ABSTRACTS

INTRODUCTION

Dyslipidemias, include hypercholesterolemia, low levels of high density lipoprotein and cholesterol. Hypercholesterolemia is a major cause of atherosclerosis and atherosclerosis induced conditions, such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. Recognition that dyslipidemia is a risk factor has led to the development of the drugs that modify cholesterol levels (Brunton et al., 2011).

Statins are one of the world’s most prescribed drugs for treatment of dyslipidemias (Soner et al., 2013). Statins treat dyslipidemias by inhibition of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase), which is a key enzyme in the cholesterol biosynthetic pathway (Sweetman, 2009). Atorvastatin is the most commonly used statins (Solomon and Freeman, 2008). Atorvastatin is an appropriate first-line lipid-lowering therapy in numerous groups of patients at low to high risk of coronary heart disease.

Atorvastatin in dosage of 10-80mg/day reduces the levels of total cholesterol, low density lipoprotein cholesterol, triglyceride and very low density lipoprotein cholesterol and increases high-density lipoprotein cholesterol in patients with a wide variety of dyslipidaemias. Aggressive reduction of serum low density lipoprotein cholesterol occurs with atorvastatin 80mg/day in patients with acute coronary syndromes (Malhotra and Goa, 2001). The first generation statins drugs, which include lovastatin, pravastatin, and simvastatin are fungal metabolites with similar chemical structures. The second generation statins drugs, which include atorvastatin, cerivastatin, and fluvastatin, are synthetic and structurally dissimilar (Slater and MacDonald, 1998). The known members of statins family have different hydrophatic profiles (Pahan, 2006). Lovastatin, simvastatin and cerivastatin are all lipophilic, whereas, fluvastatin, atorvastatin, and pravastatin are hydrophilic (Corsini et al., 1999). The most serious adverse effect of statins is muscle injury. Also a dose-dependent
increase of liver enzymes can be noted with statins use (Pasternak et al., 2002). In animals, statins intake reduces sperm parameters. The intake of statins by hypercholesterolemic patients could reduce circulating testosterone concentration and sperm quality. (Pons-Rejraji et al., 2010). Increased production of reactive oxygen species (Pal et al., 2015).

Vitamin E is one of the major antioxidants; it plays an important role in reducing oxidative stress. It is the most important hydrophobic antioxidant protecting biological molecules like DNA, proteins and lipids against reactive oxygen species (Alba et al., 2008 and Aydogan et al., 2014).

There weren't researches that proved protective effect of vitamin E against testicular involvement due to atorvastatin. So, this work aims to investigate the adverse effects of atorvastatin (80mg/d; the maximum human therapeutic dose) on the testes of the adult male albino rats and the possible protective effect of vitamin E.

**MATERIAL AND METHODS**

**Drugs:**

Atorvastatin (Liptor®) is available in tablet forms produced by SIGMA; Pharmaceutical Industries-Egypt. The human therapeutic dose is ranged from 10-80 mg/day (Brunton et al., 2011). The maximum therapeutic dose 80mg was used in this work. The equivalent therapeutic dose for adult male rat was 1.44mg/rat which calculated by using Paget and Barnes (1964). Each tablet 80mg was dissolved in 40 ml distilled water. Each rat was given 0.72 ml (contained 1.44mg of atorvastatin) orally via a gastric tube once daily.

Vitamin E is available as gelatinous capsules produced by Pharco; Pharmaceutical Company. The highest safe level of vitamin E supplements for adult human is 1000 mg/day (Pace et al., 2010) and this dose was used in this work. The equivalent therapeutic dose for adult male rat was 18mg/rat which calculated by using Paget and Barnes (1964). Each capsule which contained 1000mg present in 1ml of oily solution was dissolved in 4ml of corn oil. Each rat was given 0.09 ml (contained 18mg of vitamin E) orally via a gastric tube once daily.

**Animals:**

Fifty adult male albino rats weighing 200±20g were used in this work. The animals were fed on balanced rat chow and water. The pellets were consisted of 5% fibers, 3.5% fats, 6.5% ash and 20% proteins. They were housed under a 12/12 h light/dark cycle, with free access of water. The room temperature and humidity were maintained at 23 ±1° C and 55 ± 5%, respectively. The rats were isolated for two weeks before the beginning of the experiment for acclimatization. Throughout the experiment, the rats were housed in the animal house of faculty of medicine, Al- Azhar University. They were divided into four groups as follow:

**I-Control group (Group C; C1 and C2):** It consisted of 20 adult male albino rats. Half of them were given 0.72ml distilled water/rat orally as a single daily dose for 4 weeks (Group C1). The other half were given 0.09 ml of corn oil/ratorally as a single daily dose for 4 weeks (Group C2).

**II-Vitamin E treated group (GroupE):** It consisted of 10 adult male albino rats. Each rat was given of 0.09ml of corn oil (contained 18mg of vitamin E) orally as a single daily dose for 4 weeks.

**III-Atorvastatin treated group (GroupS):** It consisted of 10 adult male albino rats. Each rat was given 0.72ml distilled water (contained 1.44mg ofatorvastatin) orally as a single daily dose for 4 weeks.

**IV-Atorvastatin and vitamin E treated group (GroupSE):** It consisted of 10 adult male albino rats. Each rat was given the same dose ofatorvastatin as that of group E plus the dose of vitamin E as that of group E.

**Collection of the specimens and preparation for examination:**

At the end of the 4th week, the rats were anaesthetized lightly by diethyl ether inhalation and the testes were removed from the scrotum. One of the testes of each rat was used for light microscopic and morphometric studies and the other testis for transmission electron microscopic study.

