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Histopathological, Ultrastructural and Morphometric Studies on the Effect of Atorvastatin on Rat Kidney.

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ABSTRACT

Atorvastatin reduces the levels of "bad" cholesterol (LDL) and triglycerides in the blood, while increasing levels of "good" cholesterol (HDL). Accordingly, the aim of the present study was to determine the potential toxicity of the therapeutic doses of atorvastatin in male albino rats. A total of fifteen adult male albino rats were divided randomly into three groups.

The control group (group 1) did not receive any medication, while group II received atorvastatin (10 mg/kg/day for four weeks). Group III received atorvastatin (10 mg/kg/day for four weeks) then left for one month after the last dose for recovery from the drug. Kidney biopsies were taken from each rat for histopathological and ultrastructural examinations. In comparison with respective control rats, the results showed slight effects of atorvastatin on the kidney of rats, which was in the form of vacuolization, degeneration and cloudy swelling of epithelial cells in the tubules with the formation of cell debris inside the lumen of proximal convoluted tubules and diminish in urinary space of glomeruli.

These effects were partially irreversible in recovery group. Accordingly, it is highly recommended that patients who suffer from hypercholesterolemia may be advised to feed on hypocholestrolaemic food regime before prescribing statins. If patient need to prescribe a hypocholestrolaemic drugs, statin should be taken in short repeated duration with at least one month between each period to avoid side effects and make a follow up every month for laboratory measurement of kidney functions.

INTRODUCTION

Atorvastatin marketed under the trade name "Lipitor" among others, is a o99member of the drug class known as statins. Like all statins, atorvastatin works by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. It reduces the levels of "bad" cholesterol LDL and

triglycerides in the blood, while increasing levels of "good" cholesterol HDL (Mckenney, 2003).

Low plasma cholesterol levels are usually associated with reduced intracellular cholesterol and may result in decreased membrane lipid content. This, in turn, may cause physical alteration of membrane fluidity and reduce cell proliferation (Evans and Rees, 2002).

Atorvastatin is a synthetic statin characterized by a high efficacy, in part due to its longer half-life compared to other molecules of the same group (Malhotra and Goa, 2001). Compared with other medications in its class, atorvastatin provides superior efficacy, probably due to prolonged inhibition of HMG-CoA reductase (Farag *et al*., 2015).

In man, the dose of atorvastatin needed to decrease low density lipoprotein cholesterol by 30% is 10 mg/day (0.15 mg/kg/day) and the maximum dose used in therapy is 1mg/kg/day for four weeks (Lea and McTavish 1997; Farag *et al*., 2015). Due to the limitation of published works on the histological effects of atorvastatin on the mammalian kidneys, the present study was designed to give an insight on its effects, as well as on the possibility of recovery after the discontinuation of their administration to experimental animals.

MATERIALS AND METHODS

Drug dosing:

Atorvastatin (Lipitor®10mg) tablets were obtained from Pfizer Company for Pharmaceutical and Chemical Industries. The drug was dissolved in distilled water and given orally by a gastric tube. The daily single oral dose was 10mg/kg/day. This is in accordance with previous studies showing that atorvastatin delivered to normal chow-fed rats did not affect plasma cholesterol and lowered triglycerides only at 25mg/day. The maximal licensed dose of atorvastatin in patients (80mg/day~ 1mg/kg/day) achieves maximal plasma levels of 0.3–0.5 μM in humans, but only 0.03 μM in rats (Stern *et al*., 2000). Thus, the high dose atorvastatin used in our study (10 mg/kg/day) can be expected to achieve 0.3 μM, corresponding to the normal high dose regiment in humans (Schmechel *et al*., 2009).

Animals:

Fifteen adult male albino rats with average weight of 150-200gm were chosen to be the model of the present study. Rats were randomly divided into three equal groups and fed on ordinary rat diet. Rats were left for two weeks in the laboratory room before any experimental interference for acclimatization. Food and water access were available *ad libituim.*

Experimental design:

Group I: Rats received no medication and served as control group.
Group II: Ratssubjected to treatments with atorvastatin in a dose of 10mg/kg/day for four weeks.
Group III: Rats subjected to treatments with atorvastatin in a dose of 10mg/kg/day for four weeks then; they were kept for one month for recovery.

Methodology

Laboratory study:

At the end of the experimental period, rats were sacrificed by severing their neck blood vessels. Kidneys of sacrificed rats were dissected out and subjected to histological and ultrastructural procedures.

Histological study:

Light microscopic examination: Kidney samples from the three groups were cut into smaller pieces and fixed in 10% neutral buffered formalin over night at a temperature of 4°C. Tissue samples were dehydrated in ascending grades of alcohols, cleared in xylol and embedded in wax. Tissue sections of 5μm thickness were stained with: hematoxylin and eosin stain (Atwood *et al.,* 2003).Examined using a light microscope (Olympus VANOX) in Military Central Labs.

