Attenuation of the development of hypercholesterolemic atherosclerosis by thymoquinone

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Thymoquinone (TQ), derived from *Nigella sativa* seed, is an antioxidant. The present study investigated whether TQ attenuates the development of atherosclerosis, and/or reduces the serum lipid levels and oxidative stress in rabbits. New Zealand white female rabbits were assigned to four groups of six animals each: group I, control; group II, 1% cholesterol diet; group III, 1% cholesterol plus TQ (10 mg/kg/day; through a nasogastric tube) diet; and group IV, 1% cholesterol plus TQ (20 mg/kg/day; through a nasogastric tube) diet. Blood samples were collected at baseline and after four and eight weeks on the experimental diets for measurement of serum lipids, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC/HDL-C ratio and oxidative stress biomarkers

(malondialdehyde [MDA] and protein carbonyls). At the end of the eight weeks, the aorta was removed for the assessment of atherosclerotic changes, MDA and protein carbonyls. Group II animals developed atherosclerosis (45%±11% of the intimal surface of aorta was covered with atherosclerotic plaques), which was associated with an increase in the serum TC, TG, LDL-C, HDL-C, TC/HDL-C, MDA and protein carbonyls. In group III, TQ decreased serum TC, LDL-C, MDA and protein carbonyls by 26%, 29%, 85% and 62%, respectively, and aortic MDA by 73%, which was associated with a 40% reduction of the development of aortic atherosclerosis. The higher dose of TQ in group IV had effects similar to the lower dose (group III), except that this dose further decreased serum TG. It is concluded that TQ attenuates hypercholesterolemic atherosclerosis and this effect is associated with a decrease in serum lipids and oxidative stress.

Key Words: Atherosclerosis; Hypercholesterolemia; Reactive oxygen species; Thymoquinone

The role of reactive oxygen species (ROS) in hypercholesterolemia-induced atherosclerosis has been previously reported (1,2). Hypercholesterolemia stimulates polymorphonuclear leukocytes and activates vascular endothelial cells, which results in excess ROS release (3,4). In addition, hypercholesterolemia increases platelet-activating factor concentrations, which in turn increases interleukin (IL)-1 β and tumour necrosis factor- α (TNF- α) synthesis and release (5). IL-1 β , IL-8 and IL-6, TNF- α and interferon- γ are known to activate granulocytes that generate ROS (6). TNF- α also induces NADPH-oxidase enzyme to liberate ROS in the vascular endothelial cells (7).

Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone) is the bioactive constituent of the volatile oil of *Nigella sativa* seeds (8). The oil and seed constituents, in particular TQ, have shown potential medicinal properties in traditional medicine. *N sativa* seeds have long been used in folk medicine for a wide range of illnesses including bronchial asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems (9). TQ has antioxidant and anti-inflammatory activities (10), as well as lipid-lowering effects (11).

Because of these pharmacological properties, it is hypothesized that TQ may attenuate hypercholesterolemia and the

development of hypercholesterolemic atherosclerosis. The main objectives of the present study were to investigate whether TQ attenuates the development of atherosclerosis, whether this effect is associated with reduction in serum lipids and oxidative stress, and whether the effects are dose-dependent.

METHODS

Drugs

TQ (Sigma-Aldrich, USA) was dissolved in pure corn oil (Sigma-Aldrich, USA) to give a concentration of 75 mg/mL.

Animals and experimental design

Twenty-four female New Zealand white rabbits, weighing 1.8 kg to 2 kg and aged six to eight weeks, were assigned to four groups. After one week of adaptation, the rabbits were fed a regular diet (group I), a high-cholesterol diet alone (group II), or a high-cholesterol diet plus TQ 10 mg/kg/day or 20 mg/kg/day (groups III and IV) for eight weeks. The diet was prepared by Purina (USA) and did not contain any antioxidants. TQ was given through nasogastric tubes. Water was given ad libitum. The rabbits were housed in individual cages at room temperature (22°C to 24°C) and a relative humidity of 40% to 60% under a 12 h light/12 h dark cycle. The experimental protocols were approved by the Ethics Committee of the University of

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Saskatchewan, Saskatoon, Saskatchewan, and the animal care was according to the approved standards for Laboratory Animal Care. Following 18 h of fasting, blood samples (from the ear marginal vein) were collected before (zero week), and after four and eight weeks on the respective diets for measurement of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC/HDL-C and serum malondialdehyde (MDA). Serum protein carbonyls was measured only at week 8. At the end of the study (eight weeks), rabbits were anesthetized with Euthanyl (sodium pentobarbital; Bimeda-MTC Animal Health Inc, Canada) (50 mg/kg intravenously) in the marginal ear vein. Aortas were removed for the assessment of atherosclerotic changes, and measurement of aortic MDA and protein carbonyls.