**Light microscopic study:**

The testes which used for light microscopic study were fixed by immersion in Bouin’s solution for 3days. The specimens were dehydrated in ascending grades of ethyl alcohol and cleared in benzene. The specimens were impregnated for three changes in paraffin and were finally embedded in paraffin wax. The paraffin blocks were cut into serial transverse sections at 5 μm thick with a rotary microtome. Successive transverse paraffin sections were...
attached to an albumenized glass slides. The Hematoxylin and Eosin stain (Bancroft and Gamble, 2008) was used to study the testicular architectures. The Masson’s trichrome stain was used to illustrate the collagen fibers (Bancroft and Gamble, 2008). The Periodic Acid Schiff’s (PAS) reaction (Bancroft and Gamble, 1996) was also used to study the mucopolysaccharides and polysaccharides.

Transmission electron microscopic (TEM) study:

The testes which used for electron microscopic study were cut into small pieces. The specimens were immediately fixed in cold 5% glutaraldehyde and washed in 0.1 ml phosphate buffer (PH 7.2). Then, postfixed with 1% osmium tetraoxide, dehydrated and embedded in epoxy resin. The semithin sections (1μm thick) were cut on an LKB ultratome and stained with toluidine blue and examined by light microscope. Ultrathin sections (60 nm thick) were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate (Bancroft and Gamble, 2008). The ultrathin sections were examined using a transmission electron microscope (JEOL1010 EX II, Japan) at the Regional Mycology and Biotechnology Center, Al-Azhar University, Cairo, Egypt.

Morphometric study:

The image analyzer computer system Leica Qwin 500 (England) at the Regional Mycology and Biotechnology center, Al-Azhar University, Cairo, Egypt was used to evaluate the epithelial height of the seminiferous tubules of the studied groups using H&E-stained sections. The tenslides which stained with haematoxylin and eosin often albino rats were taken from equidistant points along each serially sectioned specimen. Then five seminiferous tubules were randomly selected in each slide to measure the height of germinal epithelium. Measuring the height was done by taking 4 different heights for each seminiferous tubule at magnification x 100. The mean height of the germinal epithelium of each seminiferous tubule was calculated. The cumulative mean height for each group was calculated statistically. The data was subjected to statistical analysis.

Statistical analysis:

Statistical analysis of the data obtained were expressed as mean values and standard deviations, and statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for multiple comparison (Mould, 1989). Differences were considered statistically significant at (P < 0.001).

RESULTS

-The testes of the control (Group C; C1 and C2) and vitamin E treated groups (Group E):

Light microscopic, electron microscopic, and morphometric studies of the testes of the control (Group C; C1 and C2) and vitamin E treated groups showed insignificant differences. So, their results were pooled together.

Light microscopic examination of the transverse sections of the testes of groups C (C1 and C2) and E showed that the testes were covered by testicular capsules and consisted of seminiferous tubules separated from each other by the interstitial tissues. The capsule was formed of two layers, an outer layer: the tunica albuginea and an inner layer: the tunica vasculosa. The seminiferous tubules contained numerous spermatozoa (Fig. 1a). The interstitial tissue consisted of loose connective tissue, few blood vessels and the interstitial cells of Leydig. (Fig. 2a). These seminiferous tubules had thin basal lamina, 7-8 rows of seminiferous cells, and numerous spermatozoa. The seminiferous cells consisted of spermatogonia A, spermatogonia B, Sertoli cells, primary spermatocytes, and early and late spermatids (Fig. 2a). The spermatozonia A and B were rested on the basal lamina. Spermatogonia A appeared oval in shape and had large oval lightly stained basophilic nuclei (Fig. 2a). The spermatozonia B appeared rounded in shape and had rounded deeply stained basophilic nuclei (Fig. 2a). The Sertoli cells appeared columnar in shape and their elongated lightly stained basophilic nuclei were perpendicular to the basal lamina (Fig. 2a). The primary spermatocytes were the largest cells. They appeared rounded in shape and had large rounded deeply stained basophilic nuclei (Fig. 2a). The early spermatids form about 3-5 rows. They were small rounded in shape and had lightly stained basophilic nuclei. The late spermatids had elongated deeply stained nuclei (Fig. 2a). The spermatozoa were present in the lumina and had small heads with deeply stained basophilic nuclei and elongated tails (Fig. 2a). The sections of the testes which were stained with Masson’s trichrome showed normal distribution of the collagen fibers in the testicular capsules, the basal lamina of the...
spermatogenic cells and the walls of the blood vessels (Fig. 3a). The sections of the testes which were stained with Periodic Acid Schiff’s (PAS) reaction showed normal PAS reaction in the testicular capsules, the basal lamina and the spermatogenic cells (Fig. 4a).

**Electron microscopic examination** of the testes of Groups C (C1 and C2) and E showed that the interstitial cells of Leydig had characteristic long, tortuous microvilli projecting from its plasma membrane and had ovoid nuclei with a thin layer of heterochromatin along its nuclear envelop (Fig. 5a). The basal lamina of the seminiferous tubules consisted of basement membrane and inner and outer cellular layers. The inner cellular layer consisted of a single layer of myoid cells while the outer cellular layer consisted of 1-3 layers of fibroblast cells. The basement membrane was separated from the inner myoid cells by a gap containing collagen fibers. Also a narrow gap could be hardly detected between the myoid and fibroblast cells and contained few collagen fibers (Figs. 6a, 7a, and 8a). The spermatogonia A had wide areas of contact with the basement membrane. They had large oval euchromatic nuclei. The cytoplasm contained large oval mitochondria (Fig. 6a). The spermatogonia B had large nearly rounded nuclei with large masses of heterochromatin along the nuclear envelopes and had eccentric nucleoli. The cytoplasm contained large oval mitochondria (Fig. 7a). The Sertoli cells had characteristically elongated and indented euchromatic nuclei with very thin layer of heterochromatin along the nuclear envelop and had 1-2 nucleoli. The cytoplasm was studded with rough endoplasmic reticulum and small elongated mitochondria (Figs. 5a and 8a). The primary spermatocytes had large rounded nuclei with scattered large masses of heterochromatin. The nuclei were surrounded by rims of cytoplasm which was studded with numerous small rounded mitochondria and rough endoplasmic reticulum (Fig. 9a). The early spermatids had nearly rounded euchromatic nuclei. The cytoplasm contained rough endoplasmic reticulum and characteristic small peripherally arranged mitochondria with clear matrix (Fig. 10a). The late spermatids also had euchromatic nuclei which covered by acrosomal vesicles. Also the cytoplasm contained rough endoplasmic reticulum and characteristic small peripherally arranged mitochondria with clear matrix (Fig. 11a). Different parts of the sperms tails were detected. The transverse section in the middle pieces appeared as rounded structures which had central axonemes surrounded by nine coarse fibers. These fibers were surrounded externally by zone of lightly packed elongated mitochondria which surrounded externally by plasma lemma. The principle pieces had central axonemes which surrounded by nine coarse fibers. The fibers were in turn enclosed by numerous external sheath of fibers oriented circumferentially. They had anterior and posterior columns and surrounded externally by the plasmalemma. The end pieces had central axoneme which surrounded by nine fine longitudinal fibers which surrounded externally by the plasma lemma (Fig. 12a).