Electron microscopic examination: for electron microscopic studies, small pieces $1mm^3$ of kidneys from the three groups were fixed with 3% phosphate buffered glutaraldehyde for 2 hours as a primary fixative, postfixed in 1% osmium tetroxide for one hour, tissue samples were dehydrated in ascending grades of alcohols. Embedding was done in Spurr's resin started by passing the specimens in propylene oxide-resin mixture at ratio of 3:1 (mixd well) for 1 hour.The tissues were left in propylene oxide-resin mixture at ratio of 1:1 for 1 hour, then in propylene oxide-resin mixture at ratio of 1:3 for overnight.Then, the specimens were left in fresh pure resin at room temperature for 1 hour.Specimens were transferred to gelatin capsules containing fresh resin and left in the oven at 60°C for 24 hours for polymerization. Ultrathin sections (80-90 nm) were prepared using **(**SEO ultramicrotome), and then mounted on copper grids (Hunter, 1993). Sections were stained with uranyl-acetate and lead-citrate then examined under SEO Transmission Electron Microscope in Military Central Labs**.**

Morphometric and Statistical analysis:

Morphometric analysis was carried out on three groups of rats, each group had five animals. Six reading of the urinary space of Bowman's capsule areas were taken without bias from each animal. Measurements was done on hematoxylin and eosin-stained paraffin sections using Motic Image 2000 software (version 1.3) analysis system at ×400 magnification that were taken using the light microscope model "Olympus VANOX with digital camera" at the Military Central Labs. The data was expressed as mean± SD of the urinary space of Bowman's capsule areas. These data was used to elucidate the difference between the measured values in the control and treated groups. Significance was defined as $P<0.05$ (Field, 2000). Statistical analysis of data was carried out using 'SPSS (Version 23) incorporated within the Microsoft Excel 2010 (Microsoft ® Windows 2010) software program.

RESULTS

Group I (Control Group):

Light microscopic results: Light microscopic examinations revealed normal architecture of rat kidney of control group. The renal cortex comprised of proximal convoluted tubules, distal convoluted tubules, and renal glomeruli (Fig. 1).

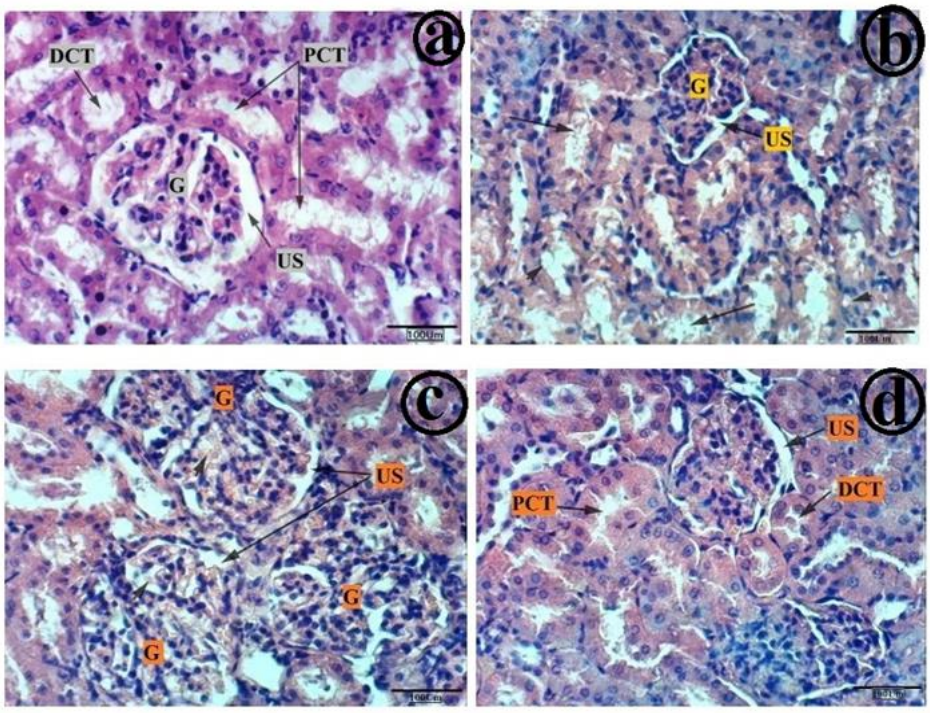

Fig. 1: Photomicrograph of renal cortex showing: a) control group showing; normal structure with normal proximal convoluted tubules (PCT), distal convoluted tubules (DCT), renal corpuscle enclosing a tuft of blood capillaries (G), and the urinary space (US). b) treated group showing vacuolization of the epithelium lining the proximal convoluted renal tubules (arrow). Few cell debris are located in the lumen of the tubules (thick arrow). Shrinked glomerulus (G) with diminished urinary space (US) is also seen. c) treated group showing; congested capillaries (arrows) and hypercellularity of the hypertrophied glomeruli (G), as well as diminishing of the urinary space (US). d) recovery group showing normal histological structure, of glomeruli (G), slightly shrinked urinary space (US) and normal proximal (PCT) and distal (DCT) convoluted tubules.

