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Efficiency of selenium in attenuating epididymal histopathological changes in hypercholesterolaemic adult rat

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Background: *Studies on sperm maturation, epididymal histology, or epididymal tubule physiology are significant parts in reproductive researches. The present study was aimed to evaluate the effect of induced hypercholesterolaemia on the epididymis of adult albino rats and to clarify the possible protective role of selenium.*

Materials and methods: *Forty adult albino Wistar rats were divided into four groups; untreated control group (group I), sham control (group II), group with induced hypercholesterolaemia (group III), group with induced hypercholesterolaemia treated with selenium 0.25 mg/kg/day (group IV).*

Results: *Histological and ultrastructural examination of the epididymal epithelial cells of hypercholesterolaemic rats (group III) showed loss of cilia with many vacuolations, fatty degenerative changes and increased collagen fibres. Morphometrically significant increase ($p < 0.0001$) in the per cent area of collagen fibres with no significant change in the optical density of periodic acid Schiff reaction ($p > 0.05$). Selenium treated group (group IV) produced marked improvement in histological, ultrastructural and morphometric results as compared with group III.*

Conclusions: *It could be concluded that hypercholesterolaemia produced deleterious effects to the epididymis and selenium could attenuate these effects.* (Folia Morphol 2015; 74, 3: 295–302)

Key words: hypercholesterolaemia, epididymis, selenium, histopathology

INTRODUCTION

Cholesterol is involved in the mechanism of sperm capacitation, acrosomal reaction and protection of spermatozoa against environmental affection [10, 29]. However, when cholesterol homeostasis is deregulated, cholesterol may become a deleterious molecule associated with several pathological abnormalities [2]. Studies on sperm maturation, epididymal histology, or epididymal tubule physiology are significant parts in reproductive researches [17]. The importance of understanding epididymal function and sperm maturation is emphasised by the fact that up to 40% of infertile men exhibit idiopathic infertility that may reflect sperm maturational disorders [9]. Epididymal dysfunction in hypercholesterolaemic animals may have detrimental effects on the cytostructural modifications and biochemical changes that occur during sperm epididymal maturation and may result both in decreased sperm motility and in the sperm morphometric abnormality (in the midpiece) found in hypercholesterolaemic animals [29]. Selenium-enriched probiotics or inorganic selenium

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supplementation had been found to ameliorate at various degrees the significant adverse effects on male fertility caused by high fatty diet [13]. The purpose of the present work was to study the epididymal histopathological changes in the hypercholesterolaemic adult albino rats and to evaluate the efficiency of selenium in attenuating these changes.

MATERIALS AND METHODS

Diet

The standard diet was prepared from casein (15%) with addition of cotton seed oil (10%), salt mixture (4%), vitamin mixture (1%), cellulose (5%) and the remainder was corn starch 65% [1]. The experimental diet was formulated using the same constituents of the standard diet except that cellulose was reduced (to 4%) with addition of cholesterol (1%) [11]. Cholesterol was purchased from El Gomhorya Co., Egypt as a pure white powder.

Animals

For this work, forty adult male Albino Sprague Dawley rats, 200–250 g body weight, were used. They were housed in separate cages (5 rats per cage) at the animal house of the Faculty of Medicine, Cairo University.

Experimental design. The rats were randomly divided into 4 equal groups (10 rats each): Group I (untreated control) was fed on the standard diet for 3 months, Group II (sham control) was fed on the standard diet (3 months) with addition of 10 mL of distilled water daily (the vehicle of selenium) via gastric gavage, Group III was fed on the experimental diet (3 months), Group IV was fed on the experimental diet for one month to induce hypercholesterolaemia [25] then selenium was given concomitantly with the experimental diet for another 2 months.

Chemicals

Selenium (Se) was supplied as sodium selenite (Na2SeO3) obtained from Sigma-Aldrich Company, imported by the Egyptian International Centre for Import in a powder form. Each 1 mg of the powder was dissolved in 10 mL saline. It was given in a dose 0.25 mg/kg/day via a gastric gavage [14].

