-converting enzyme expression and activity has been shown to increase during atherosclerosis and hyperlipidemia [9]. It is believed that the interactions between dyslipidemia and activation of neurohumoral systems, such as the RAS, may not only explain the frequent coexistence of dyslipidemia and hypertension but may also play an important role in the pathogenesis of atherosclerosis [9]. HC patients often have hypertension. Angiotensin II increases lipid uptake in cells and lipid accumulation in the vessel wall. LDL upregulates expression of the AT1 receptor in cultured vascular smooth muscle cells and in HC rabbits [10–13].

The RAS contributes considerably to a variety of CVDs and is the target of angiotensin receptor blockers (ARBs). The therapeutic value of valsartan as an antihypertensive drug is mediated by blocking AT1 receptor, which mediates most of the cardiovascular effects of angiotensin II, including oxidative stress, vasoconstriction, aldosterone secretion, renal sodium reabsorption, sympathetic stimulation, vasopressin release, cardiac and vascular cell hypertrophy, and cell proliferation. In addition, ARBs may reduce vascular cell adhesion molecules and micro albuminuria and may increase nitric oxide (NO) levels in the early stages of diabetic nephropathy [14]. Blockade of AT1 receptors by ARBs results in reduced oxidative stress and decreased breakdown of NO, and conversely, increased bioavailability of NO [7].

The benefits gained with ARBs may, at least in part, be the result of their effect on arterial inflammation and the reduction in CVD morbidity and mortality. This may also be the result of decreasing proinflammatory processes present in patients with hypertension, atherosclerosis, or heart failure [15–18].

The effects of angiotensin II and lipoproteins on atherogenic risk are not independent. Accumulating data suggest that the pathways by which angiotensin II and LDL-cholesterol lead to vascular disease may frequently overlap [19]. Interventions directed at lowering total cholesterol (TC), LDL-cholesterol, and triglyceride (TG) levels and increasing high-density lipoprotein (HDL)-cholesterol levels result in a reduction in cardiovascular events.

Statins are known as first-line agents for efficacious lipid-lowering therapy. They directly inhibit cholesterol synthesis (primarily in the liver) by blocking 3-hydroxy-methylglutaryl coenzyme (HMG-CoA) reductase, the principal rate-limiting enzyme of cholesterologenesis [20]. Statins may potentially be useful in the treatment and/or prevention of a wide range of chronic and life-threatening disorders (cancer, osteoporosis, ventricular arrhythmia, peripheral arterial disease, and lower muscle sympathetic nerve activity and oxidative stress in patients with heart failure). Thus, the benefit of statins may not be limited to cholesterol lowering and indications for their use may extend to patient populations not considered traditional candidates for this therapy [21,22].

On the basis of the previous work, the present study was designed to evaluate the possible synergistic effects of both therapies valsartan and rosuvastatin beyond their blood-lowering and cholesterol-lowering effects, and the possibility that rosuvastatin may enhance the beneficial effects of valsartan in high-fat diet-induced HC rats.

Materials and methods

Animals

Adult male albino Wistar rats weighing 200–280 g were used in the present study. They were purchased from the Animal House Colony of the National Research Center, Cairo, Egypt. Animals were housed under standardized conditions (room temperature 23 ± 2°C; relative humidity 55 ± 5%; 12 h light/dark cycle) and had free access to tap water and standard rat chow throughout the entire experimental period. All animal procedures were performed after receiving the approval of the Ethics Committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals by ‘Canadian Council on Animal Care Guidelines, 1984’.

Drugs and chemicals

Cholesterol was obtained from Panreac Quimica SA (Barcelona, Spain), cholic acid from Aldrich Chemical Co. Ltd (Gillingham, Dorset, England) peanut oil from Imtenan Health Shop Co. (Cairo, Egypt), urethane from Sigma-Aldrich Chemie (Munich, Germany), thiobarbituric acid (TBA) from Merck (Darmstadt, Germany), perchloric acid from Sigma Chemical Company (USA), trichloroacetic acid from Fluka Chemie AG (Buchs, Switzerland), total NO kit from BioAssay Systems (USA), cholesterol, TG, and HDL-cholesterol kits from Biodiagnostic (Giza, Egypt), rosuvastatin from Pharmaceutical Inc. (Peurto Rico, UK), and valsartan was obtained as gifts from the Global Napi Pharmaceutical Company (Cairo, Egypt).

Experimental design and treatment protocol

After 7 days of acclimatization to laboratory conditions, the animals were assigned randomly to experimental groups of 8–10 rats each. In one set of animals, thiouracil was included in the diet to induce HC [23–25], but it was observed that the animals lost weight and long-term mortality was high. Therefore, an attempt was made to induce HC in all other groups of animals by daily oral administration of 1 ml/100 g body weight of a cocktail containing 1 l peanut oil, 100 g cholesterol, and 100 g cholic acid over a period of 21 days without using thiouracil [26].

Animals were classified randomly according to the following design:

Group 1	Normal rats received distilled water and served as normocholesterolemic controls
Group 2	Hypercholesterolemic control group
Group 3	Hypercholesterolemic rats were treated with rosuvastatin [10 mg/kg/day, orally (p.o.)], started at the beginning of the first week and continued together with cocktail for 3 weeks [27]
Group 4	Hypercholesterolemic rats were treated with valsartan (10 mg/kg/day, p.o.), started at the beginning of the first week and continued together with cocktail for 3 weeks [28]
Group 5	Hypercholesterolemic rats were treated with valsartan (10 mg/kg/drug/day, p.o.) and rosuvastatin (10 mg/kg/drug/day, p.o.), started at the beginning of the first week and continued together with cocktail for 3 weeks

At the end of the experimental period (3 weeks), the plasma TC was evaluated to confirm the occurrence of HC.

Treatment was carried out once in the morning before supplying food to the animals to allow best absorption of the agent.

Measurement of arterial blood pressure

At the end of the treatment period (3 weeks), body weights of rats were recorded. Then, rats were anesthetized with urethane (1.5 g/kg, intraperitoneally) [29,30]; a polyethylene catheter (1.0 mm outer diameter) attached to a pressure transducer (Isotec; Hugo Sachs Elektronik, Germany) was implanted into the left carotid artery for arterial blood pressure and heart rate measurement following the method described by Krzeminski *et al.* [31]. The transducer, which was connected to a pressure coupler (Type 566; Hugo Sachs Elektronik, Germany), was mounted in an oscillographic recorder (Linear Mark VI; Graphtec Corporation, Germany).

The mean arterial blood pressure (MABP) was calculated according to the following equation:

MABP = DBP + 1/3 (SBP–DBP).

At the end of the measurement procedure, blood samples were collected directly from the carotid artery. Plasma samples were obtained by centrifugation at 3500 rpm and 8°C for 20 min (Type Z323K; Hermle Labortechnik, Germany).