Serum lipids

TC, TG and HDL-C were measured on an automated Synchron LX20 Clinical System Analyzer (Beckman Coulter Inc, USA). LDL-C was calculated. Risk ratio was estimated using the quotient TC/HDL-C.

Preparation of the aortic tissue for measurement of MDA and protein carbonyls

The aortic rings were excised at the roots of the aortic arches and kept in 10% buffered formalin for histological examination. Aortas between the origin and bifurcation to the iliac arteries were removed, cleansed of gross adventitial tissue and cut longitudinally into two halves. One half was used for assessment of atherosclerotic changes, and the other half was kept on ice for preparation of the supernatants, by a previously described method (12), for measurement of the aortic tissue MDA and protein carbonyls.

Serum and aortic MDA (thiobarbituric acid-reactive substances)

MDA levels in the aortic tissue supernatant and serum were measured as thiobarbituric acid-reactive substances by a previously described method (12,13). Thiobarbituric acid-reactive substances were extracted in a mixture of butanol and pyridine (15:1) that was separated by centrifugation. The fluorescence intensity of the butanol-pyridine solution was measured at 553 nm with excitation at 513 nm. The MDA content of the tissue was expressed as nmol/mg proteins and that of serum as nmol/mL.

Serum and aortic protein carbonyls

The protein carbonyl levels in the aortic tissue supernatant and serum were measured using a Cayman Chemical Carbonyl Protein Assay Kit (Cayman Chemical Company, USA). The absorbance was measured at wavelengths between 360 nm and 386 nm using an ELx 808TM Absorbance Microplate Reader (BioTek Instruments Inc, USA). The protein carbonyl content of the tissue was expressed as nmol/mg proteins and that of serum as nmol/mL.

Tissue protein measurement

Protein content was determined using a Modified Lowry Protein Assay Kit (Pierce Biotechnology, USA). The absorbance was measured at a wavelength of 750 nm using an ELx 808TM Absorbance Microplate Reader.

Assessment of atherosclerotic changes in the aorta

Assessment of atherosclerotic changes in the aorta was performed by using Herxheimer's solution containing Sudan IV for lipid staining, as described previously (3,14). Photographs of the stained intimal surface of the aorta were taken with a digital camera. The total and atherosclerotic areas of the intimal surface of the aorta were measured using Scion Image for Windows (Scion Corporation, USA) image analysis software. The extent of atherosclerosis was expressed as a percentage of total intimal surface area.

Histological examination

The aortic ring specimens were crosswise and embedded in paraffin. Paraffin sections of 4 μ m thickness were cut and stained with hematoxylin and eosin (H&E) and examined by light microscopy. A Sigma-Aldrich Accustain Elastic Stain Kit (Sigma-Aldrich Canada Ltd) was used to visualize elastic laminae and examine the changes in the elastic fibres by light microscopy.

Quantification and histological assessment of atherosclerotic lesions in aortic rings

Sections stained with H&E were used to compare the total cross-sectional intimal and medial areas and the intima/media thickness (I/M) ratio between the groups. Using Scion Image for Windows image analysis software, the total cross-sectional intimal area was measured between the endothelial cell monolayer and the internal elastic lamina (IEL), and the total cross-sectional medial area was measured between the external elastic lamina and the IEL. The intimal and medial surface areas, as well as the I/M ratio, were taken as measures of the severity of atherosclerosis (15).

Statistical analysis

Results were expressed as mean $\pm$ SD. Repeated-measures ANOVA was used for statistical analysis. A value of $P < 0.05$ was considered to be significant.