Methods

The experimental animals had undergone the following examinations:

Biochemical examination. A blood samples were obtained from the orbital venous plexus of the animals after 1 month from the beginning of the experiment and 3 months before scarification for measuring the serum total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides (TG). The sample collected after 1 month was to ensure the hypercholesterolaemic state in experimental diet given groups while the other sample (after 3 months) was to estimate the possible curative effect of Se. The biochemical examination was done in labs of Faculty of Medicine, Cairo University using a fully automated Olympus Au 400, Germany after formation of calibration and control for HDL cholesterol and TG. The Olympus is programmed to measure the LDL by a certain equation (LDL = TC – TG/5 + HDL). The laboratory used kits obtained from Beckman Coulter Company USA.

Histological examination. The rats were sacrificed by decapitation at the end of the last day of the experiment. The epididymis of all animal were excised and subjected to the following procedures: ***Light microscopic study:*** The epididymis of each rat was fixed in 10% neutral buffered formol saline then embedded in paraffin and sections of 5 μm thickness were prepared and stained with haematoxylin and eosin (H & E stain), Masson's trichrome stain and periodic acid Schiff (PAS) reaction [5]. ***Electron microscopic study:*** Freshly prepared sections of the epididymis of each rat were fixed in glutaraldehyde 2.5% then processed to be examined at the faculty of agriculture, Cairo University, Research centre, using Jeol Jem 1400 transmission electron microscope to study the ultrastructural changes. ***Histomorphometric study*** (image analysis computer system): It was done for the slides stained with PAS and Masson's trichrome stains. The image analysis was performed using a computer system (software Leica Quin 500) consisting of colour video camera. Colour monitor, CBU of IBM personal computer was connected to the microscope. The investigated parameters were the mean per cent area of collagen fibres in Masson's trichrome stained sections and the mean optical density of PAS reaction.

Statistical methods

The statistical software package for the social science (SPSS version 20) was used for data analysis. Data was expressed as mean ± standard deviation. Student t-test was used to compare the mean values between group III and group IV. One-way analysis of variance (ANOVA) was used to compare the mean values between all five

Table 1. The mean serum levels of total cholesterol (TC), triglycerides (TG), high and low density lipoprotein (HDL, LDL) after 1 month of the experiment among the different examined groups

Groups after 1 month	TC	TG	HDL	LDL
Group I	92.6 ± 14.9^{a}	67.6 ± 7.3^{a}	35.8 ± 4.3^{a}	67.3 ± 7.5^{a}
Group II	91.2 ± 9.8^{a}	66.2 ± 10.5^{a}	34.7 ± 5.2^{a}	66.9 ± 8.1^{a}
Group III	$281 \pm 14.1^{b**}$	$215.7 \pm 20.5^{b**}$	$13.3 \pm 3.3^{b**}$	$167.5 \pm 14.2^{b**}$
Group IV	$277.5 \pm 17.0^{b**}$	$207.9 \pm 11.1^{b**}$	$13.2 \pm 3.9^{b**}$	$187.4 \pm 11.8^{b**}$

Mean with similar superscripts (a), (b) in each column are non-significant.
*Means with different superscripts (a), (b) and (c) in each column are significant at $p < 0.05$.
**Means with different superscripts (a), (b) in each column are significant at $p < 0.001$.

Table 2. The mean serum levels of total cholesterol (TC), triglycerides (TG), high and low density lipoprotein (HDL, LDL) after 3 months of the experiment among the different examined groups

Groups after 3 months	TC	TG	HDL	LDL
Group I	92.6 ± 14.9^{a}	67.6 ± 7.3^{a}	35.8 ± 4.3^{a}	67.3 ± 7.5^{a}
Group II	91.2 ± 9.8^{a}	66.2 ± 10.5^{a}	34.7 ± 5.2^{a}	66.9 ± 8.1^{a}
Group III	$326.2 \pm 11.6^{b**}$	$268.8 \pm 17.3^{b**}$	$11.1 \pm 2.4^{b**}$	$197.3 \pm 10.5^{b**}$
Group IV	99.9 ± 8.7^{a}	$82.3 \pm 6.2^{c*}$	31.9 ± 5.5^{a}	71.4 ± 10.1^{a}

Mean with similar superscripts (a), (b), (c) in each column are non-significant.
*Mean with different superscripts (a), (b), (c) in each column are significant at $p < 0.05$.
**Mean with different superscripts (a), (b) in each column are significant at $p < 0.001$.

groups. At confidence interval of 95%, P-value was considered highly significant (**) when it was below 0.01 and significant (*) when it was below 0.05.