Animals were killed, the heart was isolated, blotted between two filter papers, weighed, and the heart weight/body weight ratio was calculated. Moreover, the liver and aorta of rats from each group were removed and fixed in 10% buffered formalin for 24 h for further histopathological examination.

Biochemical estimations

TC and TG were estimated in plasma according to the method described by Allain *et al.* [32] and Fassati and Prencipe [33], respectively. HDL was assayed according to the method of Lopes-Virella *et al.* [34].

LDL-cholesterol was calculated according to the equation of Friedewald *et al.* [35]:

LDL-cholesterol = TC-(HDL-cholesterol +TG/5).

Total NO metabolites (nitrate+nitrite) were assessed in plasma according to the method described by Bulau *et al.* [36] and Hasegawa *et al.* [37]. In this assay, cadmium quantitatively reduces nitrate to nitrite. The reaction is followed by the colorimetric detection of nitrite as an azo dye product of the Griess reaction. The Griess reaction is based on the two-step diazotization reaction in which acidified NO_2^- produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to *N*-(1-naphthyl) sulfanilic acid to form the chromophoric azo derivative, which absorbs light at 540 nm.

Lipid peroxides were estimated colorimetrically by a TBA reaction as described by Yagi [38]. TBA reacts with malondialdehyde (MDA) in acidic medium at 95°C for 30 min to form a TBA-reactive product; the absorbance of the resultant pink product can be measured at 534 nm.

Histopathological and morphometric examinations

Paraffin blocks of the liver and aorta of different groups fixed in 10% buffered formalin for 24 h were prepared and serially sectioned into 5-μm-thick sections. Staining with hematoxylin and eosin was performed for routine histopathological examination and morphometry [39].

Additional sections from each of the aorta and liver organs were stained with Masson trichrome stain [40] for better visualization of the fibrous tissue.

Morphometric analysis was carried out at the Pathology Department, National Research Center using the Leica Qwin 500 Image Analyzer (Leica Imaging Systems Ltd, Cambridge, UK), which consists of a Leica DM-LB microscope with a JVC color video camera attached to a computer system Leica Q 500IW. The length of the tunica media of the aorta was measured on a real-time image from the microscope that we visualized on the video monitor. The reading of each measurement appears in micrometers.

Statistical analysis

Results were expressed as mean±SEM. Statistical analysis of the obtained data was carried out using the SPSS (release 16.00; SPSS Inc., Chicago, Illinois, USA) statistical software and one-way analysis of variance, followed by post-hoc least significance difference. For all tests, statistical significance was set at *P* value 0.05 or less.

Results

Arterial blood pressure

The results presented in Table 1 indicated that chronic administration of cocktail (10 ml/kg/day, p.o.) for 3 weeks did not lead to any significant changes in the systolic blood pressure (SBP), diastolic blood pressure (DBP), and MABP values compared with the respective normal values. Three weeks of daily oral treatment with either rosuvastatin (10 mg/kg/day, p.o.) or valsartan (10 mg/kg/day, p.o.) did not induce any significant changes in SBP, DBP, and MABP compared with that of the corresponding normal and HC values. Concurrent treatment of HC rats with rosuvastatin and valsartan induced a mild insignificant reduction in SBP, DBP, and MABP compared with the respective normal values and with respect to the corresponding HC values.

Heart rate

In terms of the results of heart rate, the data presented in Table 1 show that the normal value of the heart rate was 283.17 ± 18.43 beats/min. Daily gavage of cocktail (10 ml/kg) for 3 weeks did not show lead to any significant change in the heart rate value compared with the normal value. Treatment of HC rats with rosuvastatin (10 mg/kg/day, p.o.), valsartan (10 mg/kg/day, p.o.), or their combination led to an insignificant change in heart rate compared with that of the normal value as well as the HC value.

Plasma lipid profile

The results presented in Table 2 indicate that following 3 weeks of daily gavage of cocktail (10 ml/kg), there was

Table 1 Effect of a 3-week oral daily administration of rosuvastatin, valsartan, or their combination on systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate in diet-induced hypercholesterolemic male rats

Groups	Parameters			
	SBP (mmHg)	DBP (mmHg)	MABP (mmHg)	Heart rate (beats/min)
Normal (distrilled water)	106.67 ± 5.42	67.50 ± 7.04	80.55 ± 4.80	283.17 ± 18.43
HC control (10 ml of coctail/kg)	103.33 ± 3.33	66.66 ± 4.40	78.83 ± 3.63	291.00 ± 19.70
HC/rosuvastatin (10 mg/kg/day)	100.00 ± 6.22	71.25 ± 5.88	81.00 ± 5.75	305.00 ± 9.08
HC/valsartan (10 mg/kg/day)	102.14 ± 5.43	75.00 ± 5.34	83.71 ± 5.33	310.67 ± 7.75
HC/Ros+Val (10 mg/kg/day/drug)	88.75 ± 5.32	56.87 ± 5.97	67.37 ± 5.58	297.37 ± 6.04

Each value represents the mean value ± SEM of the number of animals in each group (*n* = 6–8); DBP, diastolic blood pressure; HC, hypercholesterolemia; MABP, mean arterial blood pressure; Ros, rosuvastatin; SBP, systolic blood pressure; Val, valsartan.

Table 2 Effect of a 3-week daily oral administration of either rosuvastatin, valsartan, or their combination on total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol in diet-induced hypercholesterolemic male rats

Groups	Parameters			
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal (distrilled water)	67.95 ± 1.17[a,b]	120.61 ± 0.75[a,b]	23.75 ± 0.62[a]	20.12 ± 1.35[a,b]
HC control (10 ml of cocktail/kg/day)	192.75 ± 1.62[*,b]	131.13 ± 0.83[*,b]	20.32 ± 0.38[*,b]	146.10 ± 1.66[*,b]
HC/rosuvastatin (10 mg/kg/day)	163.87 ± 1.83[*,a,b]	130.67 ± 0.45[*,b]	20.13 ± 0.37[*,b]	117.22 ± 1.72[*,a,b]
HC/valsartan (10 mg/kg/day)	178.90 ± 1.73[*,a,b]	130.49 ± 0.64[*,b]	20.54 ± 0.40[*,b]	130.99 ± 1.59[*,a,b]
HC/Ros+Val (10 mg/kg/day/drug)	147.18 ± 2.13[*,a]	124.53 ± 1.02[*,a]	22.21 ± 0.51[a]	100.09 ± 2.03[*,a]

Each value represents the mean value ± SEM of the number of animals in each group (*n* = 10); HC, hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ros, rosuvastatin; TC, total cholesterol; TG, triglyceride; Val, valsartan; [a]Significantly different from the HC control value at $P \leq 0.05$; [b]Significantly different from the Ros+Val-treated value at $P \leq 0.05$; *Significantly different from the corresponding normocholesterolemic value at $P \leq 0.05$.