RESULTS

Body weight

Sequential changes of the body weight of the rabbits in the four groups at zero, four and eight weeks were documented in kilograms. Compared with baseline measurements, there was a significant increase in the body weight in all groups at eight weeks. At zero, four and eight weeks, the mean weights in group I were 1.74 $\pm$ 0.15 kg, 2.45 $\pm$ 0.08 kg and 2.92 $\pm$ 0.12 kg, respectively. In group II, the weights were 1.8 $\pm$ 0.07 kg, 2.5 $\pm$ 0.13 kg and 2.95 $\pm$ 0.09 kg, respectively, and in group III, the respective weights were 1.78 $\pm$ 0.09 kg, 2.56 $\pm$ 0.14 kg and 2.94 $\pm$ 0.22 kg. In group IV, the weights were 1.75 $\pm$ 0.1 kg, 2.45 $\pm$ 0.22 kg and 2.93 $\pm$ 0.20 kg, respectively. No significant differences in weights were observed among the groups throughout the study period.

Serum lipids

The baseline levels of serum TC in groups I, II, III and IV were 1.38 $\pm$ 0.2 mmol/L, 1.26 $\pm$ 0.3 mmol/L, 1.5 $\pm$ 0.7 mmol/L and 1.5 $\pm$ 0.3 mmol/L, respectively. The baseline levels were not significantly different among the groups. The sequential changes in serum TC in the four groups are shown in Figure 1. Serum levels of TC remained unchanged in group I with

Figure 1 Sequential changes in serum total cholesterol (TC) and triglycerides (TG) in the four groups. Results are expressed as mean $\pm$ SD. Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day. ^a $P < 0.05$, zero time versus four and eight weeks in the respective groups; ^b $P < 0.05$, group I versus other groups; ^c $P < 0.05$, group II versus groups III or IV; ^d $P < 0.05$, group III versus group IV

Figure 2 Sequential changes in serum low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in the four experimental groups. Results are expressed as mean $\pm$ SD. Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day. ^a $P < 0.05$, zero time versus four and eight weeks in the respective groups; ^b $P < 0.05$, group I versus other groups; ^c $P < 0.05$, group II versus groups III or IV

respect to zero time. Serum levels of TC were elevated in groups II, III and IV compared with their basal levels and these levels were higher compared with those in group I at weeks 4 and 8. The levels at week 8 were not different from those at week 4 in all groups, and these levels were lower in groups III and IV compared with group II.

The baseline levels of serum TG in groups I, II, III and IV were 0.79 ± 0.2 mmol/L, 1.02 ± 0.3 mmol/L, 0.62 ± 0.1 mmol/L and 0.45 ± 0.1 mmol/L, respectively. The baseline levels were not significantly different among the groups. The sequential changes in serum TG in the four groups are shown in Figure 1. Serum levels of TG remained unchanged in group I with respect to zero time. Serum levels of TG increased in groups II, III and IV compared with the initial levels and these levels were higher

compared with those in group I at weeks 4 and 8. The levels at week 8 were not different from those at week 4 in all groups and these levels were lower in group IV compared with group III.

The baseline levels of serum LDL-C in groups I, II, III and IV were 0.4 ± 0.2 mmol/L, 0.35 ± 0.2 mmol/L, 0.72 ± 0.7 mmol/L and 0.59 ± 0.3 mmol/L, respectively, and were not significantly different from each other. The sequential changes in LDL-C in the four groups are shown in Figure 2. Serum levels of LDL-C remained unchanged in group I with respect to zero time. Serum LDL-C levels increased in groups II, III and IV compared with the baseline levels and these levels were higher compared with those in group I at weeks 4 and 8. LDL-C levels at week 8 were not different from those at week 4 in all groups and these levels were lower in groups III and IV compared with group II.

Figure 3 Sequential changes in total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C) ratio and serum malondialdehyde (MDA) in the four experimental groups. Results are expressed as mean $\pm$ SD. Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day. ^a $P < 0.05$, zero time versus four and eight weeks in the respective groups; ^b $P < 0.05$, group I versus other groups; ^c $P < 0.05$, group II versus groups III or IV; ^d $P < 0.05$, group IV versus other groups

The initial levels of serum HDL-C in groups I, II, III and IV were 0.62 ± 0.2 mmol/L, 0.44 ± 0.2 mmol/L, 0.49 ± 0.2 mmol/L and 0.7 ± 0.1 mmol/L, respectively, and were not significantly different among the groups. The sequential changes in HDL-C in the four groups are shown in Figure 2. Serum levels of HDL-C remained unchanged in group I with respect to zero time. Serum HDL-C levels increased in groups II, III and IV compared with their initial levels and these levels were higher compared with those in group I at weeks 4 and 8. HDL-C levels at week 8 were not different from those at week 4 in all groups and these levels were lower in groups III and IV compared with group II at weeks 4 and 8.