**Morphometric study** of the testes of groups C (C1 and C2) and E showed no significant difference in the means of the epithelial height of the seminiferous tubules between groups C (C1 and C2) and E (P > 0.001) (Table 1 and graph 1).

-The testes of atorvastatin treated group (Group S):

**Light microscopic examination** of the transverse sections of the testes of atorvastatin treated group (Group S) showed that the majority of the testicular capsules became thick and their tunica vasculosa had severely congested and dilated blood vessels. The seminiferous tubules became widely separated and the interstitial spaces contained eosinophilic material. The basal lamina of many seminiferous tubules appeared irregular and interrupted in certain areas (Fig. 1b). Many interstitial cells of Leydig had shrunken deeply stained basophilic nuclei (Fig. 2b). The seminiferous cells of many seminiferous tubules appeared disorganized and couldn’t be recognized from each other. Some of them had vacuolated cytoplasm. Other cells had nuclear changes in the form of fading of nuclear basophilia, shrunken deeply stained basophilic nuclei and nuclear fragmentation. The spermatozoa were few in the lumina of many seminiferous tubules (Fig. 2b). The sections of the testes which were stained with Masson’s trichrome showed marked increase in the collagen fibers in the testicular capsules, the walls of the blood vessels and the basal lamina of the seminiferous tubules (Fig. 3b). The sections which were stained with Periodic Acid Schiff’s (PAS) reaction showed a mild PAS reaction in the testicular capsule, the basal lamina and in the seminiferous cells (Fig. 4b).
Electron microscopic examination of the testes of atorvastatin treated group (Group S) showed that the interstitial tissues contained numerous collagen fibers and many interstitial cells of Leydig which had irregular, heterochromatic nuclei (Fig. 5b). The basement membrane of the basal lamina appeared thick and irregular. The gaps between the basement membrane, the myoid, and fibroblast cells contained numerous collagen fibers (Figs. 6b, 7b and 8b). The spermatogonia A were slightly affected. They appeared more electron opaque and had few mitochondria with destructed cristae (Figs. 6b and 7b). The spermatogonia B had numerous intra cytoplasmic vacuoles and few mitochondria with destructed cristae (Figs. 6b, 7b). The Sertoli cells had many cytoplasmic vacuoles, dilated rough endoplasmic reticulum, mitochondria with destructed cristae and numerous fat droplets (Figs. 6b, 8b). The primary spermatocyte had numerous cytoplasmic vacuoles, swollen mitochondria with destructed cristae and dilated rough endoplasmic reticulum. Also, they had several nuclear changes as fading of nuclear chromatin in irregular nuclei with coarse masses of heterochromatin (Fig. 9b). The majority of early spermatids had intra cytoplasmic vacuoles, dilated rough endoplasmic reticulum. Few of them had shrunken nuclei with coarse masses of heterochromatin. They were separated by wide intercellular spaces (Fig. 10b). The majority of late spermatids had vacuolated cytoplasm. Few of them had nuclear changes in the form of fading of nuclear chromatin (Fig. 11b). The middle pieces of spermatozoa tail had ill-defined plasma lemma, disorganized mitochondrial sheath. Also the principle piece had ill-defined plasma lemma. The end piece appeared with ill-defined features (Fig. 12b).

Morphometric study of the testes of atorvastatin treated group (Group S) showed a highly significant decrease (P<0.001) in the means of the epithelial height in group S when compared with groups C1, C2, E and SE (Table 1 and Graph 1).

- The testes of atorvastatin and vitamin E treated group (Group SE):

Light microscopic examination of the transverse sections of the testes of atorvastatin and vitamin E treated group (Group SE) showed marked improvement in the testicular architecture in comparison to Group S (Figs 1c and 1b). The capsule, the blood vessels, the interstitial tissues and the seminiferous tubules appeared similar to those of groups C and E (Figs 1c and 1a). The lumina of many seminiferous tubules contained numerous spermatozoa. However, few interstitial eosinophilic material were still present (Fig. 1c). The majority of the interstitial cells of Leydig, the seminiferous cells and the spermatozoa appeared similar to those of groups C and E (Figs. 2c, 2a). However, few Leydig cells still had shrunken deeply stained nuclei. Also few primary spermatocytes had vacuolated cytoplasm and nuclear fragmentation (Fig. 2c). The sections of the testes which were stained with Masson’s trichrome appeared with a nearly normal distribution of the collagen fibers in the testicular capsule, the wall of the blood vessels and the basal lamina of the seminiferous tubules (Fig. 3c). The sections of the testes stained with Periodic Acid Schiff’s (PAS) reaction showed a nearly normal PAS reaction in the testicular capsule, the basal lamina of the seminiferous tubules and the seminiferous cells (Fig. 4c).