a significant increase in plasma TC, TG, and LDL levels by 183.66, 8.72, and 626.14%, respectively. However, the cocktail induced a significant decrease in HDL by 14.44% compared with the respective normal values. Oral daily administration of rosuvastatin (10 mg/kg) for 3 weeks induced a significant decrease in plasma TC and LDL by 14.98 and 19.76%, respectively, compared with the respective HC value, but did not lead to any changes in the TG and HDL values. In addition, treatment with valsartan (10 mg/kg, p.o.) for 3 weeks led to a significant decrease in plasma TC and LDL by 7.18 and 10.34%, respectively, compared with the respective HC values, but lead to any changes in the TG and HDL values. However, concurrent administration of rosuvastatin and valsartan led to a significant decrease in plasma TC, TG, and LDL by 23.64, 5.03, and 31.49%, respectively, compared with the HC value and restored the HDL to its normal value.

Plasma nitric oxide level

After 3 weeks of cocktail daily oral administration (10 ml/kg), HC rats showed a significant decrease in the plasma total nitrate+nitrite level by 17.49% to 28.15 ± 0.85 mmol/l compared with that of the normal value. Oral administration of either rosuvastatin (10 mg/kg/day) or valsartan (10 mg/kg/day) for 3 weeks restored the plasma nitrate + nitrite to its normal level in HC rats. Furthermore, concurrent administration of rosuvastatin and valsartan acted synergistically to induce a significant increase in the plasma total nitrate+nitrite level by 30.15 and 57.76% compared with the normal and HC values, respectively.

Plasma malondialdehyde level

The normal value of plasma MDA was 0.292 ± 0.005 μmol/dl (Table 3). Oral administration of cocktail (10 ml/kg) for 3 weeks induced a significant increase in the plasma MDA level by 32.53% of its normal value to 0.387 ± 0.011 μmol/dl. Treatment of HC rats with either rosuvastatin (10 mg/kg/day, p.o.) or valsartan (10 mg/kg/day, p.o.) for 3 weeks induced a significant decrease in the plasma MDA level by 9.56 and 6.71%, respectively, compared with the HC value. Three weeks of combined treatment with rosuvastatin and valsartan normalized the plasma MDA level in HC rats.

Body weight, heart weight, and heart weight/body weight ratio

Data presented in Table 4 show an insignificant decrease in the body weight and heart weight following 3 weeks of oral administration of cocktail (10 ml/kg) compared with the corresponding normal value. The ratio of heart weight/body weight of the HC rats showed a significantly higher value compared with the normal ratio. Treatment with rosuvastatin (10 mg/kg), valsartan (10 mg/kg), or their combination restored the heart weight/body weight ratio to its normal value.

Table 3 Effect of a 3-week oral daily administration of rosuvastatin, valsartan, or their combination on plasma total nitric oxide and plasma malondialdehyde in diet-induced hypercholesterolemic male rats

Groups	Parameters	
	Plasma nitrate+nitrite (μmol/l)	Plasma MDA (μmol/dl)
Normal (distrilled water)	34.12 ± 0.68[a,b]	0.292 ± 0.005[a]
HC control (10 ml of cocktail/kg/day)	28.15 ± 0.85*[,b]	0.387 ± 0.011*[,b]
HC/rosuvastatin (10 mg/kg/day)	35.34 ± 0.94[a,b]	0.350 ± 0.008*[,a,b]
HC/valsartan (10 mg/kg/day)	34.62 ± 0.82[a,b]	0.361 ± 0.004*[,a,b]
HC/Ros+Val (10 mg/kg/day/drug)	44.41 ± 0.84*[,a]	0.308 ± 0.007[a]

Each value represents the mean value ± SEM of the number of animals in each group (n = 10); HC, hypercholesterolemia; MDA, malondialdehyde; Ros, rosuvastatin; Val, valsartan; [a]Significantly different from the HC control value at $P \leq 0.05$. [b]Significantly different from the Ros+Val-treated value at $P \leq 0.05$; *Significantly different from the normocholesterolemic value at $P \leq 0.05$.

Table 4 Effect of a 3-week oral daily administration of rosuvastatin, valsartan, or their combination on body weight, heart weight, heart weight/body weight ratio, liver weight, and liver weight/body weight ratio in diet-induced hypercholesterolemic male rats

Groups	Parameters				
	Body weight (g)	Heart weight (g)	Heart weight/body weight (mg/g)	Liver weight (g)	Liver weight/body weight ×100 (g/g)
Normal (distrilled water)	228.00 ± 8.98	0.71 ± 0.03	3.11 ± 0.02[a]	7.53 ± 0.28[a]	3.30 ± 0.02[a]
HC control (10 ml of coctail/kg)	205.00 ± 4.64	0.69 ± 0.02	3.36 ± 0.04*[,b]	8.44 ± 0.13*	4.12 ± 0.10*[,b]
HC/rosuvastatin (10 mg/kg/day)	233.00 ± 5.53[a]	0.70 ± 0.02	3.00 ± 0.04[a]	7.86 ± 0.16	3.37 ± 0.04[a]
HC/valsartan (10 mg/kg/day)	219.00 ± 4.09	0.71 ± 0.01	3.24 ± 0.03	8.00 ± 0.22	3.65 ± 0.09*[,a,b]
HC/Ros+Val (10 mg/kg/day/drug)	229.00 ± 10.01	0.73 ± 0.03	3.04 ± 0.06[a]	7.82 ± 0.35	3.41 ± 0.04[a]

Each value represents the mean value ± SEM of the number of animals in each group (n = 10); HC, hypercholesterolemia; Ros, rosuvastatin; Val, valsartan; [a]Significantly different from the HC control value at $P \leq 0.05$; [b]Significantly different from the Ros+Val-treated value at $P \leq 0.05$; *Significantly different from the normocholesterolemic value at $P \leq 0.05$.

Liver weight and liver weight/body weight ratio

The results presented in Table 4 show that 3 weeks of oral administration of cocktail (10 ml/kg) induced a significant increase in liver weight and liver weight/body weight ratio by 12.08 and 24.84%, respectively. Treatment of HC rats with rosuvastatin (10 mg/kg) normalized the liver weight and liver weight/body weight ratio. However, treatment with valsartan (10 mg/kg) ameliorated the increase in the liver weight/body weight ratio, but restored the liver weight to its normal value. Combined treatment with rosuvastatin and valsartan normalized the liver weight and liver weight/body weight ratio.