The baseline values of the risk ratio TC/HDL-C in groups I, II, III and IV were 2.31 ± 0.4 , 3.04 ± 1 , 3.03 ± 1 and 2.15 ± 0.3 , respectively. The basal values were not significantly different among the groups. The sequential changes in the risk ratio in the four groups are shown in Figure 3. The risk ratio remained unchanged in group I and increased in groups II, III and IV with respect to zero time. The risk ratio values were increased to a similar extent in groups II, III and IV compared with those in group I at weeks 4 and 8. There were no significant differences among groups II, III and IV at weeks 4 and 8.

MDA levels

Serum MDA: The basal values of serum MDA in groups I, II, III and IV were 0.93 ± 0.4 mmol/L, 0.84 ± 0.3 mmol/L, 0.86 ± 0.2 mmol/L and 0.32 ± 0.1 mmol/L, respectively, and were not significantly different among the groups except in group IV, which had a lower value than the other groups. The sequential changes in serum MDA in the four groups are shown in Figure 3. The values remained unchanged in group I but increased in group II at weeks 4 and 8 and in groups III and IV at week 4 only with respect to zero time. The increases in groups III and IV were similar but lesser than those in group II at weeks 4 and 8. Serum MDA values in group II were higher than those in groups I, III and IV at four and eight weeks.

TABLE 1

Serum protein carbonyls and aortic malondialdehyde (MDA) and protein carbonyl levels in the four groups at eight weeks

	Group I	Group II	Group III	Group IV
Serum protein carbonyl content, nmol/mL	1.34 ± 0.25	$5.34 \pm 1.36^*$	$2.01 \pm 0.97^{**}$	$2.27 \pm 0.4^{**}$
Aortic MDA, nmol/mg	0.25 ± 0.06	$0.53 \pm 0.32^*$	$0.14 \pm 0.07^{**}$	$0.13 \pm 0.06^{**}$
Aortic protein carbonyl content, nmol/mg	0.40 ± 0.42	0.48 ± 0.38	0.42 ± 0.42	0.36 ± 0.11

Results are expressed as mean $\pm$ SD. ^{*} $P < 0.05$, group I versus other groups; ^{**} $P < 0.05$, group II versus groups III and IV. Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day

Aortic tissue MDA: The MDA contents of the aortic tissue in the four groups are shown in Table 1. The mean aortic MDA content from group I was 0.25 ± 0.06 nmol/mg protein. The value in group II was 2.12-fold higher than that in group I. The values in groups III and IV were 73% and 75%, respectively, lower than in group II. The 1% cholesterol diet increased the levels of aortic tissue MDA while TQ at doses of 10 mg/kg/day and 20 mg/kg/day reduced these levels by 73% and 75%, respectively.

Protein carbonyl levels

Serum protein carbonyls: Serum protein carbonyl levels in the four groups at eight weeks are shown in Table 1. The mean serum protein carbonyl level in group I was 1.34 ± 0.25 nmol/mL at the end of the eight weeks. The 1% cholesterol diet in group II increased the levels of serum protein carbonyls to a mean of 5.34 ± 1.36 nmol/mL. The values in groups III and IV were 62% and 57%, respectively, lower than in group II. The 1%

Figure 4) Representative photographs of the intimal surfaces of aortas from the four experimental groups showing Sudan IV-stained lipid deposits (dark staining in groups II, III and IV). Note the difference between the extent of atherosclerosis between group II and groups III and IV. Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day

TABLE 2
Atherosclerotic changes in the four groups

	Group I	Group II	Group III	Group IV
Atherosclerosis (% of total intimal surface)	0	45.08±11.65*	27.81±4.99*†	20.67±4.38*†
Intimal area	7.58±1.52	32.38±7.13*	15.54±6.86*†	13.31±4.23*†
Medial area	52.31±9.71	64.56±10.88	52.49±17.13	50.46±13.65
I/M ratio	0.15±0.01	0.52±0.06*	0.29±0.04*†	0.26±0.02*†

Results are expressed as mean ± SD. Intimal (I) and medial (M) surface areas are expressed as $\times 10^3 \mu\text{m}^2$. * $P < 0.05$, group I versus other groups; † $P < 0.05$, group II versus groups III and IV. Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day

cholesterol diet increased the levels of serum protein carbonyls by 3.89-fold compared with the regular diet (group I), while TQ in groups III and IV produced inhibition of serum protein carbonyl levels by approximately 1.5-fold compared with the control group.

