Toxicology and Industrial Health
2015, Vol. 31(5) 467–474

DOI: 10.1177/0748233712472523
tih.sagepub.com

Protective role of L-carnitine and vitamin E on the testis of atherosclerotic rats

Afrah F Salama[1], Safwat M Kasem[2], Ehab Tousson[1,3] and Mohammed KH Elsisy[1]

Abstract

Atherosclerosis is a condition caused by lipid build-up and inflammation in the arteries, so hyperlipidemia is the major reason for atherosclerosis. Testis was found to be negatively affected by hyperlipidemia which leads to its impaired functions. Vitamin E and L-carnitine have well-known lipid-lowering and antioxidative activities. Triton WR 1339 is a non-ionic detergent, which induces severe hyperlipidemia by inhibition of lipoprotein lipase. The present study evaluates the protective role of vitamin E and L-carnitine on the testis in atherosclerosis and detects the most effective choice for protection against atherosclerosis; vitamin E, L-carnitine or a combination of both. A total of 80 albino male rats were divided into eight groups (10 rats for each group): control (G_1), triton (G_2), L-carnitine (G_3), triton + L-carnitine (G_4), vitamin E (G_5), triton + vitamin E (G_6), L-carnitine + vitamin E (G_7) and triton + L-carnitine + vitamin E (G_8). Data showed a significant increase in the levels of total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), 17 beta hydroxysteroid dehydrogenase (17 β HSD), testicular catalase and malondialdehyde (MDA) in G_2 when compared with G_1, whereas high-density lipoprotein cholesterol (HDL-C), serum testosterone, testicular 17 ketosteroid reductase (17 KSR), total thiol and glutathione-*S*-transferase (GST) data showed a significant decrease in G_2 when compared with G_1. Treatment with L-carnitine or/and vitamin E helps in improving the adverse effect of triton; also the histological changes confirm this finding. So the present study recommends all people to include L-carnitine and vitamin E in their diet to be protected against atherosclerosis.

Keywords

Testes, triton, atherosclerotic L-carnitine, vitamin E, lipid profile, oxidative stress.

Introduction

Atherosclerosis is an inflammatory disease. High plasma concentration of cholesterol, in particular, those of low-density lipoprotein cholesterol (LDL-C) is one of the principal risk factors for atherosclerosis (Martinez-Martos et al., 2011). The etiology of atherogenesis involves two major pathogenic processes: inflammation and lipid delivery and accumulation at the site of the lesion. Lipids, abundant constituents of both the vascular plaque and lipoproteins, play a pivotal role in atherosclerosis (Bethesda, 1993). Low-density lipoprotein (LDL) localized in the subendothelial space becomes entrapped by extracellular matrix proteins and may be subject to oxidative modification by endothelial cells, smooth muscle cells and resident monocyte and macrophages (Hazfil and Stocker, 1993; Herttuala et al., 1989; Savenkova et al., 1994). Chemically modified LDL, but not native LDL, was readily internalized by tissue macrophages and this pathway of LDL internalization was not subject to normal regulatory mechanisms (Esterbauer et al., 1992; Jialal and Grundy, 1992; Steinbrecher et al., 1990). At the beginning of the 1990s, apolipoprotein E and LDL receptor-deficient mice, derived by homologous

[1] Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt
[2] Department of Medical Biochemistry, Faculty of Medicine, Tanta University, Tanta, Egypt
[3] Department of Chemistry, Tanta University, Tanta, Egypt

Corresponding author:
Ehab Tousson, Department of Zoology, Tanta University, Tanta 002040, Egypt.
Email: toussonehab@yahoo.com

recombination techniques (Ishibashi et al., 1993; Plump et al., 1992; Zhang et al., 1992), were shown to develop arterial lesions that progress from foam cell-rich fatty streaks to fibro-proliferative plaques with lipid and necrotic cores, similar to the evolution of human lesions (Ishibashi et al., 1994; Nakashima et al., 1994; Reddick et al., 1994). Carnitine is a conditionally essential nutrient that plays a vital role in energy production and fatty acid metabolism. Carnitine (β-hydroxy-γ-*N*-trimethyl aminobutyric acid) is widely distributed in food from animal sources but there is limited availability in plants (Kendler, 1986).