Histopathological and morphometric examinations

Aortic pathologic changes

Histopathological examination of the aorta sections of the control group showed no histopathological changes and normal tunica media thickness of 31.93 ± 0.74 mm (Table 5) as shown in Figures 1 and 2. However, aortic sections from HC rats showed straightening and thickening in tunica intima with fat deposition (Fig. 3). Tunica media showed moderate thickening (37.27 ± 0.94 mm) (Table 5), with disorganization of muscle fibers, irregular nuclei, and numerous lipid clefts (Fig. 4).

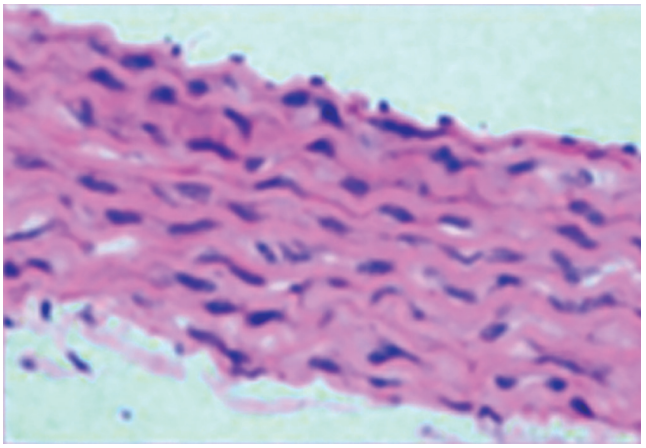
On treatment with rosuvastatin, the aorta showed a significant decrease in the thickness of tunica media reaching 30.72 ± 1.19 mm (Table 5), and a marked reduction in lipid clefts. No disorganization of muscle fibers and no irregular nuclei were observed; waviness of the tunica intima was regained. A reduction in the thickness of tunica adventitia was also observed (Fig. 5). Treatment with valsartan did not induce any improvement in tunica media as well as lipid clefts;

Table 5 Effect of a 3-week oral daily administration of rosuvastatin, valsartan, or their combination on tunica media thickness in diet-induced hypercholesterolemic male rats

Groups	Parameter (tunica media) (μm)	
	Mean ± SEM	% of normal value
Normal (distrilled water)	31.937 ± 0.741[a]	100.00[a]
HC control (10 ml of cocktail/kg/day)	37.278 ± 0.947*,[b]	116.72*,[b]
HC/rosuvastatin (10 mg/kg/day)	30.726 ± 1.194[a]	98.27[a]
HC/valsartan (10 mg/kg/day)	39.217 ± 1.437*,[b]	122.79*,[b]
HC/Ros+Val (10 mg/kg/day/drug)	28.220 ± 0.730[a]	88.36[a]

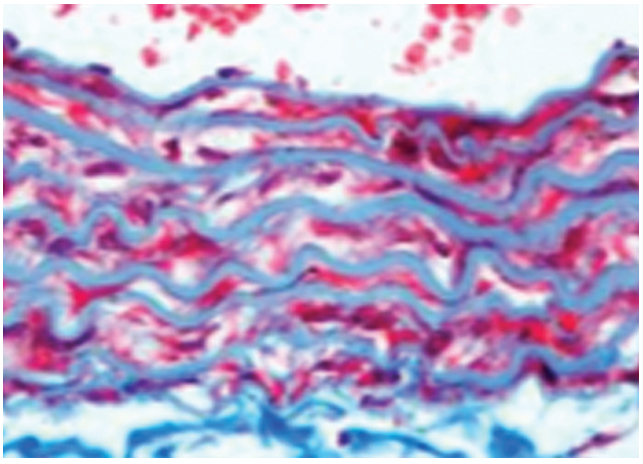
Each value represents the mean value±SEM of the number of animals in each group ($n = 10$); HC, hypercholesterolemia; Ros, rosuvastatin; Val, valsartan; [a]Significantly different from the HC control value at $P \leq 0.05$; [b]Significantly different from the Ros+Val-treated value at $P \leq 0.05$; *Significantly different from the normocholesterolemic value at $P \leq 0.05$.

Figure 1



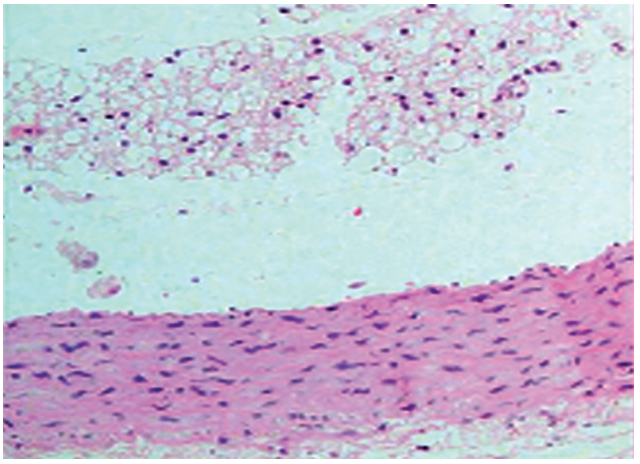
Histological section of normal rat aorta (hematoxylin and eosin, ×400).

Figure 2



Histological section of normal rat aorta (Masson trichrome, ×400).

Figure 3



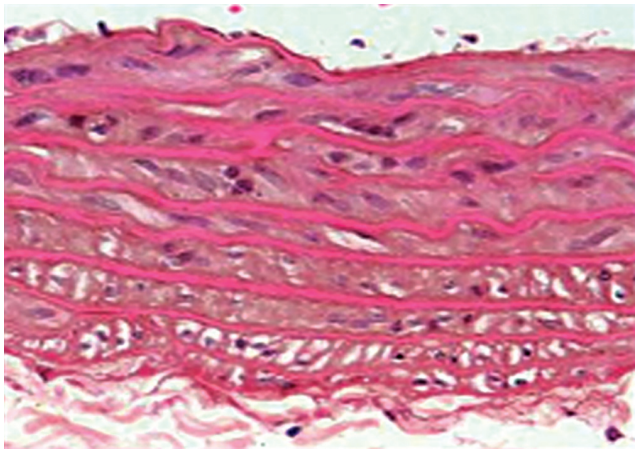
Histologic section of the aorta of hypercholesterolemic rat showing straight intima and foamy macrophages within the lumen of the aorta (hematoxylin and eosin, ×100).

disorganized muscle fibers and irregular nuclei were still observed (Fig. 6). The effect of treatment with both rosuvastatin and valsartan together was more marked than the effect of each drug alone, in the form of a significant reduction in thickness of tunica media reaching 28.22 ± 0.73 mm (Table 5), and tunica adventitia, absence of disorganization in muscle fibers and nuclear irregularity, marked reduction in lipid clefts, and regaining of the waviness of the tunica intima (Fig. 7).