Aortic tissue protein carbonyls: Aortic tissue protein carbonyl levels of the four groups are summarized in Table 1. The mean protein carbonyl content of the aortic tissue from group I was 0.40 ± 0.42 nmol/mg protein. The values in all groups were similar.

Histological effects

Extent of atherosclerotic plaque formation: Representative photographs of the atherosclerotic changes in the intimal surface of the aortas from each group are shown in Figure 4 and the results are summarized in Table 2. There were no atherosclerotic changes in the intimal surface of the aortas from group I. Significant areas of the intimal surfaces of aortas from groups II ($45.08 \pm 11.65\%$), III ($27.81 \pm 4.99\%$) and IV

Figure 5) Representative photographs of the microscopic changes from the four groups stained with hematoxylin and eosin. Note the normal arterial wall structure in group I (original magnification $\times 10$), the increased intimal thickness in group II that involves most of the aortic circumference and the lesser thickness that involves much smaller portions of the aortic circumference in groups III and IV (original magnification $\times 4$). Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day

($20.67 \pm 4.38\%$) were covered with atherosclerotic plaques. Administration of TQ at doses of 10 mg/kg/day and 20 mg/kg/day reduced the development of atherosclerosis by 40% and 53%, respectively. No significant difference was found between the effects of the two doses of TQ.

Microscopic changes in the aortic segments: Representative photographs of the histological sections of the aorta stained with H&E and the Accustain Elastic Stain Kit from the four groups are shown in Figures 5 and 6, respectively.

In Figure 5, the control group (group I) specimens showed normal arterial wall structure with no atherosclerotic changes. Group II showed moderate to severe atherosclerotic lesions. Administration of TQ at doses of 10 mg/kg/day and 20 mg/kg/day with the 1% cholesterol diet, in groups III and IV, showed only mild localized areas of atherosclerosis.

In Figure 6, group I specimens were intact throughout the entire wall of the artery. Group II showed diffuse damage in the IEL at the sites of the atherosclerotic changes. Administration of TQ at doses of 10 mg/kg/day and 20 mg/kg/day with the 1% cholesterol diet, in groups III and IV, showed only focal damage in the IEL while most of the wall showed intact IEL.

Changes in intimal and medial thicknesses in the aortic segments: The intimal and medial surface areas and ratios are summarized in Table 2. The intimal and medial cross-sectional areas and the I/M ratio values in group I were $7.6 \pm 1.5 \times 10^3 \mu\text{m}^2$, $52.3 \pm 9.7 \times 10^3 \mu\text{m}^2$ and $0.15 \pm 0.01 \times 10^3 \mu\text{m}^2$, respectively. Both the intimal cross-sectional area and I/M ratio values were increased by 4.3- and 3.5-fold, respectively, in group II while the medial cross-sectional area remained unchanged. Administration of TQ reduced the intimal cross-sectional area and I/M ratio by 52% and 45%, respectively, in group III, and by 60% and 50%, respectively, in group IV. No significant differences were found between the effects of the two doses of TQ.

DISCUSSION

TQ doses used in the present study were similar to those used by other investigators. Based on that, there was no significant difference in the body weight gain among the studied groups, indicating that the various interventions did not affect the body weight. The high-cholesterol diet induced a significant increase in the serum levels of TC, TG, LDL-C, HDL-C and the TC/HDL-C ratio. Similar changes in the lipid profile were reported in several studies (4,16). Administration of TQ at a dose of 10 mg/kg/day reduced the levels of the TC, LDL-C and HDL-C. However, the TC/HDL-C ratio did not show any change due to the concomitant decreases in both TC and the HDL-C levels. The TG levels were not affected by TQ administration. No previous data are available on the effects of TQ on lipid profile in rabbits. However, Zaoui et al (11) reported that *N sativa* seed oil has a lipid-lowering effect in rats. TQ was also reported to produce significant reductions in the levels of TC, TG, LDL-C and HDL-C in albino rats, but with no linear dose- or time-dependent effect (17). The mechanism of these hypolipidemic effects is unknown.