L-Carnitine (the biologically active stereoisomer) is absorbed from foods via both active and passive transport across enterocyte (intestinal cell) membranes (Rebouche, 2004). Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet. The major biologic role of vitamin E is to protect polyunsaturated fatty acids (PUFAs) and other components of cell membranes and LDL from oxidation by free radicals (Duthie, 1993). In the testes, oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells (Hales et al., 2005; Martinez-Martos et al., 2011; Purohit, 2007) as well as the capacity of the germinal epithelium to differentiate normal spermatozoa.

Studies on animals and humans suggest that androgen deficiency is associated with increased triglycerides (TGs), total cholesterol (TC) and LDL-C (Naughton et al., 2001). Tyloxapol (Triton WR-1339) is a non-ionic surfactant whose study is of practical and theoretical interest. It has been shown that it increases the concentration of plasma lipid when injected into rats. The increase has been interpreted as triton inhibits hydrolysis of lipoproteins by LPLase in various tissues to accumulate the lipids in blood (Friedman and Byers, 1957; Levine and Saltzman, 2006). Therefore, the aim of the present study was to investigate the effect of protective role of L-carnitine and vitamin E on the testis of atherosclerotic rats.

Materials and methods

The experiment was performed on 80 male albino rats aged 8–9 week (*Rattus norvegicus*) weighing 120 ± 10 g. The rats were kept in the laboratory for 1 week before the experimental work and maintained on a standard diet and water available *ad libitum*. The temperature in the animal room was maintained at 23 ± 2°C with a relative humidity of 55 ± 5% and a 12:12 h light–dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were randomly and equally divided into eight groups (10 animals each).

Group 1: (G_1) Control group in which rats were injected intraperitoneally with saline with a dose of 2 ml/100 g body weight for 2 weeks.

Group 2: (G_2) The rats were injected intraperitoneally with Triton WR 1339 dissolved in saline with a dose of 2 ml/100 g body weight for 2 weeks for induction of hyperlipidemia and atherosclerosis.

Group 3: (G_3) The rats were injected intraperitoneally with L-carnitine only with a dose of 300 mg/kg body weight/day dissolved in saline for 3 weeks (Seccombe et al., 1987).

Group 4: (G_4) The rats daily received L-carnitine (300 mg/kg body weight/day) dissolved in saline and administrated intraperitoneally for 1 week, then triton with the same dose as G_2 and L-carnitine with a dose of 300 mg/kg body weight/day were injected together for another 2 weeks (Steinbrecher et al., 1990).

Group 5: (G_5) The rats received vitamin E orally with a dose of 0.04 g/kg body weight/day dissolved in oil for 3 weeks (Robert et al., 1999).

Group 6: (G_6) The rats received 0.04 g/kg body weight/day of vitamin E dissolved in oil and administrated orally for 7 days, then triton with the same dose as G_2 and vitamin E with a dose of 0.04 g/kg body weight/day were injected together for another 2 weeks (Robert et al., 1999).

Group 7: (G_7) The rats were injected with both L-carnitine and vitamin E.

Group 8: (G_8) The rats were injected L-carnitine (intraperitoneal injection (IP)) with the same dose as G_3 and vitamin E (orally) with the same dose as group V together for 1 week, then triton with the same dose as G_2 and L-carnitine with the same dose as G_3 and vitamin E with the same dose as G_5 were injected together for another 2 weeks.

At the end of the experimental period, rats were euthanized with IP injection of sodium pentobarbital and subjected to a complete necropsy after 10–12 h of fasting. Blood samples were individually collected by cardiac puncture from each rat in non-heparinized glass tubes to estimate some biochemical parameters

Table 1. Changes in lipid profiles (cholesterol, triglyceride, HDL and LDL) in different groups under study.