Liver pathologic changes

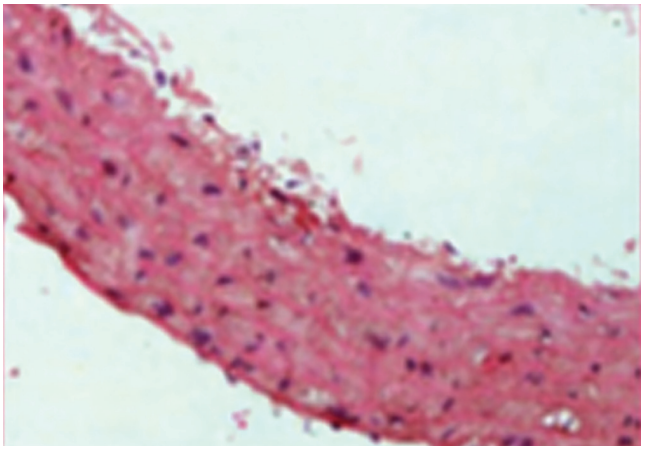
Light microscopic examination of liver sections from the control group (normocholesterolemic rats) showed normal liver architecture in the form of hexagonal hepatic lobules with a central vein in the center of each lobule (Figs. 8 and 9). Light microscopic examination of liver sections from rats fed a high-cholesterol diet showed prominent fatty changes. Marked microvesicular vacuolar degeneration was observed within each hepatocyte. The entire hepatocyte showed a bubbling appearance within the cytoplasm, together with a centrally located nucleus. Necrotic areas were observed within hepatic lobules together with moderate inflammatory cellular infiltrate (Fig. 10). Moderate congestion was observed within the central vein. Moderate portal tract fibrosis was observed, with scattered fibrous tissue septae extending from portal tracts into hepatic lobules. Occasional portal-to-portal bridging fibrosis

Figure 4



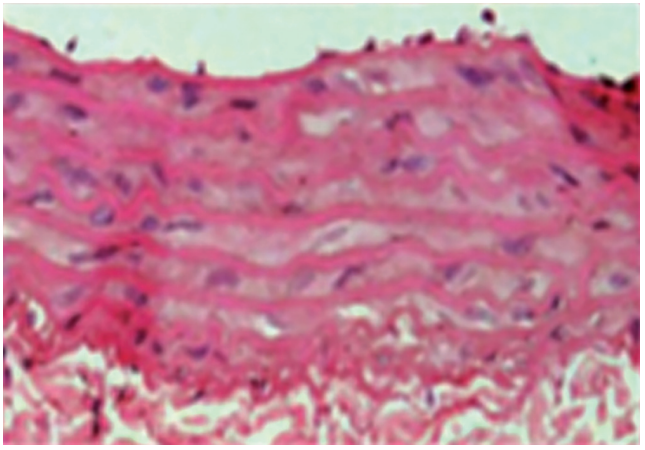
Histologic section of the aorta of hypercholesterolemic rat showing lipid clefts, disorganized muscle fibers, irregular nuclei, and increased thickness in the tunica media (hematoxylin and eosin, ×400).

Figure 5



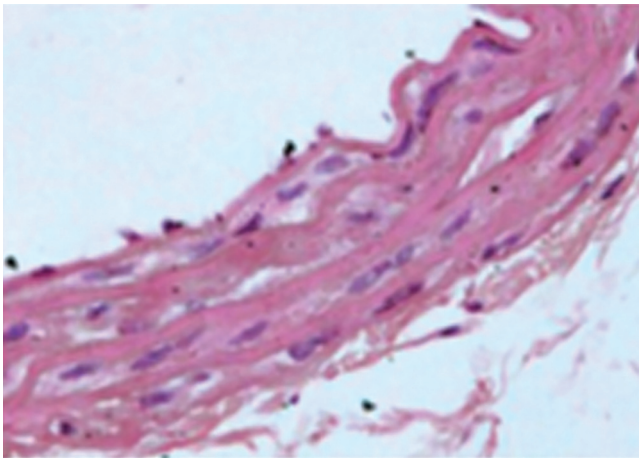
Histological section of the aorta of a rosuvastatin-treated hypercholesterolemic rat showing a marked reduction in lipid clefts, a moderate reduction in the thickness of media, and no disorganization of muscle fibers (hematoxylin and eosin, ×400).

Figure 6



Histologic section of the aorta of a valsartan-treated hypercholesterolemic rat showing a moderate reduction in the thickness of media. Lipid clefts could still be observed (hematoxylin and eosin, ×400).

Figure 7



Histologic section of the aorta of hypercholesterolemic rat treated with both rosuvastatin and valsartan showing a marked reduction in the thickness of media and no disorganization of muscle fibers. Waviness in the tunica intima was regained (hematoxylin and eosin, ×400).

was observed. Fibrous tissue strands were observed in between hepatic cords (Fig. 11).

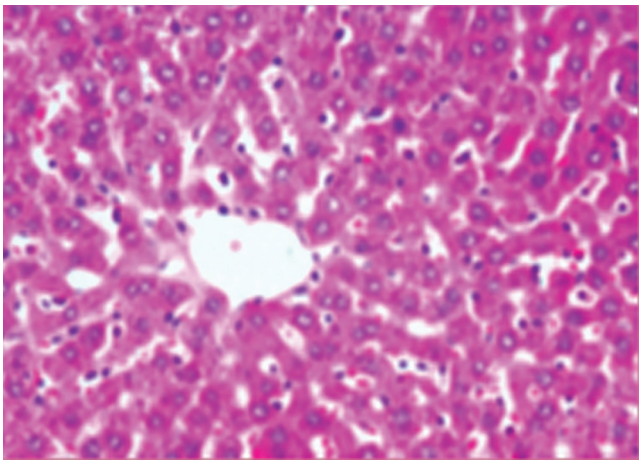
Treatment of HC rats with rosuvastatin induced a decrease in the hepatocytes' fatty microvesicular vacuolar degeneration (Figs. 12 and 13). Treatment of HC rats with rosuvastatin or valsartan induced a decrease in central vein congestion, decrease in portal tract fibrosis, and decrease in fibrous tissue strands in between hepatocytes, and a reduction in inflammatory cellular infiltrate together with a reduction in necrosis (Figs. 14 and 15). Menwhile, rosuvastatin showed a more marked ameliorative effect than that caused by valsartan.

Combined treatment of HC rats with both rosuvastatin and valsartan exerted a more marked effect than the effect of each drug alone in the form of a marked decrease in central vein congestion, portal tract fibrosis, and fibrous tissue strands in between hepatocytes, a marked decrease in inflammatory cellular infiltrate, a marked decrease in necrosis, and a marked decrease in the hepatocytes' fatty microvesicular vacuolar degeneration, being markedly reduced at the periphery of lobules and less reduced adjacent to the central vein (Figs. 16 and 17).