Consistent with previous reports (2,4,16), the present study demonstrated the coexistence of severe hyperlipidemia, enhanced ROS activity and atherosclerosis in this rabbit model. The study showed for the first time, to our knowledge, the increased levels of serum protein carbonyls in rabbits fed a high-cholesterol diet. Protein carbonyls are a marker of irreversible damage due to enhanced ROS activity (18), which validates this model for the study of ROS injury. The extent of atherosclerosis in the present study was 45% in the 1% cholesterol-fed group of rabbits. The cholesterol diet also increased the intimal cross-sectional area and I/M ratio 4.3 and 3.5 times, respectively, compared with the control. The intimal cross-sectional area and I/M ratio were considered previously as markers for the severity of atherosclerosis (15). Finally, the cholesterol diet induced marked destruction to the IEL. In the current study, the hypercholesterolemic atherosclerosis was associated with increases in the serum and aortic MDA and protein carbonyls, suggesting an increase in the levels of ROS. Several mechanisms were postulated to explain the role of ROS in the pathogenesis of atherosclerosis. One of these mechanisms is the oxidation of LDL molecules to highly immunogenic oxidized LDL molecules in the presence of excess ROS (19). Oxidized LDL molecules are found in the subendothelial layers and help activate monocytes that are transformed into macrophages, upregulating their scavenger receptors and toll-like receptors that then phagocytose them. With progressive accumulation of oxidized LDL, macrophages modulate their phenotype, turning them into foam cells. Foam cells are the principal component of the fatty streaks, the first step in atheromatous plaque formation (20). ROS may also induce atherosclerosis by producing endothelial cell injury (21) and modulation of adhesion molecules (22).

The antiatherogenic effect of TQ may be due to its antioxidant and/or anti-inflammatory effects. Previous *in vitro* and *in vivo* studies reported that TQ is a potent superoxide anion scavenger (10). TQ was also found to inhibit the cyclooxygenase and lipoxygenase enzymes (23). Cyclooxygenase enzymes generate prostaglandins and thromboxane from arachidonic acid, while lipoxygenase enzymes catalyze the formation of leukotrienes (24). Oxygen radicals are produced during the synthesis of prostaglandins and leukotrienes (25). In the

Figure 6 Representative photographs of the microscopic changes from the four groups stained with elastic fibre stain. Note the black-stained elastic fibres, the relatively well-defined internal elastic lamina (IEL) and the poorly defined external elastic lamina (EEL) (original magnification $\times 4$). Group I: normal diet; group II: 1% cholesterol diet; group III: 1% cholesterol diet plus thymoquinone 10 mg/kg/day; group IV: 1% cholesterol diet plus thymoquinone 20 mg/kg/day

present study, the use of TQ decreased the levels of the serum and aortic MDA and the serum protein carbonyls, suggesting a decrease in the levels of the ROS. The higher dose of TQ did not further lower the levels of the serum or aortic MDA, or the serum protein carbonyls. Results from previous experiments also showed that increasing TQ dose over 10 mg/kg/day was not more beneficial in protecting rats against cisplatin-induced nephrotoxicity (26). The present study is the first to demonstrate the effects of TQ on aortic MDA and protein carbonyls in hypercholesterolemic atherosclerotic rabbits. In other models, TQ administration significantly reduced the brain tissue MDA in ischemia-reperfusion injury (15), the hepatic MDA in carbon tetrachloride-induced hepatotoxicity (27) and the renal MDA in gentamicin-induced nephrotoxicity (28). The decrease in the levels of serum and aortic ROS induced by TQ is probably due to antioxidant properties of TQ (10). In the current study, the antiatherosclerotic effect of TQ (10 mg/kg/day) was manifested in group III by a 40% decrease in the extent of atherosclerosis, a 52% decrease in the intimal surface area and a 45% decrease in the I/M ratio compared with group II. It also minimized hypercholesterolemia-induced damage in the IEL of the arteries. The 20 mg/kg/day dose showed 53%, 60% and 50% decreases in the atheromatous and intimal areas and I/M ratio, respectively, in group IV.

CONCLUSION

TQ reduced the development of atherosclerosis and this was associated with reductions in serum lipids and oxidative stress. Increasing the dose of TQ from 10 mg/kg/day to 20 mg/kg/day was not associated with a further improvement. Further testing in humans is suggested.

Limitations of the study

Caution should be exercised to interpret our results. Although our data indicate that reduction of oxidative

stress and/or hyperlipidemia is associated with attenuation of atherosclerotic plaque development, it is plausible that TQ may have other functions independent of its effect on the ROS system. Further work is necessary to identify the cause of reduction in lipid parameters by TQ. Of further interest, it would be important to study the macrophage content of atherosclerotic lesions in rabbits with and without TQ treatment. Our data also do not address the potential toxicity of TQ.

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