Ultrastructural results

Glomeruli: Electron microscopic examination of the glomerulus revealed normal structure with normal glomerular basement membrane (filtration barrier), and podocytes with podocyte feet. Mesangial cells are located between the capillaries which were rich in red blood cells (Fig. 2). Normal filtration barriers comprising endothelium with open fenestrae was underlined by fused basement membrane, and processes of podocytes (Fig. 2).

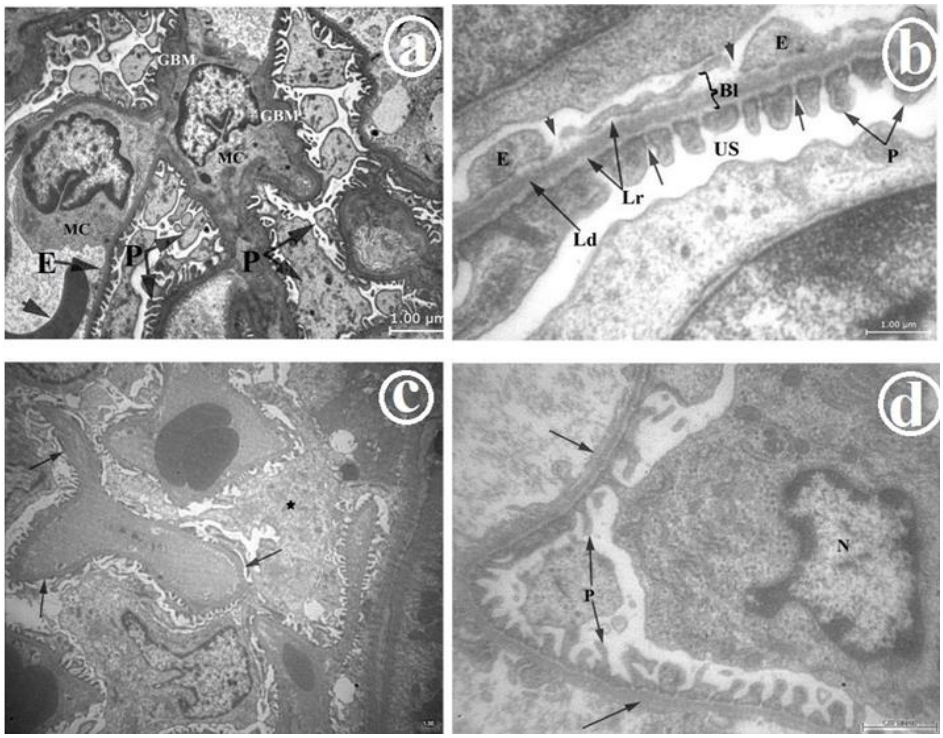

Fig. 2: Electron micrograph of part of glomerulus showing: a) control group showing; normal glomerulus basement membrane (GBM), several podocytes with feet processes (P), mesangial cells (MC), endothelial cells (E) and erythrocyte (arrow head) inside the capillary. b) control group showing; normal filtration barrier with endothelium (E), open fenestrae (arrowhead), fused basal laminae (BL) of endothelial cells, and the processes of podocytes (P). The basement membrane consists of a central lamina densa (Ld) bounded on both sides by a light-staining laminae rara (Lr). Arrows indicate the thin diaphragms crossing the filtration slits. c) Treatment group showing; focal fusion of podocyte (⬟), effacement of secondary podocytes processes in few places (arrows), and mesangium cell with large indented nucleus (N). d) Recovery group showing; normal filtration membrane (arrows) with normal podocyte processes (P). (Bar = 1.00 Um).

Proximal convoluted tubules: Electron microscopic examination of the cells of the proximal convoluted tubule revealed simple-cubical or low-columnar epithelium. This epithelium possesses a conspicuous brush border on its luminal surface composed of closely packed microvilli. Each cell of the proximal tubules contained a large oval and centrally located nucleus. Mitochondria are elongated in shape with outer membrane and multiple lamellae, occupied most of the basal and lateral portions of the cytoplasm, and were parallel to the long axis of the cell. The basal membrane of these cells were highly enfolded (Fig. 3).

Distal convoluted tubules: The cells of the distal convoluted tubules in electron microscopic preparations possessed cuboidal epithelial cells with densely packed long mitochondria oriented vertically among the numerous deep infoldings of the basolateral plasma membrane. The nucleus was rounded in shape and euchromatic in structure. There were moderately abundant populations of apical cytoplasmic microvilli. Vacuoles of endocytic apparatus at the apical region of the cells were also detected (Fig. 4).