Discussion

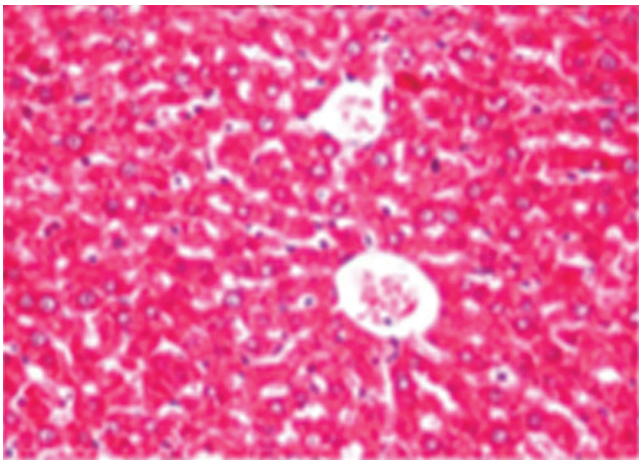
HC was established successfully in the present model, where daily oral administration of cocktail, containing 100 g cholesterol and 100 g cholic acid in 1 l peanut oil, at a dose 10 ml/kg for 3 weeks resulted in a significant increase in plasma TC, TG, and LDL-cholesterol by 183.66, 8.72, and 626.14%,

Figure 8



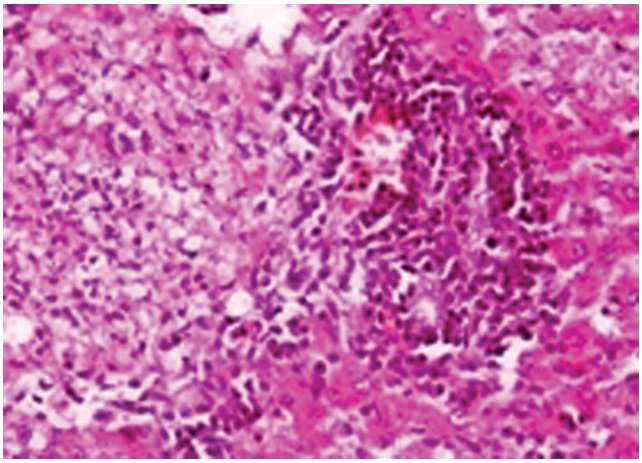
Histological section of normal rat liver (hematoxylin and eosin, ×400).

Figure 9



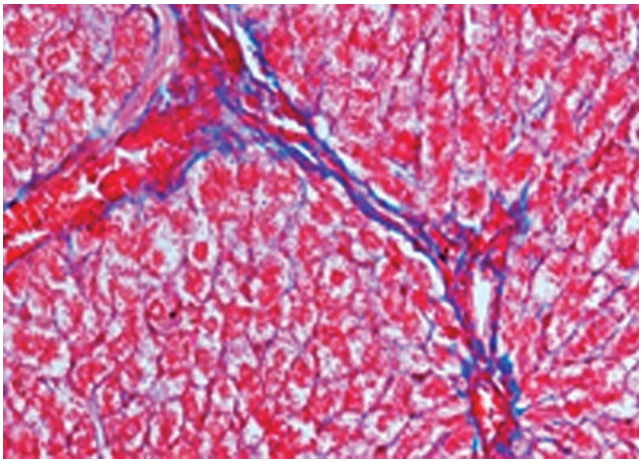
Histological section of normal rat liver (Masson trichrome, ×400).

Figure 10



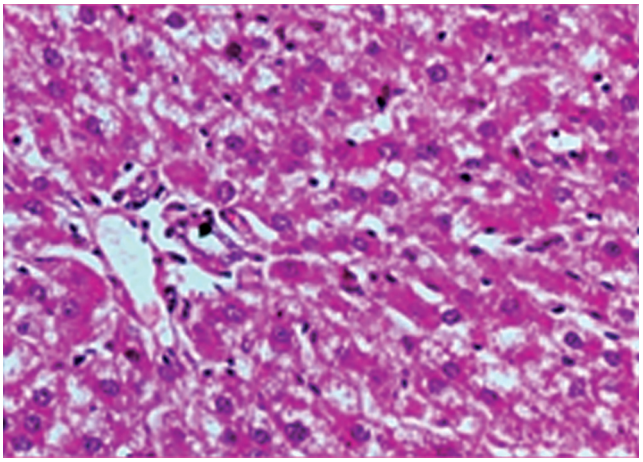
Liver histological sections of diet-induced hypercholesterolemic rats showing a wide area of hepatocyte necrosis with adjacent inflammatory cell aggregate (hematoxylin and eosin, ×100)

Figure 11



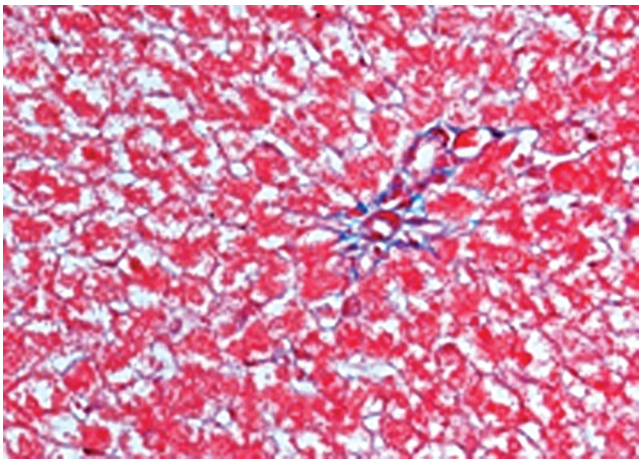
Liver histological sections of diet-induced hypercholesterolemic rats showing portal-to-portal bridging fibrosis and extensive microvesicular vacuolar degeneration within hepatocytes (hematoxylin and eosin, ×400).

Figure 12



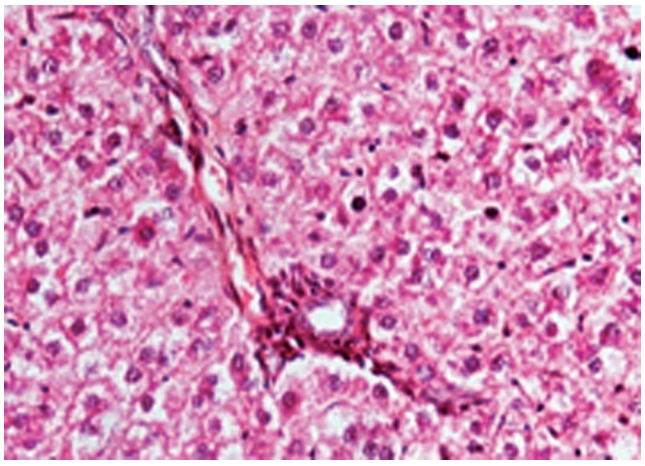
Liver histological sections of diet-induced hypercholesterolemic rats treated with rosuvastatin showing reduced vacuolar degeneration within hepatocytes. Scattered hepatocytes showing eosinophilic cytoplasm devoid of vacuoles (hematoxylin and eosin, ×400).