Electron microscopic examination of the testes of atorvastatin and vitamin E treated group (Group SE) showed marked improvement of many cells in comparison to those of the group S. However, few interstitial cells of Leydig still had heterochromatic nuclei (Fig. 5c). The basal lamina and the basement membrane became thin, regular with few depositions of collagen fibers (Figs. 5c, 6c, 7c and 8c). Few spermatogonia A still had mitochondria with destructed cristae (Fig. 6c). Also few spermatogonia B still had small intracellular spaces (Fig. 7c). The Sertoli cells (Figs. 5c and 8c) and the primary spermatocytes (Fig. 9c) appeared nearly similar to those of groups C and E (Figs. 8a and 9a). Few early and late spermatids still had few cytoplasmic vacuoles (Fig. 10c). The majority of the late spermatids appeared more or less similar to those of groups C and E (Figs. 11a, 11c). The different pieces of the sperm tail appeared more or less similar to groups C and E. However few middle pieces still had ill-defined plasma lemma (Figs. 12a, 12c).

Morphometric study of the testes of group SE showed an insignificant difference in the mean of the epithelial height between groups SE, C1, C2, and E (P>0.001) (Table 1 and graph 1).
(Fig. 1a): Photomicrograph of transverse section of the testis of the control and vitamin E treated groups shows the testicular capsule which is formed of tunica albuginea (Ta) and tunica vasculosa (Tv). It also shows the seminiferous tubules (ST) which contain numerous spermatozoa (Z). The seminiferous tubules are separated by the interstitial tissues (IT). (H & E X 100)

(Fig. 1b): Two photomicrographs of transverse section of the testes of atorvastatin treated group shows the tunica albuginea (Ta) and the tunica vasculosa (Tv) which contains severely congested and dilated blood vessels (BV). The interstitial tissues (IT) contain eosinophilic material (star). The basal lamina (BL) of the seminiferous tubules appear irregular and interrupted in certain areas. (H & E X 100)

(Fig. 1c): Photomicrograph of transverse section of the testis of atorvastatin and vitamin E treated group shows marked improved in the testicular architecture. The blood vessels (BV), the seminiferous tubules (ST) and the interstitial tissues (IT) have normal appearance. The lumena of seminiferous tubules contain numerous spermatozoa (Z). However, few interstitial eosinophilic material still present (star). (H & E X 100)
(Fig. 2a): Photomicrograph of transverse section of the testis of the control and vitamin E treated groups shows interstitial cells of Leydig (ICL) and blood vessels (BV). The seminiferous tubules have thin basal lamina (BL). The tubules have 7-8 rows of seminiferous cells; the spermatogonia A (SA), the spermatogonia B (SB), the Sertoli cells (Se), the primary spermatocytes (PS), the early (ES) and the late (LS) spermatids and spermatozoa (Z). (H & E X 400)

(Fig. 2b): Photomicrograph of transverse section of the testis of atorvastatin treated group shows that the interstitial tissues (IT) contain interstitial eosinophilic material (star). Few interstitial cells of Leydig (ICL) have shrunken deeply stained basophilic nuclei (2 arrows). These seminiferous cells appear disorganized and couldn’t be recognized from each other. Some of them have vacuolated cytoplasm (V). The other cells have nuclear changes in the form of fading of nuclear basophilia (head arrow), shrunken deeply stained basophilic nuclei (→) and nuclear fragmentation (F). The spermatozoa appear few in number. (H & E X 400)

(Fig. 2c): Two photomicrographs of transverse section of the testis of atorvastatin and vitamin E treated group shows that few interstitial cells of Leydig (ICL) still have shrunken deeply stained nuclei (2 arrows). Few primary spermatocytes have fragmented nuclei (F), and vacuolated cytoplasm (V). The Sertoli cells (Se), the spermatogonia A (SA), the spermatogonia B (SB), the primary spermatocytes (PS) and the early (ES) and the late (LS) spermatids and the blood vessel (BV) have normal appearance. (H & E X 400)

(Fig. 3a): Photomicrograph of transverse section of the testis of the control and vitamin E treated groups shows normal distribution of the collagen fibers in the tunica albuginea (Ta), the basal lamina (BL) of the seminiferous tubules and the walls of the blood vessels (BV). (Masson’s trichrome X 200)

(Fig. 3b): Two photomicrographs of transverse section of the testis of atorvastatin treated group shows marked increase in the collagen fibers in the tunica albuginea (Ta), the walls of blood vessels (BV) and the basal lamina (BL) of the seminiferous tubules. (Masson’s trichrome X 200)

(Fig. 3c): Photomicrograph of transverse section of the testis of atorvastatin and vitamin E treated group shows normal distribution of the collagen fibers in the tunica albuginea (Ta), the wall of the blood vessels (BV) and the basal lamina (BL) of the seminiferous tubules. (Masson's trichrome X 200)
(Fig. 4a): Photomicrograph of transverse section of the testis of the control and vitamin E treated group shows normal PAS reaction in the tunica albuginea (Ta), the basal lamina (BL) and the seminiferous cells. (PAS X 400)

(Fig. 4b): Two photomicrographs of transverse section of the testis of atorvastatin treated group show a mild PAS reaction in the tunica albuginea (Ta), the basal lamina (BL) and the seminiferous cells. (PAS X 400)

(Fig. 4c): Photomicrograph of transverse section of the testis of atorvastatin and vitamin E treated group shows normal PAS reaction in the tunica albuginea (Ta), the basal lamina (BL) and the seminiferous cells. (PAS X 400)