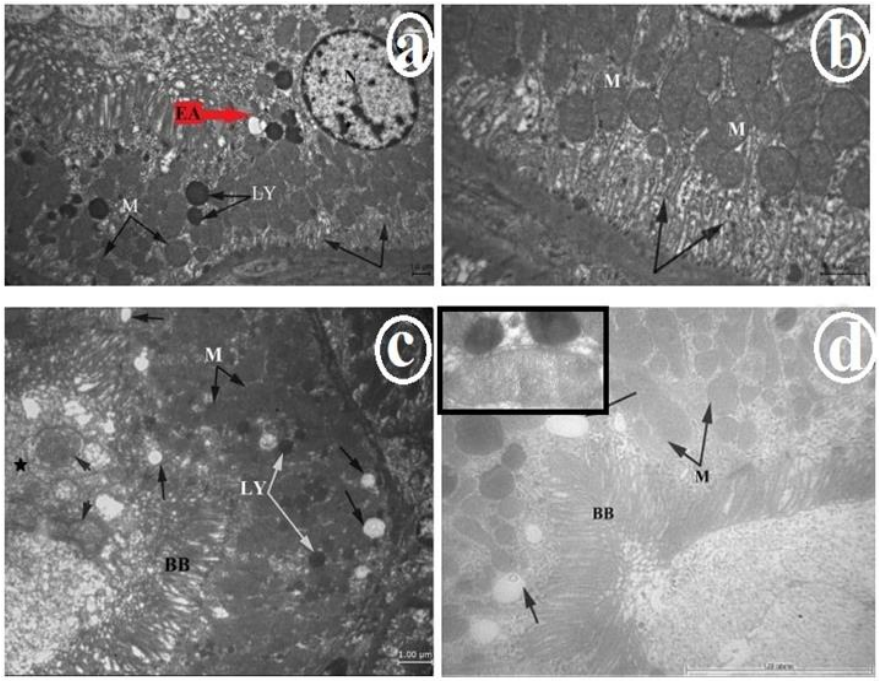

Fig. 3: Electron micrograph of part of proximal convoluted tubule showing: a) control group showing; normal proximal tubules with endocytotic apparatus (EA) fused with lysosomes (LY). b) higher magnification from figure a showing; Normal mitochondria (M) with outer membrane and multiple lamellae and concentrated at the base of the cell and are arranged parallel to the long axis of the cell. The basal membrane of these cells is highly enfolded (arrows).C) treatment group showing some vacuoles (arrows), brush border (BB), some lysosomes (LY), many mitochondria (M), exfoliation (arrow heads), and cell debris ( ) are also seen inside the lumen. d)recovery group showing; some vacuoles (arrows), and well developed brush border (BB), and normal mitochondria (M) with intact outer membranes and multiple lamellar cristae. **(Bar = 1.00 Um).**

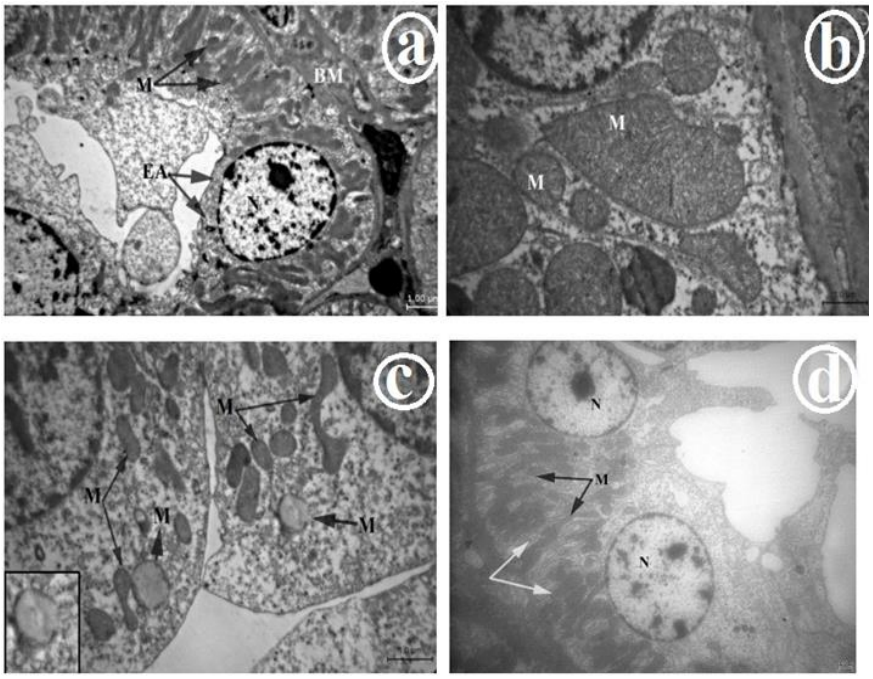

Fig. 4: Electron micrograph of part of distal convoluted tubule showing: a) control group showing; moderately abundant apical cytoplasmic microvilli (arrows), basolateral membranes (Bm) forming numerous infoldings. Vacuoles of endocytic apparatus appear at the apical region of the cells (EA). The nucleus (N) is rounded in shape and euchromatic in structure, and contains one nucleolus. b) control group showing; normal mitochondria (M) with their outer double membrane and multiple lamellas. c) treated group showing: distal convoluted tubule lacking microvilli. Degenerated mitochondria (M) in the form of irregular and opaque with short cristae are also seen. d) recovery group showing: showing apical cytoplasmic projections (arrow), amplification of the basolateral membranes (white arrow), mitochondria (M), the nuclei (N) and nucleoli. **(Bar = 1.00 Um).**

Collecting tubules: Electron microscopic examination of the cells of collecting tubules revealed the presence of the two characteristic types of epithelial cells; the principal cells and intercalated cells. Principal cells were relatively low cuboidal cells. They had many regular basolateral plasma membrane enfoldings, which contain few organelles, and a lightly stained cytoplasm, which is responsible for the original designation of principal cells in microscopic studies as light cells. Intercalated cells contained many mitochondria and a more darkly stained cytoplasm, as they are designated as dark cells (Fig. 5).