RESULTS

Biochemical results

The present work revealed elevation of serum level of LDL, TC and TG as well as decline in level of HDL after 1 month of the experiment in the experimental diet groups. This ensures hypercholesterolaemia in group III and IV (Table 1). Selenium after 3 months of the experiment produced marked improvement in the serum level of LDL, HDL and TG of group IV as compared with group III (Table 2).

Histological results

Group I and II (control groups): The histological and ultrastructural findings of the normal and sham control groups were similar. The epididymis of the control groups revealed regularly arranged tubules lined with pseudo-stratified ciliated epithelium. Its lining cells were formed of principal cells which were columnar ciliated and basal cells with basal nuclei The connective tissue between the tubules contained many smooth muscle fibres, blood vessels and many capillaries (Figs. 1A, B, 2A). The control epididymis specimens showed a thin layer of collagen fibres in

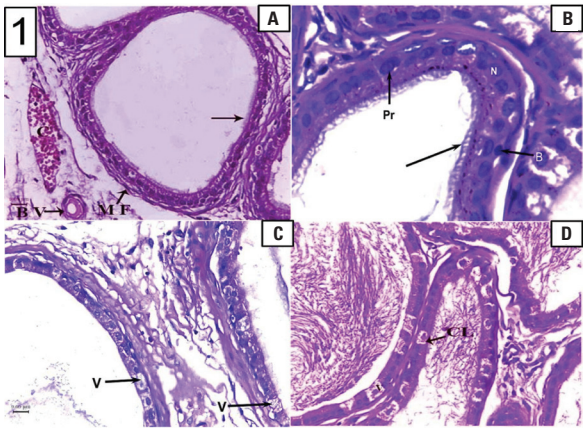

Figure 1. A photomicrograph of a section from a rat epididymis of the: **A.** Sham control group showing the epididymis with ciliated mucosa (arrow). Smooth muscle fibres (MF), blood vessels (BV) and capillaries (C) are present in the connective tissue septa (H & E × 400); **B.** The untreated control group showing the epididymis with ciliated mucosa (arrow). The mucosa is formed of principal cells (Pr) and basal cells (B). The latter contains basally located nuclei (N) (H & E × 1000); **C.** Group III showing loss of the cilia of the epididymal cells of the tubules. The lining cells of the epididymis exhibiting cytoplasmic vacuolation (V), appearing as clear cells; **D.** Group IV showing mostly intact epididymal mucosal cells with largely preserved cilia. Some mucosal cells are showing cytoplasmic vacuolation (V), appearing as clear cells (CL). The lumen is full of sperms (H & E × 400).

the interstitial tissue around the tubules in Masson's trichrome stained sections (Fig. 3A). Using PAS reaction for studying the carbohydrate content of the

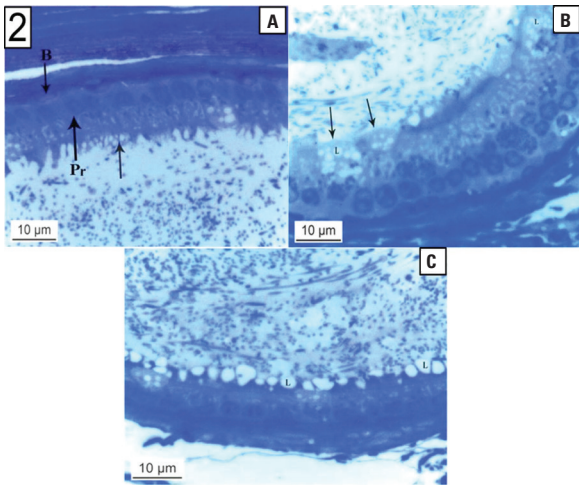

Figure 2. A photomicrograph of a semi-thin section from a rat epididymis of the: **A.** Sham control group showing the epididymal tubule with well apparent cilia (arrow). The mucosa is formed of principal cells (Pr) and basal cells (B). The latter contains basally located nuclei.; **B.** Group III showing degeneration of the cilia (arrows) of the cells of the epididymal tubules with lipid droplets (L) in their cytoplasm; **C.** Group IV showing apparently regular epididymal tubular cells with appearance of some lipid droplets (L) in between the cilia (toluidine blue × 1000).