(Fig. 5a): Electron micrograph of the testis of the control and vitamin E treated group shows that the interstitial cell of Leydig (ICL) has characteristic long, tortuous microvilli projecting from its plasma membrane. Its nucleus appears euchromatic and has a thin layer of heterochromatin along its nuclear envelop. Notice the presence of the basal lamina (BL), the spermatogonia B (SB) and the Sertoli cell (Se). (X 5000)

(Fig. 5b): Electron micrograph of the testis of atorvastatin treated group shows that the interstitial tissues contain numerous collagen fibers (C) and the nucleus of interstitial cell of Leydig (ICL) has large masses of heterochromatin. The basal lamina (BL) has...
thick and irregular basement membrane (BM) and numerous collagen fibers (C) between the basement membrane, myoid (Mc) and fibroblast (Fc) cells. Notice that the Sertoli cell (Se), the primary spermatocytes (PS) and the spermatogonia A (SA) are slightly affected. (X 5000)

(Fig. 5c): Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the interstitial cell of Leydig (ICL) still has heterochromatic nucleus. Notice that the basal lamina (BL), the Sertoli cell (Se) and the spermatogonia B (SB) have normal appearance. (X 5000)

(Fig. 6a): Electron micrograph of the testis of the control and vitamin E treated group showsthe basal lamina (BL) which consists of thin and regular basement membrane (BM), myoid cell (Mc) and collagen fibers (C) in the gap between the basement membrane and myoid cell. The spermatogonia A (SA) has large oval euchromatic nucleus (N). Its cytoplasm contains large oval mitochondria (M). Notice the presence of part of the spermatogonia B (SB) and the primary spermatocyte (PS). (X 10000)

(Fig. 6b): Two electron micrographs of the testis of atorvastatin treated group show that the basement membrane (BM) of the basal lamina (BL) appears thick and irregular, the gaps between the basement membrane, the myoid (Mc) and fibroblast (Fc) cells contain numerous collagen fibers (C). The spermatogonia A (SA) have normal euchromatic (N) and its cytoplasm contains mitochondria with destructed cristae (M). The spermatogonia B (SB) have nearly normal heterochromatic nucleus (N) and numerous intra cytoplasmic vacuoles (V). Notice the presence of parts of two adjacent Sertoli cells (Se) which have normal euchromatic nucleus (N) and large intracytoplasmic vacuoles (V). (X 10000)

(Fig. 6c): Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the basement membrane (BM) of the basal lamina (BL) becomes nearly regular and thin. Also the collagen fibers (C) nearly appear normal. The spermatogonia A (SA) has normal euchromatic nucleus (N) and its cytoplasm still contains few mitochondria with destructed cristae (M). Notice the presence of part of the primary spermatocyte (PS) which has normal mitochondria (M). (X 10000)
(Fig. 7a): Electron micrograph of the testis of the control and vitamin E treated group shows that the spermatogonia B (SB) have large nearly rounded nuclei with characteristic large masses of heterochromatin which present mainly along the nuclear envelopes and have eccentric nucleolus (Nu). Their cytoplasm contain small oval and rounded mitochondria (M) and free ribosomes (R). The basal lamina (BL) consists of basement membrane (BM), myoid cell (Mc) and 1-3 layers of fibroblast cells (Fe). The basement membrane is separated from the myoid cells by a gap containing collagen fibers (C). (X 10000)

(Fig. 7b): Two electron micrographs of the testis of atorvastatin treated group shows that the spermatogonia B (SB) have large numerous intracytoplasmic vacuoles (V). The spermatogonia B and A (SA) have mitochondria with destructed cristae (M). The basal lamina (BL) has irregular basement membrane (BM) and numerous collagen fibers (C) in the gap between the basement membrane and myoid cell (Mc) (X 10000)

(Fig. 7c): Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the spermatogonia B (SB) still have small intracytoplasmic vacuoles (V). The basement membrane (BM) of the basal lamina (BL) appears thin and nearly regular. Notice the presence of part of the primary spermatocyte (PS) which has normal appearance. (X 10000)
**Fig. 8a:** Electron micrograph of the testis of the control and vitamin E treated group shows that the Sertoli cell (Se) has characteristically elongated and indented euchromatic nucleus (N) with very thin layer of heterochromatin along the nuclear envelop and has rounded nucleolus (Nu). The cytoplasm contains small elongated mitochondria (M). Notice the presence of the basal lamia (BL) which consists of thin and regular basement membrane (BM), myoid cell (Mc) and collagen fibers (C) in the gap between the basement membrane and the myoid cell. (X 10000)

**Fig. 8b:** Two electron micrographs of the testis of atorvastatin treated group shows parts of the Sertoli cells (Se) which have intra cytoplasmic vacuoles (V), dilated rough endoplasmic reticulum (RE), mitochondria with destructed cristae (M) and numerous fat droplets (Fd). It also shows the basal lamina (BL) which has thick and irregular basement membrane (BM) and numerous collagen fibers (C) in the gap between the basement membrane, the myoid (Mc) and the fibroblast (Fc) cells. (X 10000)

**Fig. 8c:** Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the Sertoli cell (Se) has normal nucleus (N) and small elongated mitochondria (M). The spermatogonia B (SB) have normal appearance. The basal lamina (BL) has thin and nearly regular basement membrane (BM) and few collagen fibers (C) (X10000)

**Fig. 9a:** Electron micrograph of the testis of the control and vitamin E treated group shows that the primary spermatocyte have large rounded nucleus (N) with scattered large masses of heterochromatin. It has a rim of cytoplasm which studded with small rounded mitochondria (M). Notice the presence of head of sperms (HS). (X 10000)

**Fig. 9b:** Two electron micrographs of the testis of atorvastatin treated group shows that the cytoplasm of the primary spermatocytes (PS) contain numerous vacuoles (V), swollen mitochondria with destructed cristae (M) and dilated rough endoplasmic reticulum (RE). Also one primary spermatocyte has fading of nuclear chromatin (head arrow) and the other one has irregular and shrunken nucleus (N) with large masses of heterochromatin (→). (X 10000)