Groups Parameter	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
Cholesterol (mg/dl)	94.7 ± 2.7	630.7 ± 10.2[a]	85.9 ± 2.6[a]	305.9 ± 10.1[a]	79.5 ± 3.0[a]	280.9 ± 5.8[a]	70.4 ± 3.2[a]	269.6 ± 4.6[a]
Triglyceride (mg/dl)	53.5 ± 3.0	1205.5 ± 27.1[a]	47.1 ± 2.6[a]	666.3 ± 10.6[a]	42.9 ± 2.5	382.3 ± 17.8[a]	37.5 ± 3.0[b]	279.1 ± 8.7[a]
HDL (mg/dl)	42.1 ± 2.3	22.9 ± 2.6[a]	44.5 ± 2.42[a]	31.0 ± 2.6[a]	50.9 ± 2.5[a]	34.6 ± 2.2[a]	51.8 ± 2.6[a]	34.7 ± 2.2[a]
LDL (mg/dl)	56.9 ± 2.3	459.6 ± 9.3[a]	49.0 ± 3.0[a]	293.3 ± 6.7[a]	47.7 ± 2.2[a]	273.3 ± 4.6[a]	43.2 ± 2.6[a]	254.4 ± 4.6[a]

HDL: high-density lipoprotein; LDL: low-density lipoprotein.
[a]Statistically significant at $p \leq 0.05$.
[b]Statistically significant at $p \leq 0.01$.
Each reading represents Mean ± SD.

in serum. Also testes were quickly removed and weighed after removing the surrounding connective tissues carefully and then washed in saline, one testis from each rat was frozen at −20°C, homogenized and assayed for activities of testicular functions and some of the oxidative stress markers. The rest of the testes was fixed for histological study.

Serum was separated by centrifugation at 3000 r/min for 15 min in room temperature; the serum was separated and kept in a clean stopper glass vial at −20°C until assay. The levels of lipid profile and testosterone of different groups under study were estimated.

Lipid profiles such as cholesterol, TGs and LDL were assayed using commercial kits (Spinreact, Santa Coloma, Spain). High-density lipoprotein (HDL) was assayed using a commercial kit (Human, Wiesbaden, Germany).

Testicular functions (testosterone, 17 ketosteroid reductase (17 KSR) and 17 beta hydroxysteroid dehydrogenase (17 β HSD)). Testosterone was assayed using a commercial kit that was supplied by Coat-A-Count testosterone radioimmuno assay (RIA) from Diagnostic Systems Laboratories (DSL, Webster, Texas, USA). Activity of steroidogenic 17-ketosteroid reductase was estimated by the method of Katryna and Anita (1980). Activity of steroidogenic 17 β HSD was estimated by the method of Bogovich and Payne (1980).

Oxidative stress markers such as malondialdehyde (MDA), glutathione-*S*-transferase (GST), total thiol and catalase activity (CAT) in testes tissue were estimated for different groups under study. Activity of GST was estimated through the formation of the adduct, due to conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) according to the method of Habig et al. (1974). CAT was estimated according to the method of Xu et al. (1997). MDA was estimated by the method of Lahouel et al. (2004). Total thiol of testes was estimated by the method of Sedlak and Lindsay (1986).

Testes were fixed in 10% neutral-buffered formalin for 24 h, dehydrated, cleared and embedded in paraffin. Serial sections of 5 µm thick were cut by means of rotary microtome (Litz). Sections were processed for haematoxylin and eosin staining (Bancroft and Cook, 1994). All stained slides were viewed using an Olympus microscope and images were captured using a digital camera (Cannon 620). Brightness and contrast were adjusted using the Adobe Photoshop software (version 4.0.1; Adobe Systems, Mountain View, California, USA).

Statistical analysis: One-way analysis of variance (ANOVA) was used to assess significant differences among treatment groups. The Dunnett test was used to compare all groups against the control group and shows the significant effect of treatment. The criterion for statistical significance was set at $p < 0.05$ or $p < 0.01$ (GraphPad in Stat Software).