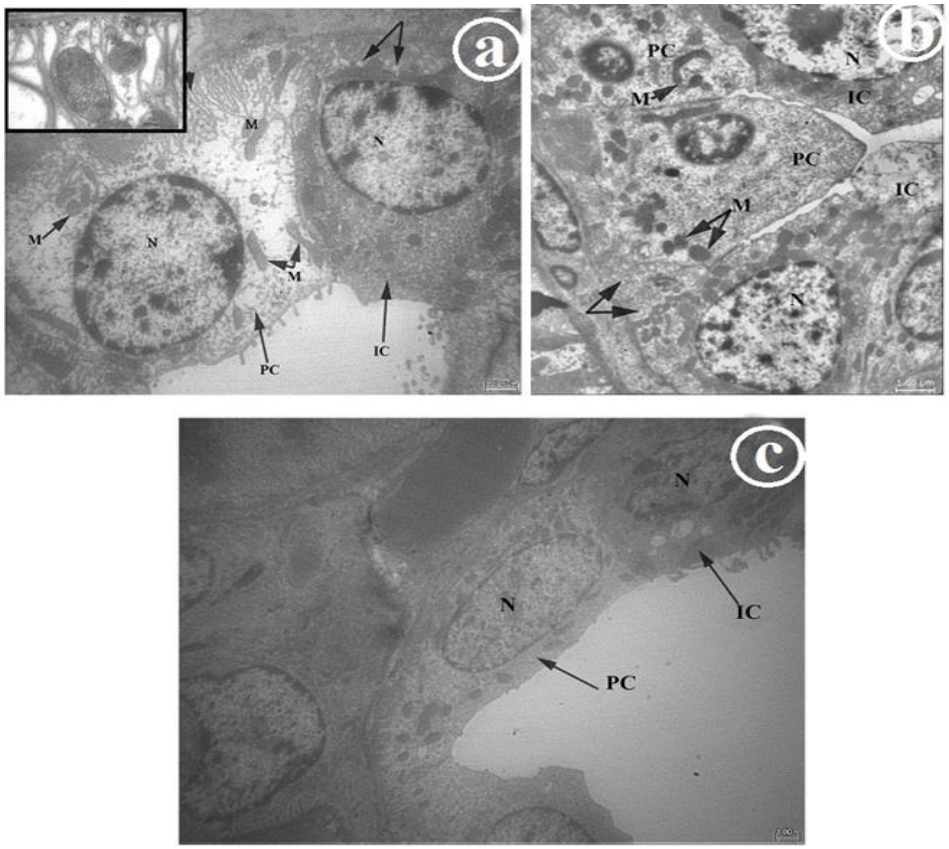

Fig. 5: Electron micrograph of part of convoluted tubule showing: a) control group showing; principal cells (PC), with lightly staining cytoplasm, euchromatic nucleus (N), and basal membrane containing some tortuous infoldings (arrows). Intercalated cells (IC) with ovoid and basal located nuclei (N). Mitochondria (M) are small irregular and randomly oriented, and the basal membrane is slightly enfolded.b) treated group showing; principal cells (PC), with lightly staining cytoplasm, euchromatic nucleus (N), and basal membrane containing some tortuous infoldings (arrows). Intercalated cells (IC) with ovoid and basal located nuclei (N). Mitochondria (M) are small irregular and randomly oriented, and the basal membrane is slightly enfolded. c) recovery group showing; normal ultrastructure of principal cell (PC) and intercalated cell (IC). **(Bar = 1.00 Um).**

Group II (atorvastatin-treated group for four weeks):

Light microscopic results: Light microscopic examination of group II revealed renal cortex with vacuolization of the epithelium lining the proximal convoluted tubules and few cell debris inside their lumen. Shrinked glomeruli with diminished urinary space (Fig. 1), as well as hypertrophy of glomerular tufts with hypercellularity and congestion were also seen (Fig. 1).

Ultrastructural results:

Glomerulus: Electron microscopic examination of the glomeruli of group II showed focal fusion of podocytes and effacement of secondary processes of podocytes in few places were also seen (Fig. 2).

Proximal convoluted tubule: Electron microscopic examination of the cells of the proximal convoluted tubule of group II revealed brush border, the presence of many vacuoles, a number of lysosomes and normal as well as degenerated mitochondria. The lumen of these tubules contained cell debris (Fig. 3).