**Fig. 9c:** Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the primary spermatocyte (PS) has normal heterochromatic nucleus (N) with eccentric nucleolus (Nu) and its cytoplasm contains numerous small rounded mitochondria (M) with normal appearance. Notice the presence of part of spermatid (S) with its characteristic peripherally arranged mitochondria with clear matrix (M). (X 10000)
(Fig. 10a): Electron micrograph of the testis of the control and vitamin E treated group shows that the early spermatids (ES) have nearly rounded euchromatic nuclei (N). Its cytoplasm characterized by the presence of peripherally arranged small mitochondria with clear matrix (M). (X 10000)

(Fig. 10b): Two electron micrographs of the testis of atorvastatin treated group shows that the early spermatids (ES) have intra cytoplasmic vacuoles (V) and dilated rough endoplasmic reticulum (RE). One spermatid has irregular and shrunken nuclei with condensed masses of heterochromatin (N). The nucleus of the other one has normal appearance. Notice the presence of intercellular spaces (star). (X 10000)

(Fig. 10c): Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the early (ES) and late spermatids (LS) still have few cytoplasmic vacuoles (V). Also their nuclei (N) appear normal. (X 10000)

(Fig. 11a): Electron micrograph of the testis of the control and vitamin E treated group shows that the late spermatids (LS) have euchromatic nuclei (N) which are covered by acrosomal
vesicles (AV). The cytoplasm characterized by presence of peripherally arranged small mitochondria with clear matrix (M).\textit{(X 10000)}

(Fig. 11b): Two electron micrographs of the testis of atorvastatin treated group shows that the late spermatids (LS) have vacuolated cytoplasm (V). One spermatid has nucleus with fading of its chromatin (head arrow). The other one has normal nucleus (N). \textit{(X 10000)}

(Fig. 11c): Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the late spermatid (LS) has normal euchromatic nucleus (N) and its cytoplasm characterized by presence of peripherally arranged small mitochondria with clear matrix (M).\textit{(X 10000)}

(Fig. 12a): Electron micrograph of the testis of the control and vitamin E treated groups shows different parts of spermatozoa tail. The middle pieces (Mp) have central axonemes which are surrounded by nine coarse fibers. These fibers are surrounded by zone of lightly packed, elongated mitochondria (M) which surrounded externally by plasma lemma (PL). The principle piece (Pp) has central axoneme which is surrounded by nine coarse fibers. It has anterior and posterior columns and plasma lemma (PL). The end pieces (Ep) have central axonemes which are surrounded by nine fine longitudinal fibers and the plasma lemma (PL). \textit{(X 30000)}

(Fig. 12b): Two electron micrographs of the testis of atorvastatin treated group shows that the middle pieces (Mp) of spermatozoa tail have ill-defined plasma lemma and disorganized mitochondrial sheath (M). Also the principle piece (Pp) has ill-defined plasma lemma (PL). The end pieces (Ep) are ill-defined. \textit{(X 30000)}

(Fig. 12c): Electron micrograph of the testis of atorvastatin and vitamin E treated group shows that the principle (Pp) and the end (Ep) pieces of the sperm tail have normal appearance. While the middle piece (Mp) still has ill-defined basal lamina (PL). \textit{(X 30000)}

Table 1: It shows the means and standard deviation of the epithelial height of the seminiferous tubules. It shows one way ANOVA statistical analysis between Groups C (C1 and C2), E, S and SE. Post Hoc analysis using LSD test was applied to investigate the significance between the individual groups (P<0.001).

<table>
<thead>
<tr>
<th>Variables</th>
<th>C1 vs C2</th>
<th>C1 vs E</th>
<th>C1 vs S</th>
<th>C1 vs SE</th>
<th>C2 vs S</th>
<th>C2 vs SE</th>
<th>S vs E</th>
<th>SE vs E</th>
<th>S vs SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value</td>
<td>0.889</td>
<td>0.994</td>
<td>0.000</td>
<td>0.390</td>
<td>0.083</td>
<td>0.000</td>
<td>0.318</td>
<td>0.000</td>
<td>0.394</td>
</tr>
</tbody>
</table>
Group C1: Albino rat testes of control group1.
Group C2: Albino rat testes of control group2
Group E: Albino rat testes of vitamin E treated group.
Group S: Albino rat testes of atorvastatin treated group.
Group SE: Albino rat testes of atorvastatin and vitamin E treated group.

**DISCUSSION**

In the present work oral administration of atorvastatin for adult male albino rats for 4 weeks (Group S) lead to various deleterious changes in their testes. There was congestion and vasodilatation in the blood vessels of the testes and wide interstitial spaces with eosinophilic material indicating oedema. This finding is in line with Fraunfelder. (2004) who reported that ocular hemorrhage has been linked to statins use. Also, Amarenco et al. (2006) stated that 80mg of atorvastatin per day was associated with increased rates of haemorrhagic stroke in a large randomized controlled trial of patients with previous stroke or transient ischemic attack. Also, Dinoop et al. (2013) stated that atorvastatin failed to provide a protection against vancomycin induced renal damage. Renal histopathology revealed tubular oedema and degeneration, glomerular congestion and nephritis. In addition, Parker et al. (2003) stated that statin had vasorelaxation effect. Moreover, Liberopoulos and Mikhailidis. (2006) reported that headaches were the commonest side effects associated with the use of statins. The possible cause of the congestion and vasodilatation effects of atorvastatin can be explained by Undas et al. (2014) who reported that statin therapy might affect several steps of the blood coagulation cascade, including decreased thrombin generation and therefore, decreased fibrinogen cleavage and platelet activation. Also, Bax et al. (2003) stated that statin had been shown to attenuate vasoconstriction by increasing endothelial nitric oxide (NO) activity.