Figure 13



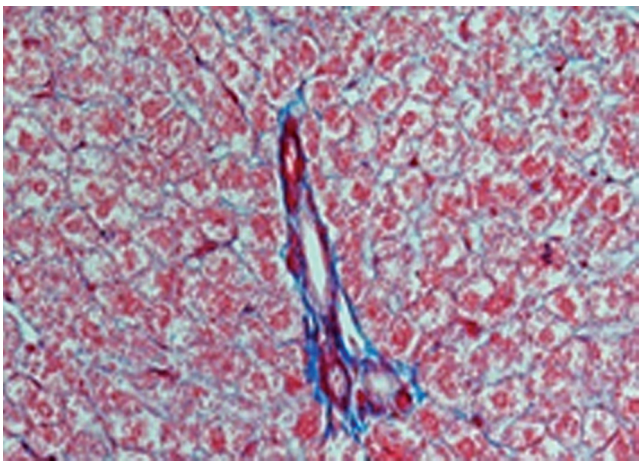
Liver histological sections of diet-induced hypercholesterolemic rats treated with rosuvastatin showing thin and less dense fibrosis around the central vein and markedly reduced portal tract fibrosis (Masson trichrome, ×400).

Figure 14



Liver histological sections of diet-induced hypercholesterolemic rats treated with valsartan still showing microvesicular vacuolar degeneration within hepatocytes. A thin fibrous septum was observed extending within the hepatic lobule with adjacent inflammatory cell aggregate (hematoxylin and eosin, ×400).

Figure 15

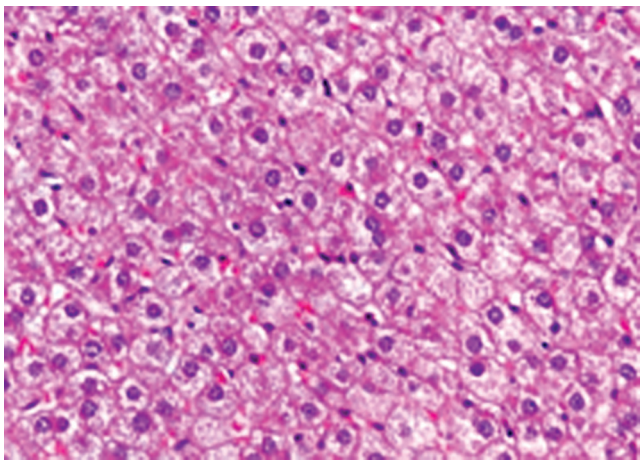


Liver histological sections of diet-induced hypercholesterolemic rats treated with valsartan showing markedly reduced fibrosis around the portal tract and in between hepatocytes (Masson trichrome, ×400).

respectively, which was associated with a significant decrease in HDL-cholesterol by 14.44% compared with the respective normal values, but no significant changes in arterial blood pressure and heart rate were observed. These results were in agreement with the studies by Al-Numair [41] and Kumar *et al* [42]. The changes observed may be attributed to the better cholesterol absorption favored by cholic acid supplementation. Moreover, feeding of rats with cholic acid and cholesterol downregulated the transcription of the cholesterol-7a-hydroxylase (CYP7A1) transgene [43], the rate-determining enzyme in the biosynthetic pathway of bile acids from cholesterol in the liver, for its excretion into the bile, which accounts for about 50% of the daily cholesterol excretion [44].

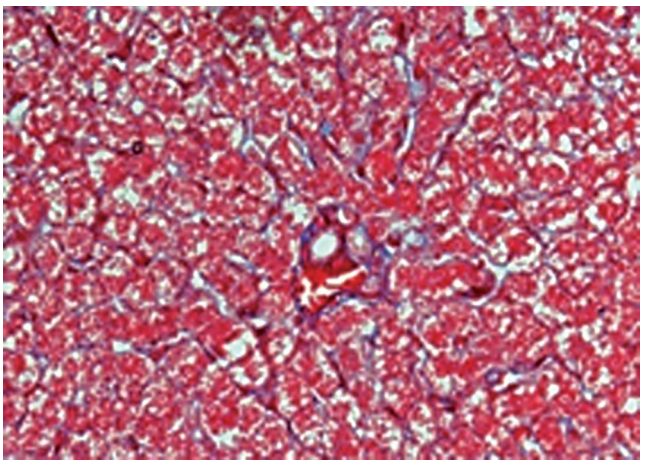
The increase in LDL-cholesterol was explained by the study of Berg *et al*. [45], which showed that uptake of LDL-cholesterol is dependent on receptors in the plasmatic membrane and these are reduced when the cell has enough cholesterol. In addition, Tebib *et al*. [46] found that the activity of lipoprotein lipase enzyme was augmented in HC animals. Lipase converts very low-density lipoprotein into LDL-cholesterol, which would lead to an increase in the serum concentration of LDL-cholesterol. Increased TG and decreased HDL-cholesterol levels in HC rats may be attributed to decreased activity of lipoprotein lipase [47].

Figure 16



Liver histological sections of diet-induced hypercholesterolemic rats treated with both rosuvastatin and valsartan showing a marked decrease in microvesicular vacuolar degeneration (hematoxylin and eosin, ×400).

Figure 17



Liver histological sections of diet-induced hypercholesterolemic rats treated with both rosuvastatin and valsartan showing a marked decrease in fibrosis around the portal tract and in between hepatocytes (Masson trichrome, ×400).

Moreover, several lines of evidence support a synergistic potentiation of endothelial dysfunction and cardiovascular risk by augmented angiotensin II and LDL levels. The result of the present study showed that plasma nitrate+nitrite was markedly reduced in HC rats, indicating impaired endothelial function. These results are in accordance with those of Amin and Abd El-Twab [48]. This could be attributed to the increased endothelial production of oxygen free radicals through activation of NAD(P)H-dependent oxidases, which rapidly degrade NO molecules [49]. LDL and oxidized LDL upregulate AT1 receptor gene expression in vascular cells [11,50]. Furthermore, oxLDL itself can induce the expression of NAD(P) H oxidase and superoxide anion formation [51]. In addition, HC reduced the synthesis of NO in endothelial cells through transcriptional inhibition of the endothelial NO synthase gene, post-transcriptional mRNA destabilization, and competitive inhibition of NO generation by NO synthase [52–54].