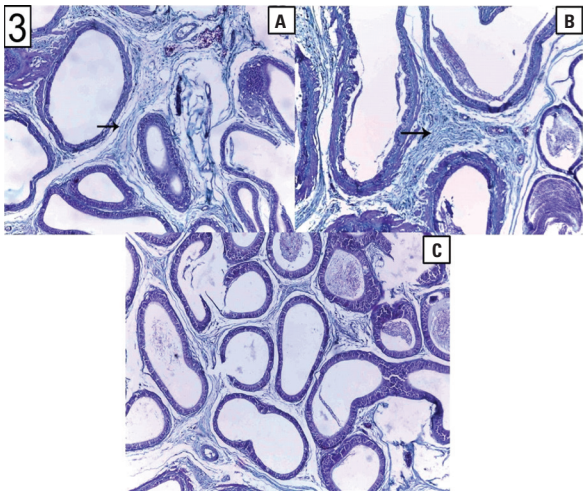

Figure 3. A photomicrograph of a section of a rat epididymis from the: **A.** Untreated control group showing the epididymial tubules with normal pattern of collagen fibres deposition (arrow) in the interstitial tissue; **B.** Group III showing the epididymis with increased amount of collagen fibres deposition (arrow) in the interstitium; **C.** Group IV showing mild increase in collagen fibres deposition in between the epididymal tubules (Masson's trichrome × 100).

control epididymis, the cytoplasm of the epididymal cells contained considerable amounts of glycogen as shown by their strong positive reaction. It appeared as very small granules aggregated together to fill the

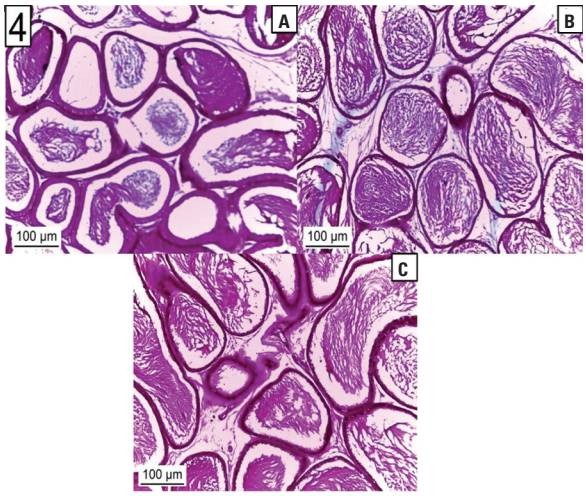

Figure 4. A photomicrograph of a section from a rat epididymis of the: **A.** Untreated control group showing the epididymal tubules with strong positive periodic acid Schiff (PAS) reaction. The lumen is full of sperms; **B.** Group III showing the epididymal tubules with a moderate PAS reaction; **C.** Group IV showing the epididymal tubules with moderate to strong PAS reaction (PAS × 100).

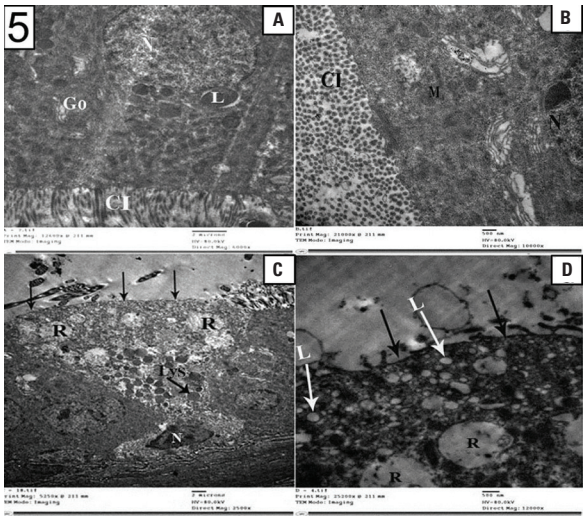

Figure 5. An electron micrograph of rat epididymis from the: **A.** Untreated control group showing the principal cells of the epididymis with regular nucleus (N). Lipid droplets (L) and Golgi apparatus (Go) are also seen within the cytoplasm. The cilia (CI) of the cells appear as tubular prolongation from the cells (EM × 6000); **B.** Sham control group showing the principal cells of the epididymis with regular nucleus (N) and abundant mitochondria (M). The cilia (CI) of the cells are cut transversely (EM × 10000); **C.** Group III showing part of the lining cells of the epididymis with shrunken nucleus (N). Lysosomes (Lys) and areas of rarefaction (R) appear in the cytoplasm. Absence of the cilia (arrows) of the principal cells is noticed in this section (EM × 2500); **D.** Group III showing the lining cells of the epididymis with absence cilia (arrows). Lipids droplets (L) with their regular shapes and areas of rarefaction (R) appear in the cytoplasm (EM × 12000).