Distal convoluted tubule: Electron microscopic examination of the cells of the distal convoluted tubule from the kidney of group II, revealed the absence of microvilli, as well as the presence of irregular opaque mitochondria, and degenerated cytoplasmic areas (Fig. 4).

Collecting tubule: Electron microscopic examination of the cells of the collecting tubules from the kidney of group II displayed intercalated cells with ovoid heterochromatic and basal located nuclei. The luminal cell surface lost their microvillar appearance. Mitochondria were small irregular and randomly oriented. Principal cells possessed normal nuclei and slightly enfolded basal membranes (Fig. 5).

Group III (recovery of group II for one month):

Light microscopic results: Light microscopic examination of group III revealed renal cortex with normal glomerular structure. However, some glomeruli had slightly shrinked urinary space. Both proximal and distal convoluted tubules were normal (Fig. 1).

Ultrastructural results:

Glomeruli: Electron microscopic examination of the glomeruli of group III showed normal filtration membrane with normal podocyte processes (Fig. 2).

Proximal convoluted tubule: Electron microscopic examination of cells of the proximal convoluted tubule from the kidney of group III revealed the presence of some vacuoles, well developed brush border, and normal mitochondria with their outer double membrane and multiple lamellas (Fig. 3).

Distal convoluted tubules: Electron microscopic examination of cells of the distal convoluted tubule from the kidney of group III revealed the presence of apical microvilli, and extensive amplification of the basolateral membranes that were forming numerous invaginations. Mitochondria were concentrated at the base of the cell, and arranged laterally in parallel to the long axis of the cell. The nucleus was rounded in shape and euchromatic in structure, and contained one or more nucleoli (Fig. 4).

Collecting tubule Electron microscopic examination of cells of the collecting tubule from the kidney of group III revealed normal ultrastructure of principal cell and intercalated cell (Fig. 5).

Statistical Analysis: The difference between measurements of the urinary space of Bowmen's capsule of the control group compared to the atorvastatin-treated and recovery groups is shown in Table 1, and Fig. 6. These data are represented as the mean ± SD ($P< 0.05$).

In control group, the mean area of the urinary space was 17243.51 ± 3086.087 μm^2. In group II there were a marked drop in the mean area of the urinary space of this group (4341.65 ±923.59 μm^2), where the difference was highly significant in compared to the control group ($p< 0.05$).

In group III, the mean area of the urinary space (5660.28 ± 1244.15 μm2), was slightly increased in comparison to group II which were given the same dose of

atorvastatin, but left for four weeks for recovery. However, this value was still highly significantly different than the control group (P< 0.05, Table 1& Fig. 18).

Table 1: Summary of the mean area ± SD o of urinary space of Bowman's capsule of the control group and atorvastatin-treated groups.

Groups	Treatment	Urinary space of Bowman's capsule (μm^2)
Group I	Control	17243.51 ± 3086.087
Group II	Treated with atorvastatin for four weeks	4341.65 ±923.59**
Group III	Recovery group	5660.28 ± 1244.15**
- Each value represents the mean ± SD of three rats (six readings of each). Highly Significantly different as compared to control, at P < 0.05 **		

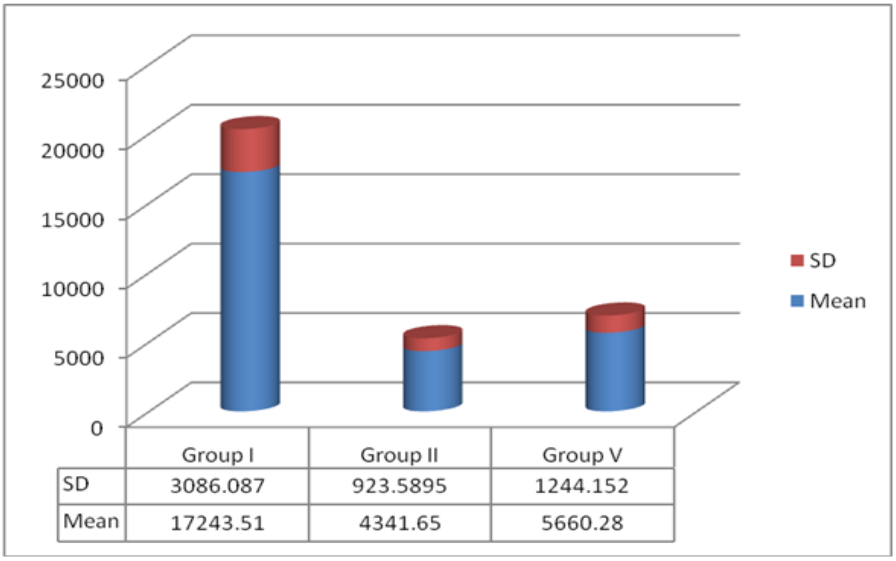

Fig. 6: The mean area ± SD o of urinary space of Bowman's capsule of the control group and atorvastatin-treated groups.