Results

Lipid profile

Results of the lipid profile (cholesterol, TGs, HDL and LDL) clarified the very strong effect of triton WR 1339 on lipids where the data showed a significant increase in levels of TC, TGs and LDL-C in G_2 when compared with G_1, this elevation decreased in G_4 and G_6 when compared with G_2, where G_6 was less than G_4. Among all triton-treated groups, G_8 had the lowest levels of TC, TGs and LDL-C. On the other hand, HDL cholesterol data showed a significant decrease in G_2 when compared with G_1. Data of G_4 and G_6 showed a significant increase in HDL when compared with G_2, where G_6 was higher than G_4 (Table 1).

Table 2. Effects of Triton WR 1339, L-carnitine and vitamin E on the levels of testicular functions (serum testosterone, 17 KSR and 17 β HSD) in the testes in different groups under study.

Groups Parameter	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
Serum testosterone (ng/ml)	4.43 ± 0.3	0.4 ± 0.04[a]	4.8 ± 0.2[b]	1.1 ± 0.1[a]	5.1 ± 0.6[a]	1.8 ± 0.2[a]	5.1 ± 0.3[a]	2.7 ± 0.2[a]
17 KSR (unit/min/mg protein)	10.1 ± 0.1	1.2 ± 0.1[a]	10.2 ± 0.06	1.4 ± 0.03[a]	10.3 ± 0.1[b]	1.9 ± 0.02[a]	10.4 ± 0.1[a]	2.0 ± 0.08[a]
17 β HSD (unit/min/mg protein)	3.0 ± 0.1	8.3 ± 0.2[a]	2.9 ± 0.1	8.1 ± 0.1[a]	2.7 ± 0.08[a]	7.8 ± 0.1[a]	2.5 ± 0.07[a]	7.7 ± 0.3[a]

17 KSR: 17 ketosteroid reductase; 17 β HSD: 17 beta hydroxysteroid dehydrogenase.
[a]Statistically significant at $p < 0.05$.
[b]Statistically significant at $p < 0.01$.

Table 3. Effect of different treatments (testicular total thiol, testicular MDA, testicular GST and testicular catalase) in different groups under study.

Groups Markers	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
Testicular total thiol (mM/g)	135.9 ± 6.5	73.7 ± 4.6[a]	141.6 ± 4.6	79.4 ± 3.5[a]	146.2 ± 3.5[a]	87.5 ± 4.3[a]	147.3 ± 6.1[a]	92.7 ± 3.9[a]
Testicular MDA (nmol/g)	82.2 ± 4.4	487.1 ± 11.5[a]	76.2 ± 5.2	423.0 ± 6.7[a]	70.3 ± 5.0[a]	356.2 ± 8.7[b]	64.3 ± 6.2[a]	291.7 ± 9.5[a]
Testicular GST (nmol/min/g)	1.35 ± 0.03	0.52 ± 0.02[a]	1.4 ± 0.02[b]	0.55 ± 0.03[a]	1.42 ± 0.02[a]	0.62 ± 0.02[a]	1.5 ± 0.03[a]	0.7 ± 0.04[a]
Testicular catalase (μmol/min/g)	1.81 ± 0.03	5.0 ± 0.2[a]	1.75 ± 0.04	4.3 ± 0.3[a]	1.6 ± 0.04[a]	3.3 ± 0.3[a]	1.44 ± 0.03[a]	3.0 ± 0.2[a]

MDA: malondialdehyde; GST: glutathione-*S*-transferase.
[a]Statistically significant at $p < 0.05$.
[b]Statistically significant at $p < 0.01$.

Testicular functions

Table 2 shows a significant reduction in the level of serum testosterone and testicular 17 KSR in G_2 when compared with control. Data of G_4 and G_6 showed a significant increase in the level of serum testosterone and testicular 17 KSR when compared with G_2, where group 6 was higher than G_4. G_8 was the highest among all triton-treated groups. On the other hand, a significant elevation in 17 β HSD in G_2 was observed when compared with control. This elevation decreased in G_4 and G_6 when compared with G_2, where G_6 was less than G_4. Among all triton-treated groups, G_8 had the lowest level.