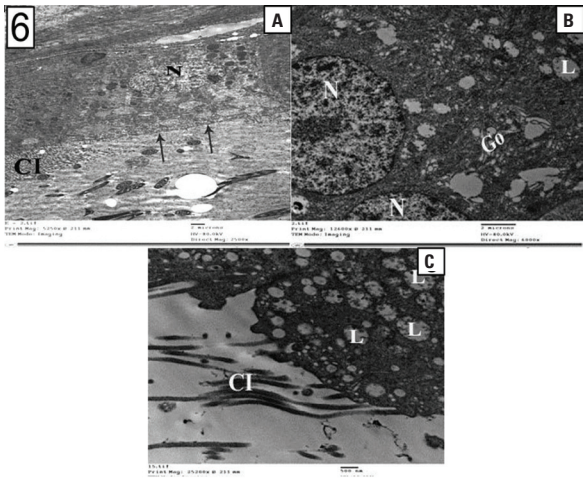

Figure 6. An electron micrograph of rat epididymis from group IV showing apparently normal lining cells of the epididymis with: **A.** Regular nuclei (N), abundant organelles and well apparent cilia (CI) are also seen. Other cells are showing absence of cilia (arrows) and cytoplasmic rarefaction (EM × 2500); **B.** The cells appear with regular nuclei (N) and abundant organelles such as Golgi apparatus (Go). Few lipid droplets (L) could be seen (EM × 6000); **C.** Preservation of the cilia (CI) and many lipid droplets (EM × 12000).

whole cytoplasm. The lumen of the tubules was full of sperms (Fig. 4A). Electron microscopic study of the control groups revealed that the principal cells of the epididymis were regular with rounded nuclei and abundant chromatin material. The cytoplasm showed widely spread organelles such as mitochondria and Golgi apparatus. The cilia appeared as a long projection from the luminal border of the cells or as a circles in transverse cut sections (Fig. 5A, B).

Group III (hypercholesterolaemic group): On examination of the epididymis of this group, the epithelium showed loss of its cilia and the cytoplasm of the lining cells displayed vacuolations and appeared as clear cells (Fig. 1C) with fatty degeneration in the form of lipid droplets (Fig. 2B). The collagen fibres in Masson's trichrome stained sections were increased in the interstitial tissue around the tubules and blood capillaries (Fig. 3B). The epididymal cells in the epididymal tubules of the current group showed moderate PAS reaction (Fig. 4B). Electron microscopic study revealed the principal cells of the epididymis with absence of their cilia (Fig. 5C). Many lysosomes appeared as regular shaped bodies with different densities (Fig. 5C). Fatty degeneration (Fig. 5D), areas of cytoplasmic rarefaction and nuclear changes in the form of indentation as well as shrinkage were noticed in the cells of this group (Fig. 5C, D).

Group IV (hypercholesterolaemic group treated with selenium): The epididymis of this group revealed apparent normally arranged tubules lined with pseudo-stratified ciliated epithelium. Its lining cells were formed of principal cells and basal cells. Most of the cells appeared as regular and uniform cells with rounded and darkly stained nuclei (Figs. 1D, 2C). The cytoplasm exhibited moderate vacuolations appearing as clear cells (Fig. 5D). Some specimens presented regular epididymal tubular cells with appearance of lipid droplets in between the cilia (Fig. 1D). The collagen fibres displayed mild increase around epididymal tubules in Masson's trichrome stained sections (Fig. 3C). The epididymal cells showed a moderate to strong PAS reaction (Fig. 4C). Electron microscopic study revealed that the lining cells of the epididymis had regular rounded nuclei with widely spread organelles such as Golgi apparatus (Fig. 6A, B). Few cells showed absent cilia and rarefaction of the cytoplasm (Fig. 6A) while others showed preservation of the cilia with many lipid droplets (Fig. 6C).