Masson's trichrome stain showed increase in the collagen fibers deposition in the testicular capsule, the wall of the blood vessels and the basal lamina. Also, electron microscopic examination showed increase in the collagen fibers in the interstitial tissues and the basal lamina. These data indicating that atorvastatin has inflammatory and fibrotic effects. This finding is supported by Schaefer et al. (2004) who reported that the rats which received mild and high dose of cerivastatin (0.5 and 1(mg/kg)/day had necrosis and inflammation in skeletal muscles. Also, Lancut et al. (2004) stated that atorvastatin administration in maximal dosage (80mg/kg) produced fibrinosis within the intercellular spaces of the muscles of albino rat under electron microscope examination. Additionally, Westwood et al. (2005) reported that simvastatin at maximum tolerated dose of 80mg/kg/day induced inflammatory cell infiltration in the skeletal muscles of the rat. The possible cause of inflammation and fibrosis that induced by atorvastatin can be explained by Stevens and Lowe. (2000) who stated that the degenerated muscle fibers which induced by atorvastatin release.

Graph (1): Bar chart representing the means of the epithelial height of the seminiferous tubules of the studied groups.
different inflammatory mediators that lead to mononuclear cellular infiltration. Also, Pal et al. (2015) reported that administration of atorvastatin altered the pro oxidant-antioxidant status of the liver by reducing intercellular glutathione level, antioxidant enzymes activities and increasing intracellular lipid peroxidation. The lipid peroxidation induced oxidative damage to proteins and nucleic acids leading to increased collagen formation.

On the other hand this finding is contrary to Mizugushi et al. (2004) who stated that atorvastatin 20 mg significantly decreased tissue transforming growth factor-beta, resulting in a decrease in renal tubular damage and interstitial fibrosis. Also, Barsante et al. (2005) said that oral treatment with atorvastatin 1-10 mg/kg from days 10 to 15 after arthritis induction ameliorated the histopathological findings of joints. This was mirrored by an effective blockade of neutrophil influx.

In addition, Xue-mei et al. (2008) reported that simvastatin 5-20 mg/kg attenuated bleomycin-induced pulmonary fibrosis, as indicated by decreased lung collagen accumulation. Moreover, Zhao et al. (2010) reported that statins had cardioprotective effects that were associated with their anti-fibrotic effects in mice.

Moreover, in the present work atorvastatin induced variable degrees of degeneration and necrosis in the seminiferous cords and interstitial cells of Leydig. The degeneration and necrosis were in the form of cytoplasmatic vacuoles, dilated rough endoplasmic reticulum, swollen mitochondria with destructed cristae, and nuclear changes in the form of karyolysis (fading of the basophilia of the nuclear chromatin), pyknosis (shrunken nuclei with presence of masses of heterochromatin more than normal) and karyorrhexis (nuclear fragmentation). Also, there were decrease in the epithelial height, the number of sperms and glycogen contents in the testis. These findings coincide with Nakahara et al. (1998) who said that when simvastatin or pravastatin were administered to the rabbits revealed different pathological changes; muscle fiber necrosis and degeneration, autophagic vacuoles, mitochondrial swelling and disruption and hypercontraction of the myofibrils. Also, Zarabska et al. (2003) stated that the high dose of atorvastatin 2.8 mg/24 hours induced degenerative changes in many cells of the suprarenal gland. In addition, Lancut et al. (2004) reported that six-week administration of atorvastatin (80 mg/kg) for albino rats produced invagination in the nuclear envelope and numerous cytoplasmic vacuoles. Maximal dosage of atorvastatin (800 mg/kg) revealed numerous vacuoles in the perinuclear spaces, and between myofibrils and swelling of the mitochondria. Moreover, Westwood et al. (2005) stated that simvastatin at maximum tolerated dose of 80 mg/kg/day induced muscle necrosis in the rat.

Also, these findings are in line with Ozek et al. (2009) stated that simvastatin treatment induced a significant decrease in lipids, nucleic acid, protein and glycogen content. Also, Pons-Rejraji et al. (2014) observed that a 5-month atorvastatin intake (10 mg/d) induced deleterious effects on testicular, prostatic and epididymal functions in human. It also decreased the total number of spermatozoa in ejaculate and sperm vitality and increased morphological abnormalities.

The possible causes of the previous deleterious effects of atorvastatin on the histological structure of the testes of the adult male albino rat (GroupS) can be explained by Westwood et al. (2005) and Otruba et al. (2011) who stated that inhibition of 3-hydroxy-3-methyl glutaryl coenzyme A pathway deprived the cell from important antioxidant molecules such as ubiquinone or coenzyme Q10. Also, Sirvent et al. (2008) stated that statins had a direct effect on the respiratory chain of the mitochondria. It is proposed that mitochondrial impairment lead to a mitochondrial calcium leak. Both mitochondrial and calcium impairments may account for apoptosis process, oxidative stress, and muscle remodeling and degeneration. In addition, Carvalho et al. (2004) reported that statins inhibit the HMG-CoA reductase which result in low intracellular cholesterol levels. Low intracellular cholesterol levels modulate fluidity of cell membranes, that in turn altered the Na/K pump function and lead to degeneration of the membranous organelles of muscle fibers. In addition, Parker et al. (2003) stated that statins induced upregulation of endothelial nitric oxide synthase (e-NOS). However, changing the balance between localized NO and O(2-) fluxes could lead to oxidant stress and cellular injury through the formation of reactive secondary oxidants such as peroxynitrite and increasing the potential for damage to muscles and other tissues. Moreover, Das and Indira (2015) thought that the testicular alterations observed in
Atorvastatin treated rats might be due to reduced testosterone levels since testosterone is needed for the normal morphology of the testis.