Oxidative stress markers

Table 3 shows a significant increase in levels of testicular catalase and MDA in G_2 when compared with G_1, this elevation decreased in G_4 and G_6 when compared with G_2, where G_6 was less than G_4. Among all triton-treated groups, G_8 had the lowest levels of catalase and MDA. On the other hand, total thiol and GST data showed a significant decrease in G_2 when compared with G_1. Data of G_4 and G_6 showed a significant increase in both when compared with G_2, where G_6 was higher than G_4. Among all triton-treated groups, G_8 was the highest.

Histopathological study showed that the cycle of spermatogenesis was regular in all male rats in G_1, G_3, G_4 and G_7, respectively (Figure 1(a) to (c) and g). The structural components of the testis were seminiferous tubules and interstitial cells known as Leydig cells. Two types of cells were identified in rat seminiferous tubules, the Sertoli cells and the spermatogenic cells. The Sertoli cells were found resting on the thin basal lamina (basement membrane), whereas the spermatogenic cells were arranged in many layers (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms). The lumen in the seminiferous tubules in G_4 and G_7 was revealed to be fully packed with sperms (Figure 1(c) and (g)).

However, the light microscopy examination of the testes of the triton group (G_2) revealed marked irregular disturbance in spermatogenesis cycles with severe morphological changes such as degeneration of germinal epithelium and sloughing of germ cells into the tubular lumen (Figure 1(d)). Vacuolar degenerative changes also appeared in the cytoplasm of the