Histomorphometric results

Per cent area of collagen fibres. The mean per cent area of collagen fibres in group III (23 ± 5.93) as measured by image analyser exhibited significant increase as compared with that of the control group (13.35 ± 4.55) (Table 3). In group IV, the mean per cent area of collagen fibres (11.45 ± 4.19) exhibited significant decrease as compared with that of group III (Table 3).

Optical density. The mean optical density of group III (30.34 ± 2.6) as measured by image analyser showed no significant change as compare with that of the control group (32.44 ± 5.18) (Table 4). The mean optical density of group IV (34.12 ± 16.33) as measured by image analyser showed no significant change as compared with that of group III (Table 4).

DISCUSSION

Diet-induced hyperlipidaemia has been found to influence sperm lipid composition and adversely affect semen quality including sperm concentration, motility, capacitation, and acrosomal reaction [19]. The present study revealed that the epididymis was injured in group III (induced hypercholesterolaemia group). There was loss of the cilia of principal cells, fatty degeneration and increased number of clear cells. This was similar to the observation of Abdelmalik [1]

Table 3. Statistical difference in the mean values of the per cent area of collagen fibres in Masson's trichrome stained sections of the epididymis among the different examined groups

Statistical profile	Group I	Group II	Group III	Group IV
Mean ± standard deviation	13.35 ± 4.55	13 ± 3.7	23 ± 5.93	11.45 ± 4.19
ANOVA	$p \leq 0.001$**			
t test between groups III and IV			$p \leq 0.004$*	

*$p \leq 0.05$ significant; **$p \leq 0.001$ highly significant

Table 4. Statistical difference in the mean values of the optical density of periodic acid Schiff (PAS) reaction of the epididymis among the different examined groups

Statistical profile	Group I	Group II	Group III	Group IV
Mean ± standard deviation	32.44 ± 5.18	32.0 ± 4.8	30.34 ± 2.6	34.12 ± 16.33
ANOVA	$p \leq 0.816$			
t test between groups III and IV			$p \leq 0.18$	

$p > 0.05$ not significant

who revealed that hypercholesterolaemia resulted in an increased number of clear cells suggesting concomitant increased phagocytosis of the degenerated epithelium and abnormal sperms. Ultrastructural examination of the epididymis of group III showed the epithelial cells containing many lysosomes which appeared as vesicles of different sizes, forms and densities, however, some cells exhibited nuclear shrinkage and indentation. These results were concordant with Abdelmalik [1] who reported only fat accumulation around the tubules and thickening of the smooth muscle fibres. The latter author also revealed that in hypercholesterolaemic rats, principal cells contained the usual organelles with only few lumens of the epididymal tubules contained few degenerated sperms. The latter author assumed that the epididymis has been a target of cholesterol toxicity in hypercholesterolaemic rats that might have a detrimental effect on sperm maturation process. Many previous studies clarified the important influence of epididymal epithelium in sperm maturation. Cooper and Yeung [8] and Chabory et al. [6] suggested that several small water soluble components secreted by the epididymal cells into epididymal fluid (myo-inositol, L-carnitine, taurine, glutamate). This fluid was taken up by spermatozoa during post-testicular maturation, to function as a reserve of intracellular osmolytes against the osmotic challenges that spermatozoa later experience at ejaculation. Moreover, the high concentration of organic compounds in epididymal fluid created high osmolality of epididymal fluid leading to dehydrating spermatozoa as a means of enforcing sperm quiescence [7]. Therefore, it could be postulated that any alteration in epididymal epithelium and its secretory function might influence male infertility.

In the present study, there was a significant increase in the collagen fibres in the epididymis of rats of induced hypercholesterolaemia as compared with that of control group. This finding might indicate hypercholesterolaemia induced fibrosis in the epididymis. Dissimilar to this finding, Emrich de Abreu et al. [12] reported only hepatic steatosis in response to induced hypercholesterolaemia with no increase in liver fibrosis as detected by Masson's trichrome. This could be explained by their short duration (8 weeks) compared with 12 weeks duration of the present work and this longer duration might be enough for fibrosis to occur. The deleterious effects of hypercholesterolaemia on the epididymis in this work were complementary to that found by Lancellotti et al. [16] who reported that fat increment (0.05% cholesterol) in standard diet promoted a significant increase in serum and sperm membrane cholesterol. This ultimately altered membrane-coupled sperm specific functions: osmotic resistance, acrosomal reaction and sperm capacitation in rabbits. These changes were also associated with a reduction in motility percentage and appearance of abnormal sperm morphology. In an attempt to explain the mechanism by which hypercholesterolaemia produced deleterious effects on male fertility, Rejraji et al. [21] reported that lipid homeostasis is of particular importance in germ cells.