In the present work concomitant administration of atorvastatin and vitamin E (Group SE) revealed that vitamin E ameliorated the deleterious effects of atorvastatin on the testes (Group S). Where most of the seminiferous tubules nearly retained their normal architecture. Also, morphometric study showed no significant difference in means of epithelial height between groups SE, C1, C2, and E (P > 0.001). Moreover, the testicular capsule, the blood vessels and the collagen fibers became nearly similar to the groups C and E. This finding is in agreement with Momeni et al. (2012) who reported that vitamin E ameliorated the adverse effects of sodium arsenite on epididymal sperm number and some morphometrical parameters of the adult rat testis. Also, Oyyemini et al. (2015) stated that vitamin E improved the reduction in sperm characteristics, hormone levels and testicular alterations observed in nicotine treated rats. The possible cause of protective effect of vitamin E on the testicular structure of atorvastatin treated rats was explained by Al Damegh. (2012) who stated that vitamin E had been shown to suppress lipid peroxidation in testicular microsomes and mitochondria and to reverse the detrimental effects of oxidative stress on testicular function.

CONCLUSION

It could be concluded that atorvastatin 80 mg/day induced various deleterious changes in the histological structure of the testes of adult male albino rat. The concomitant administration of vitamin E with atorvastatin alleviated the deleterious changes.

RECOMMENDATIONS

1- We found that vitamin E alleviated the deleterious effects of atorvastatin on the testes of the adult male albino rats so the vitamin E is a useful agent in the patients who are in need to use the maximum therapeutic dose of atorvastatin.

2- We found that atorvastatin (80 mg/day; the maximum therapeutic dose in human) induced fibrosis in the testes of adult male albino rat. This finding was in agreement with many authors who used the same or higher doses. On the other hand, this finding was contrary to many authors who used lower doses. So from this view, we recommend many researches about the fibrotic effect of atorvastatin by using different doses.

REFERENCES


Bax, L.; Mali, W. P. and Busken, E. (2003): The benefit of stent placement and pressure and lipid lowering for the prevention of renal dysfunction caused by atherosclerosis ostial stenosis of the renal


الملخص العربي

الخلفية: أثار الفاسفاتين هو الخط الأول في خفض الدهون لعلاج المرضى الذين يعانون من أمراض القلب الناجمة. تتكون الاستبان في الحيوانات، يقلل من خصائص الأحماض الدهنية حيث أن الأورافاساتين يؤدي إلى زيادة إنتاج الأكيسيين التفاعلية الضارة. فيتامين هد٠ يلعب دوراً هاماً في الحد من الإجهاد التكيذي.

إن الهدف من هذا البحث هو التحقق من وجود أثار سلبية لأثار الفاسفاتين (80 مجم /جمرة العلاج العلاجية القصوى للإنسان) على الخصائص لذكور الفئران البضائع والأنثى الوقاني الممكن كيف يتم. استخدم في هذا العمل خمسمائتيار من ذكور الفئران البضائع البالغين، وقسمتهم إلى 4 مجموعات: المجموعة (المجموعة C1) وتم اعطاء نصفهم 0,72 مل من الماء المقطر (المجموعة C2) و مجموعة فيتامين ه المعنوي (المجموعة C3) ونصفهم الآخر 0,09 مل من زيت النتر (المجموعة C1) و مجموعة الأروفاساتين المعالجة (المجموعة C4) و مجموعة الأروفاساتين غير المعالجة (المجموعة C1) و تم اعطاءهم 0,72 مل من الماء المقطر محتوي على 1,44 مجم ومجموعة الأروفاساتين و فيتامين SE) للمعالجة (المجموعة C1) و مجموعة الأروفاساتين غير المعالجة (المجموعة C1) اعطاءهم نفس جرعة مجموعة الأروفاساتين و فيتامين ه. تم إعطاء العلاجات كجزء يومية واحدة بالدمية عند 4 أسابيع. تم استخراج الخصائص في نهاية الأسبوع الرابع. و تم إعادة الدراسة بواطعة المجهر الضوئي، والمجهر الإلكتروني والدراسة المورفومترية. أظهرت الدراسة بواسطة المجهر الضوئي، والمجهر الإلكتروني أن الأروفاساتين أحدث أثاراً سلبياً مختلفة على الهيكل النسيجي للخصائص لذكور الفئران البضائع البالغين. وكانت هذه الأثار في شكل احتقان وتسع في الأوعية الدموية و ارتفاع الخلايا. كما كانت هناك زيادة في أليف الكولاجن. بالإضافة إلى ذلك كانت هناك دوراً مغيرة من انخفاض ونخر الخلايا المنوية والخلايا الخالية من نوع لينين كان الاكتشافات تتوافق مع نتائج المقدمة والمقدمة المكددة. وتتقبل النتائج المقدمة ومقدمة المكددة من الإجابة المؤيدة للخلايا المنوية في الحيوانات، وهي تشير إلى اختلافات في النواة مثل تفاعل النواة الأورافاساتين الدوائي وتغطية النواة الأخرى. وت認め أن النتائج في خصائص النوى المعتدلة للنواة أيضاً كان هناك خلافات في نمو الخلايا المنوية وحيويتيات الجلوكوجين. أظهرت الدراسة المورفومترية عن وجود فرق معنوي بين المونوات الحساسية لارتفاع الخلايا المنوية للمجموعة C2 المضادة الكيميائية ونظام الفاسفاتين في هذه البحث يؤدي إلى تخفيف العديد من الأثار السرية للأروفاساتين على الخصائص حيث أن ذلك عن الدراسات بواسطة المجهر الضوئي والمجهر الإلكتروني. وأيضاً أظهرت الدراسة المورفومترية عن وجود فرق معنوي بين المونوات الحساسية لارتفاع الخلايا المنوية للمجموعة C2 C1، SE) بالمجموعات C2، C1، SE. 

عند درجة معنوية أكبر من 0,001 E