DISCUSSION

Hyperlipidemia is a common metabolic disorder results from genetic predisposition interacting with an individual`s diet. The most important lipoproteins are LDL (low density lipoprotein) and HDL (high density lipoprotein). Excess LDL cholesterol contributes to the blockage of arteries, which eventually leads to heart attack (Pessayre *et al*., 2001). Statins are the most widely used lipid-lowering agents and best tolerated drugs for treating hyperlipidemia. Inhibiting the key enzyme in cholesterol biosynthesis (HMG-CoA reductase) in the liver induces an increased hepatic uptake of plasma LDL with a reduction in circulating levels (Alberts *etal*., 1980).

Skeletal muscles, kidney and liver were reported to be affected during statins therapy. Generalized muscular aches are the commonest adverse effect in humans associated with statin therapy occasionally leading to a frank myotoxicity, which ranges from mild myopathy to frank rhabdomyolysis (Farmer and Torre-Amione, 2012).

Rhabdomyolysis is a syndrome resulting from destruction of skeletal muscles, as the effect of statins with a number of different etiologies may cause acute renal

failure via direct toxic effects on tubular epithelium or by introducing intratubular cast formation(Evans and Rees, 2002).

In the present work, kidney cortex of rats treated for four weeks with 10mg atorvastatin, revealed signs of changes in tubules at both light and electron microscopic levels.These changes were in the form of vacuolization, degeneration and cloudy swelling of epithelial cells in thetubules. These changes were associated with the formation of cell debris inside the lumen of proximal convoluted tubules, and diminish in urinary space of glomeruli. Moreover, hypercellulaty and congestion of glomeruli were also detected. Vacuolization of the cells of the proximal convoluted tubules is related to cellular hydration and sodium retention for purposes of water balance. This may be comparable to osmotic nephrosis which is an example of intracellular vacuolization (Dickenmann *et al.*, 2008). In the present work, atorvastatin treated rats revealed the formation of cell debris inside the lumen of proximal convoluted tubules at both light and electron microscopic levels. The Occurrence of hyaline droplets representing protein overload in kidney tubules of rats has assumed toxicological importance in recent years. This is due to the observations that an increasing number of diverse chemical substances can invoke a marked increase in eosinophilic protein droplets within the proximal convoluted tubule of rats, as the first step in a nephropathic sequence(Hard and Snowde, 1991). In diseases ranging from glomerulonephritis and hypertension, renal damage frequently leads to cellular proliferation mainly mesangial (Keane *et al.*, 1993) and epithelial cells (Vrtovsnik *et al.*, 1997), that triggers a chronic mechanism which can cause permanent loss of nephrons. These studies most likely support the formation of hyaline casts in the kidney tubules in statins-treated groups.

In fact, the total glomerular filtration rate depends upon the structural integrity and the area of the glomeruli (Ossani *et al.*, 2009). Measuring glomerular area or volume is widely employed in human and experimental biology (Ossani *et al.*, 2009). The present work revealed significant differences in the areas of urinary space among the studied animal groups. It has also been reported that statins may promote apoptosis that mediates the resolution of glomerular hypercellularity and glomerular scarring in experimental mesangial proliferative nephritis (Buemi *et al.*, 2002).

In the present study, mitochondria of the kidney tubules became opaque and irregular in shape. The mitochondrion plays a central role in regulating apoptosis by cytochrome c release into the cytosol, which then forms an “apoptosome”. Some reports indicated that statins induce apoptosis by an increase caspase-9 and caspase-3 activity together with pyknosis, chromatin marginalization, and formation of dense bodies (Campos-Pereira *et al.*, 2012). Notably, proximal tubular cells do not use glucose for their energy production(Balaban and Mandel, 1988)but depend primarily on fatty acid oxidation. Fatty acid oxidation is mostly performed by mitochondria, which therefore have a central role in these cells. Mitochondrial production of ATP, which is essential for generating the energy-dependent ion gradients that drive renal tubular reabsorption, is impaired in acute kidney injury (Hall *et al.*, 2011). This can cause massive and life-threatening losses of fluids, electrolytes, and low-molecular weight nutrients, a dysfunction known as renal Fanconi’s syndrome (Che *et al.*, 2014).

In the present work, the kidney of four weeks atorvastatin-treated rats revealed signs of changes at electron microscopy. These changes were in the form of lacking of brush border and completely distorted microvilli of some surface areas of distal

and collecting tubules. In fact, most of the sodium and water of the glomerular filtrate is reabsorbed in the kidney by the cells of the proximal convoluted tubules. The water is reabsorbed through the cell membrane which covers the microvilli and by means of tubular invaginations (Carroll, 2006). Du *et al.* (2004) hypothesized that flow-dependence of kidney proximal tubule Na+ reabsorption is signaled by the microvilli. The "brush-border" microvilli serve a mechanosensory function in which fluid dynamic torque is transmitted to the actin cytoskeleton and modulates Na+ absorption in kidney proximal tubules. Accordingly, the disorganization of brush border and completely distorted microvilli of kidneys tubules, reported in the present work, may lead to dysfunction of reabsorption of salt and water, and consequently may lead to kidney failure.