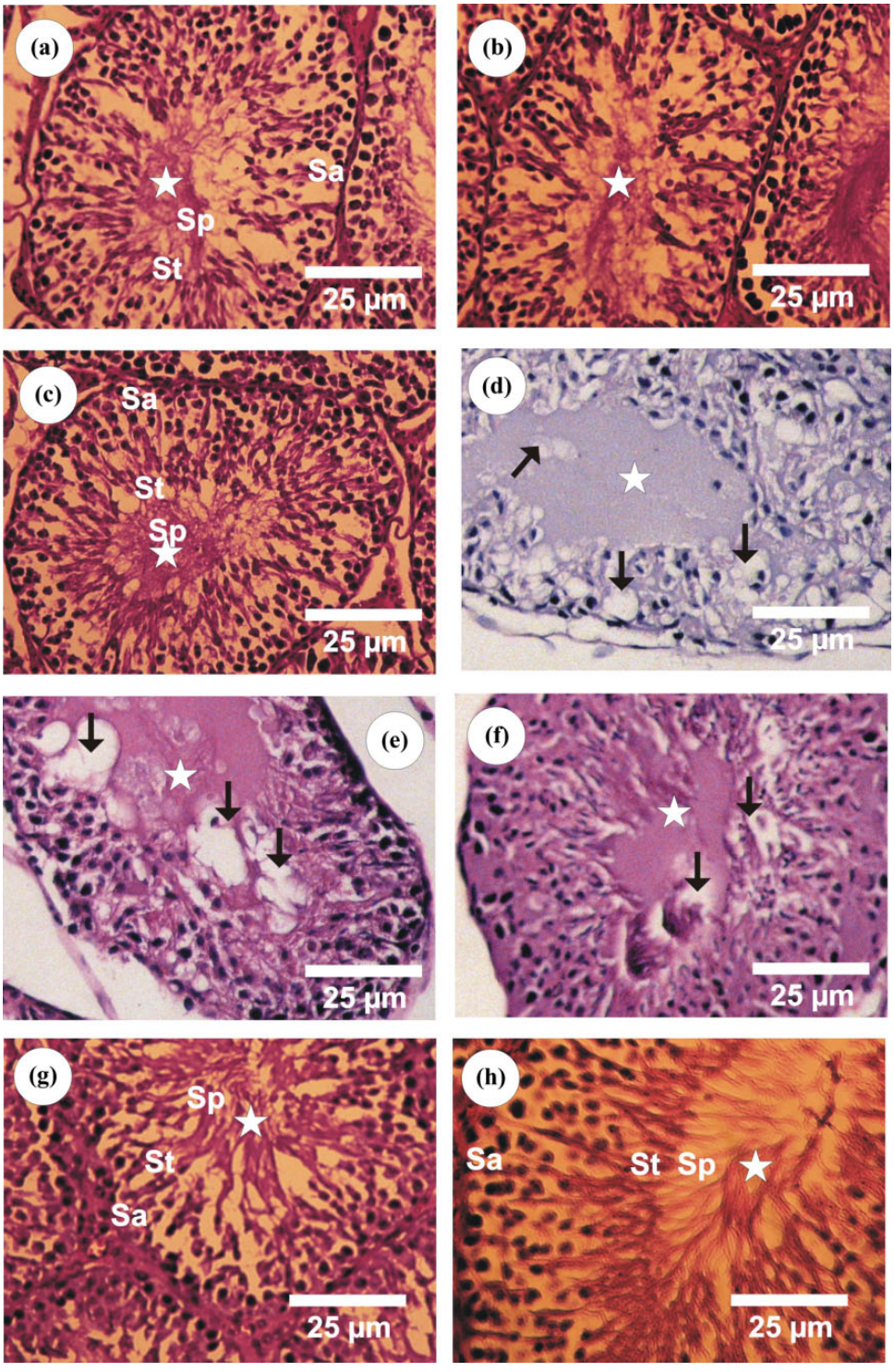

Figure 1. Light micrographs of rat testes stained by haematoxylin and eosin stains. (a) to (c) Normal histological structure of seminiferous tubules with fully packed sperms (stars) in lumen in the testes of control, L-carnitine and vitamin E, respectively. (d) and (e) Seminiferous tubules in rat testes of the triton group showing marked irregular disturbance in spermatogenesis cycles with severe morphological changes such as degeneration of germinal epithelium (arrows) and sloughing of germ cells into the tubular lumen. (f) Mild vacuolar degenerative changes appeared in the cytoplasm of seminiferous tubules in rat testes post-treated with L-carnitine after triton administration. (g) Regular distribution in spermatogenesis cycles with a few vacuolar degenerative changes in the cytoplasm of seminiferous tubules in rat testes post-treated with vitamin E after triton administration. (h) Seminiferous tubules in rat testes of the L-carnitine and vitamin E group showing normal histological structure of seminiferous tubules with a regular cycle of spermatogenesis. Sp: sperms, St: spermatids and Sa: spermatogonia.

spermatogenic epithelium and in the Sertoli cells. A significant severe decrease in the number of spermatogenic cells in the seminiferous tubules, in addition to little Leydig cell numbers, was also found (Figure 1(d)). Figure 1(e) shows that the accumulation of spermatogenic and Leydig cells increased in the seminiferous tubules in rats post-treated with L-carnitine after triton administration (G_4) when compared with the triton group (G_2). Furthermore, abnormal distribution of spermatozoa was seen in the lumen of the seminiferous tubules without significant increase in the number of spermatocytes. On the other hand, mild vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and in the Sertoli cells (Figure 1(e)). Seminiferous tubules in the testes of rats that were treated with vitamin E after triton administration (G_5) revealed regular distribution in spermatogenesis cycles with only a few vacuolar degenerative changes in the cytoplasm of the spermatogenic epithelium although the seminiferous tubules were fully packed with sperms (Figure 1(f)). Seminiferous tubules in the testes of rats that were treated with L-carnitine and vitamin E together after triton administration (G_8) revealed normal structure of the seminiferous tubules, regular distribution in spermatogenesis cycles, narrow lumen of seminiferous tubules fully packed with sperms and increased number of Leydig cells (Figure 1(h)).