Oxidative stress was obvious in HC control rats, indicated by the significant increase in MDA levels; these results were in agreement with those of Martinet *et al.* [55] and Ónody *et al.* [56]. Mahfouz and Kummerow [57], and Al-Numair [41] reported a decrease in the respective enzyme mRNA expressions and the antioxidant defense system after cholesterol-feeding stress associated with an increase in the MDA level.

In relation to the relative liver weight to body weight, there was a significant increase in the HC group compared with the normal group. These results might be attributed to the accumulation of fat in the liver cells. These results were confirmed by histopathological examination, which showed vaculations of tunica media and narrowing in the lumen as well as focal necrosis of tunica intima and tunica media associated with the infiltration of a few leukocytic cells in the aorta and fatty changes of hepatocytes. These results were in agreement with the studies of Rezq and El-Khamisy [58], who reported that aortic and liver histopathological changes and the increase in liver weight could be a result of their higher fat content (fat/liver).

Treatment with rosuvastatin (10 mg/kg) for 3 weeks led to a significant decrease in the plasma TC and LDL-cholesterol level, but did not induce any changes in arterial blood pressure, heart rate, plasma TG, and HDL level compared with their corresponding HC values. These findings were in agreement with the results of Shepherd [59], who reported that administration of statins to HC rats led to an inhibition in cholesterol production in rat liver by blocking HMG-CoA reductase, but did not impact intestinal cholesterol absorption. As a result, hepatocytes became depleted of cholesterol and responded by increasing LDL-cholesterol clearance from the blood through upregulation of hepatic LDL-cholesterol receptors and decreasing the entry of LDL-cholesterol into the circulation [60].

Moreover, rosuvastatin normalized the plasma nitrate+nitrite level, heart weight/body weight ratio, liver weight, and liver weight/body weight ratio and ameliorated the effect of HC diet on the plasma MDA level, indicating improved endothelial function and decreased oxidative stress. These results were in

agreement with those of Wilson *et al.* [61], Bolayirli *et al.* [62], Sakabe *et al.* [63], and Ali *et al.* [64]. Furthermore, lipid-independent scavenging of reactive oxygen species and reduction in superoxide anion (O_2^-) formation were induced *in vitro* and *in vivo* by fluvastatin [65–67], thus reducing NO degradation and increasing its bioavailability. In the same group, histopathological studies of the aorta and liver showed marked improvement after administration of rosuvastatin compared with the HC group. These results were in agreement with the study of Kim *et al.* [68], who reported that despite higher plasma cholesterol, fat accumulation of liver tissue in the statin-treated group appeared less than that in the HC group. Furthermore, endothelium of aorta tissue of the control appeared more rumpled than that of the group treated with statin.

Valsartan is usually used as an antihypertensive drug; it improves renal function and also induces a reduction in the progression of atherosclerosis lesions. In the current study, valsartan (10 mg/kg) treatment induced a significant decrease in plasma TC and LDL-cholesterol, but did not induce any changes in plasma TG, HDL, MABP, and heart rate. Ran *et al.* [69] reported that in experimental models, as well as some human studies, ARBs had shown the ability to affect lipid metabolism in a modest but significant manner.

More precisely, ARBs improved the overproduction and accumulation of TG in the liver, in experimental models, through mechanisms independent of their hypotensive action. The lipid-lowering property of ARB is possibly because of numerous different mechanisms. It is well known that some ARBs partially activate peroxisome proliferator-activated receptor-γ, which regulates lipid metabolism and partially reduces TC and LDL-cholesterol [11,70–72]. In addition, valsartan treatment of HC rats led to a significant decrease in plasma MDA level and was accompanied by increased plasma nitrite and nitrate levels, reflecting improved endothelial function and reduced oxidative stress. The study of Harrison [73,74] and Wassmann *et al.* [75] showed that AT1 receptor antagonism can improve endothelial function and reduce oxidative stress, which may lead to an increased bioavailability of NO, during HC, irrespective of lipid lowering or blood pressure reduction. Furthermore, AT1 receptor blocker therapy reduced NAD(P)H oxidase subunit expression in arteries [76], thus resulting in reduced free radical production and oxidative stress, which was shown by decreased plasma MDA level.

The present data indicated that valsartan normalized liver weight and heart weight/body weight ratio; meanwhile, it ameliorated the effect of HC diet on liver weight/body weight ratio, which could be attributed to the observed decrease in fatty changes in valsartan-treated HC rats. These findings are in agreement with the study of Ibrahim *et al.* [77], which showed that the degree of hepatic steatosis, inflammation, and fibrosis were significantly decreased in the telmisartan-treated group compared with the cholesterol-fed group.

The present study showed that 3 weeks of daily gavage of rosuvastatin (10 mg/kg/day) combined with valsartan (10 mg/kg/day) induced a mild and insignificant reduction in arterial blood pressure without affecting the heart rate, normalized plasma HDL, and significantly decreased the plasma TC and LDL to a greater extent than monotherapy with each drug and ameliorated the effect of HC diet on the plasma TG level in HC rats. These results were in agreement with the findings of a previous study of Nickenig [78] that indicated that a combination of a statin with antihypertensive therapy led to a significant additive effect on HC; moreover, our previous study showed that concurrent administration of rosuvastatin and valsartan in normal rats induced a mild and insignificant reduction in arterial blood pressure. Some clinical studies have shown a close relationship between AT1 receptor density and plasma LDL-cholesterol, and an association of the use of statins to lower cholesterol with AT1 receptor downregulation [79,80].

In addition, concurrent administration of rosuvastatin and valsartan in diet-induced HC rats induced a significant increase in the plasma nitrate+nitrite level compared with the normocholesterolemic and HC values. Moreover, the combined treatment normalized the plasma MDA level with respect to the effect of rosuvastatin or valsartan alone. These results were in agreement with the study of Rueckschloss *et al.* [76], who showed that combined treatment by statins and AT1 receptor blockers has the potential to reduce oxidative stress and endothelial dysfunction, as well as those of Lunder *et al.* [81], who showed that low-dose atorvastatin or losartan and especially their combination increases the expression of nitric oxide synthase 3 and decreases the expression of vasoactive-related genes endothelin receptor type A, which play a major role in improving arterial function. Furthermore, combined treatment normalized liver weight, heart weight/body weight, and liver weight/body weight ratios. These could be attributed to the observed decrease in fatty changes in HC rats treated with rosuvastatin and valsartan and this was shown by the improvement in histopathological changes induced by a high-cholesterol diet in the aorta and liver of HC rats.

In conclusion, this study suggests that coadministration of rosuvastatin and valsartan in a high-fat diet-induced HC rats ameliorated the increase in the plasma TC and TG and restored HDL to its normal value. The combined treatment acted synergistically to increase the total plasma NO level and it also restored the plasma MDA level to its normal value. These explain why administration of rosuvastatin and valsartan could produce synergistic effects against CVDs.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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