These cells undergo different modifications in lipid concentrations, especially cholesterol, desmosterol and phospholipids during their differentiation. These modifications are important to maintain membrane fluidity and ability to fertilisation. Hypercholesterolaemia may also stimulate oxygen radical production and increase lipid peroxide levels in various tissues of the rabbits [20]. Lipid peroxides are cytotoxic to spermatozoa causing sperm morphological change [23]. Diet-induced hyperlipidaemia in rabbits has been found to influence sperm lipid composition and adversely affect semen quality including sperm concentration, motility, capacitation, and acrosomal reaction [19, 22].

In the present work, group IV, Se improved the histological and ultrastructural changes in the epididymis as compared with induced hypercholesterolaemia group III. The principal cells regained its cilia and most of them appeared with minor histopathologic changes. However, some principal cells exhibited moderate vacuolation and rarefaction of the cytoplasm with partial loss of their cilia. In this respect, Sharma and Agarwal [26] mentioned that antioxidants protected sperm from lipid peroxidation which leads to the loss of membrane fluidity necessary for sperm function and fusion with the oocyte. Moreover, in hyperlipidaemia, lipid peroxides are an important source of reactive oxygen which has been found to be extremely cytotoxic to male gonads [24]. The latter author also reported that lipid peroxides were found to be extremely cytotoxic to human spermatozoa as a result of lipid peroxides decomposition into highly toxic aldehydes which migrate from one site to another in the body.

The results of the current study regarding the changes produced by selenium against hypercholesterolaemia, agreed to those of Abdelmalik [1] who declared that Se-enriched probiotics or inorganic Se supplementation had ameliorated at various degrees the significant adverse effects on male fertility caused by high fatty diet [1]. Also, it was found that vitamin E (antioxidant) produced significant improvement regarding the mean fertility index, sperm cell count, sperm motility, sperm viability and abnormal forms of sperms against the harmful effects of hypercholesterolaemia on the testis [25].

The present work showed that, in group IV, Se produced nearly restoration of the normal serum levels of LDL, HDL and TG. This was in agreement with the findings of Lizuka et al. [18] who observed that administration of Se suppressed the amount of TG, TC, free fatty acid and LDL cholesterol in the serum of rats fed with high cholesterol diet. Co-administration of Se and ascorbic acid provided protection against alcohol-induced oxidative stress and hyperlipidaemia [4, 27]. Selenium is an essential trace element that is an integral part of many selenoproteins [3]. It has been observed that Se has an antiatherogenic action and suppresses peroxidation of lipids [15]. The effect of Se on nicotine induced hyperlipidaemia was investigated in rats by Sreekala and Indira [28]. They revealed that nicotine intake caused an increase in concentration of TC, TG, free fatty acids, phospholipids and LDL compared with control group. Co-administration of Se along with nicotine reduced the levels of lipids compared with nicotine group. This reduction was attributed by the latter authors to reduction in the biosynthesis of lipids as evidenced by the reduced activity of HMGCoA reductase and lipogenic enzymes.

Human male infertility might be associated with altered lipid metabolism in seminal plasma [16]. It has been reported that high plasma levels of TC and/or TG are associated with testicular dysfunction and poor semen quality in humans, which may lead to male infertility [22]. The clinical importance of the current study is to understand epididymal structure and function and the role of hypercholesterolaemia in sperm maturation. Such data may help in understand and to emphasize the fact that up to 40% of infertile men exhibit idiopathic infertility that may reflect sperm maturational disorders [9]. Also to elucidate the role of Se in minimising hypercholesterolaemic effects on the epididymis.

CONCLUSIONS

From the previous results, it could be concluded that hypercholesterolaemia produced deleterious effects to the epididymis and Se could attenuate these deleterious effects.

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