Discussion

The results of the present study explained the harmful effects of atherosclerosis on testis through severe hyperlipidemia, ischemia and oxidative stress. Additionally, the present study proved the effective role of both L-carnitine and vitamin E together in prevention and protection against atherosclerosis besides relief of its complications. The results of the present work showed a significant increase in concentrations of cholesterol and TGs of all triton-treated groups, in which rats were intravenously injected with Tyloxapol when compared with control. In the current study, the results of oxidative stress markers (GST, MDA, CAT and total thiol) showed an aggressive oxidative stress in testis. Martinez-Martos et al. (2011) reported a significant decrease in serum circulating levels of testosterone which was observed after induction of hypercholesterolemia during investigation of diet-induced hypercholesterolemia impaired testicular steroidogenesis in mice through the renin–angiotensin system where the bioactive peptides of the renin–angiotensin system localized in the gonads play a key role in the relation between cholesterol and testosterone by modulating steroidogenesis and inhibiting testosterone production. Additionally, Purohit (2007) has done a considerable work on hyperlipidaemia in relation to body metabolism and function status in the animal kingdom but the relationship between hyperlipidaemia and testicular function revealed that cholesterol diet may have a significant effect on the intratesticular androgen levels either by inhibiting the Leydig cell function or by inhibiting the hypothalamo–pituitary axis where cholesterol treatment resulted in severe degeneration in spermatogonia, primary and secondary spermatocytes and spermatids and the number of interstitial cell types, that is fibroblasts, immature and mature Leydig cells also showed a significant reduction in all the treated groups. In agreement with these studies, the results of the present study showed a significantly increased 17 β HSD/KSR ratio in G_2 (triton) than G_1 (control) which also may explain why serum testosterone is severely decreased. The most important thing to be discussed is the great amelioration of testis function by administration of L-carnitine and vitamin E especially together as a combination. The study of Robert et al. (1999) demonstrated that increased dietary vitamin E lowers concentrations of plasma LDL-C, has an inhibitory effect on early atherosclerosis, increases the lag phase of LDL oxidation and decreases the rate of LDL oxidation in the hypercholesterolemic hamster, relative to the control. In addition, according to Seccombe et al. (1987) L-carnitine administration clearly prevented the development of early atherosclerotic lesions by decreasing the levels of plasma cholesterol and TGs. In conclusion, vitamin E and L-carnitine have lipid lowering and antioxidative properties through which they are able to resist atherosclerosis. Testes in rats that were injected intraperitoneally with triton for 2 weeks showed many abnormalities in testes represented in marked irregular disturbance in spermatogenesis cycles with severe morphological changes such as degeneration of germinal epithelium and sloughing of germ cells into the tubular lumen. The present study is in agreement with the study of Purohit (2007), who reported the presence of correlation between hyperlipidaemia and testicular function in albino rats. Treatment with L-carnitine or/and vitamin E helps in improving the adverse effect of hyperlipidemia and atherosclerosis; also the histological study confirms this finding.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Bancroft JD, Cook HC (1994) *Manual of histological techniques and their diagnostic application*. Churchill Livingstone, Edinburgh, London, New York, Tokyo. P 23–26.

Bethesda M (1993) National Cholesterol Education Program. Recommendations for improving cholesterol measurement. A Report from the Laboratory Standardization Panel of the National Cholesterol Education Program. National heart, lung and blood institute. (NIH) publication 30: 93–95.

Bogovich K, Payne A (1980) Purification of rat testicular microsomal 17-ketosteroid reductase evidence that 17-ketosteroid reductase and 17 beta-hydroxysteroid dehydrogenase are distinct enzymes. *Journal of Biological Chemistry* 225: 5552–5559.

Duthie G (1993) Lipid peroxidation. *European Journal of Clinical Nutrition* 47: 759–764.

Esterbauer H, Gebicki J, Puhi H and Jurgens G (1992) The role of lipid peroxidation and antioxidants in the oxidative modification of LDL. *Free Radical Biology and Medicine* 13: 341–390.

Friedman M, Byers S (1957) Mechanism underlying hypercholesterolemia induced by Triton WR-1339. *American Journal of Physiology* 190: 439–445.

Habig W, Pabst M and Jakoby W (1974) Glutathione-S-transferase the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249: 7130–7139.

Hales D, Allen J and Shankara T (2005) Mitochondrial function in Leydig cell steroidogenesis. *Annals of the New York Academy of Sciences* 1061: 120–134.

Hazfil U, Stocker R (1993) Oxidation of low density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high uptake form for macrophages. *Biochemical Journal* 90: 165–172.

Herttuala Rosenfeld M, Parthasarathy S, Glass C, Sigal E, Witztum J, et al. (1989) Colocalization of 15-lipoxygenase mRNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. *Proceedings of the National Academy of Sciences of the United States of America* 86: 1046–1050.

Ishibashi S, Brown M, Goldstein J, Gerard R, Hammer R and Herz J (1993) Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *Journal of Clinical Investigation* 92(2): 883–893.

Ishibashi S, Goldstein J, Brown M, Herz J and Burns D (1994) Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. *Journal of Clinical Investigation* 93(5): 1885–1893.

Jialal I, Grundy S (1992) Influence of antioxidant vitamins on LDL oxidation. *Annals of New York Academy of Sciences* 669: 2327–2348.

Katryna B, Anita P (1980) Purification of rat testicular microsomal 17-ketosteroid reductase. evidence that 17-ketosteroid reductase and 17β-hydroxysteroid dehydrogenase are distinct enzymes. *Journal of Biological Chemistry* 255: 5552–5559.

Kendler B (1986) Carnitine an overview of its role in preventive medicine. *Preventive Medicine* 15: 373–390.

Lahouel M, Boulkour S, Segueni N and Fillastre J (2004) The flavonoids effect against vinblastine, cyclophosphamide and paracetamol toxicity by inhibition of lipid-peroxidation and increasing liver glutathione concentration. *Pathologie Biologie (Paris)* 52: 314–322.

Martinez-Martos JM, Arrazola M, Mayas MD, Carrera-Gonzalez MP, Garcia MJ and Ramirez-Expsito MJ (2011) Diet-induced hypercholesterolemia impaired testicular steroidogenesis in mice through the renin–angiotensin system. *General Comparative Endocrinology* 173: 15–19.

Nakashima Y, Plump A, Raines E, Breslow J and Ross R (1994) ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arteriosclerosis and Thrombosis* 14(1): 133–140.

Naughton C, Nangia A and Agarwal A (2001) Pathophysiology of varicoceles in male infertility. *Human Reproduction* 7: 473–481.

Plump A, Smith J, Hayek T, Aalto-Setala K, Walsh A, Verstuyft J, et al. (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 71(2): 343–353.

Purohit A (2007) A correlation between hyperlipidaemia and testicular function in albino rats. *Congress of the European Atherosclerosis Society* 76: 10–13.

Rebouche C (2004) Kinetics, pharmacokinetics and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Annals of New York Academy of Sciences* 1033: 30–41.

Reddick R, Zhang S and Maeda N (1994) Atherosclerosis in mice lacking apo E. evaluation of lesional development and progression. *Arteriosclerosis and Thrombosis* 14(1): 141–147.

Robert N, Carl L and Wilson T (1999) vitamin E reduces plasma low density lipoprotein cholesterol, LDL oxidation and early aortic atherosclerosis compared with

black tea in hypercholesterolemic hamsters. *Nutrition Research* 19(8): 1201–1214.

Savenkova M, Mueller D and Heinecke J (1994) Tyrosyl radical generated by myeloperoxidase a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein. *Biological Chemistry* 269: 394–400.

Seccombe D, James L, Hahn P and Jones E (1987) L-carnitine treatment in the hyperlipidemic rabbit. *Metabolism* 35: 1192–1196.

Sedlak J, Lindsay R (1986) Estimation of total protein-bound and non-protein sulfhydryl groups in tissue with Elman's reagent. *Analytical Biochemistry* 24: 192–205.

Levine S, Saltzman A (2007) A procedure for inducing sustained hyperlipemia in rats by administration of a surfactant. *Journal of Pharmacological and Toxicological Methods* 55: 224–226.

Steinbrecher U, Zhang H and Lougheed M (1990) Role of oxidatively modified LDL in atherosclerosis. *Free Radical Biology and Medicine* 9: 155–168.

Xu J, Yuan X and Lang P (1997) Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. *Chinese Environmental Chemistry* 16: 73–76.

Zhang S, Reddick R, Piedrahita J and Maeda N (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258(5081): 468–471.