In the present work, some components of the filtration barrier were also affected, such as podocytes which appeared with some focal fusion of their secondary processes or infrequently disappeared. These changes may induce dysfunction in the glomerular filtration barrier, which in turn may cause proteinuria (Tryggvason *et al.,* 2006). Although proteinuria is a useful marker of kidney damage associated with hypertension, it is itself a risk factor for the progression of renal disease (Atkins *et al.,* 2005; Flack *et al.,* 2010). Because podocytes serve as the final barrier against urinary loss in normal glomeruli, any change in podocytes structure or function may be intimately associated with proteinuria and consequent glomerular sclerosis (Doublier *et al.,* 2003). Podocytes are terminally differentiated cells that line the outer aspect of the glomerular basement membrane. It therefore forms the final barrier to protein loss, which explains why podocyte injury is typically associated with marked proteinuria. Indeed, all forms of nephrotic syndrome arecharacterized by abnormalities in the podocytes (Mundel and Shankland, 2002). In addition, podocytes play a major role in establishing the selective permeability of the glomerular filtration barrier, which explains why podocyte injury is typically associated with marked albuminuria. Podocytes are highly differentiated cells with limited capability to undergo cell division in the adult, and the loss of podocytes is a hallmark of progressive kidney disease (Greka and Mundel 2012). Podocytes damage was accompanied by tubule interstitial cell activation and injury (Joles *et al.,* 2000). It was recently reported that the degree of podocyte damage determines the glomerulus ability to undergo repair instead of glomerulosclerosis (podocytes depletion hypothesis) (Wiggins, 2007).

One study revealed that treatment with cerivastatin in 40 patients with chronic glomerulonephritis reduces the amount of urinary protein excretion and urinary podocyte excretion in normotensive hypercholesterolemic chronic glomerulonephritis patients, a potential marker of podocyte injury (Nakamura *et al.,* 2002). In addition, Akahori *et al.* (2005) revealed, it is possible that the loss of podocyte nephrin is the transition step from an obesity-related glomerular change to the pathogenesis of proteinuria. It has also been reported that statin may induce hematuria, proteinuria, and microalbuminuria, this is thought to be secondary to statin interference with the tubular reabsorption of albumin (van der Tol *et al.,* 2012; Robles *et al.,* 2013).

However, adverse effects and intolerance of statins depend on the specific prescribed molecule and on patient characteristics (Mancini *et al.,* 2016). Controversially, Casey *et al.* (2005) demonstrated impairment in functional, biochemical, and structural parameters of kidney in the diabetic nephropathy group, which was diminished by treatment with pravastatin. Moreover, it has been generally accepted that hyperlipidemia is involved in the pathophysiological mechanisms that

accelerate progression of renal failure (Kasiske *et al.*, 1990; Agarwal & Curley, 2005). Because lipid deposition can directly damage the glomerular basement membrane so, lowering LDL cholesterol level and triglycerides by statins may be beneficial for the kidney.

Shepherd *et al.* (2007) suggest that atorvastatin may be nephron protective. Subanalysis of the Treating to New Targets study investigated how intensive lipid lowering with 80mg of atorvastatin affects renal function when compared with 10 mg in patients with coronary heart disease. Their result revealed that glomerular filtration rate improved in both treatment groups but was significantly greater with 80 mg thanwith 10 mg, suggesting this benefit may be dosage related data (Shepherd *et al.*, 2007). İşeri *et al.* (2007) revealed that simvastatin reduces the extent of both kidney and liver damage and preserves both kidney and liver functions, in Sprague–Dawley rats of either sex. They concluded that simvastatin is beneficial in cisplatin-induced kidney and liver dysfunction and organ damage in rats via prevention of lipid peroxidation and tissue fibrosis, preservation of antioxidant glutathione, and suppression of neutrophil infiltration. Moreover, simvastatin treatment prevents glomerulosclerosis independent of the lipid-lowering effects (Zhang *et al.*, 2008). The beneficial effect of simvastatin might be mediated by the effect of anti-inflammatory action through a reduction of transcription factor nuclear factor kappa B activation, and inflammatory mediators (Zhang *et al.*, 2008).

In fact, most of the controversial studies have been reported to use statins as clinical trials for the treatment of the side effects of other drugs. For example, İşeri *et al.* (2007) used simvastatin for treating nephrotoxicity induced by cisplatin, Cormack-Aboud *et al.* (2009) used rosuvastatin to protect kidney against adriamycin and puromycin aminonucleoside induced apoptosis in podocytes, and Panonnummal *et al.* (2013)used atorvastatin for treating nephrotoxicity induced by vancomycin in rats.

Taking together these studies, it could be suggested that statins may improve physiological mechanisms in patient with nephropathy and coronary heart disease, though their direct effects on cholesterol and triglyceride by lowering their concentrate. In other cases, statins may act against the factors that cause nephrotoxicity such as cisplatin, adriamycin and vancomycin. However, induction of statins to individuals with normal lipid profile may induce histopathological changes in the kidneys as reported in the present study.

CONCLUSION

Atorvastatin 10mg/kg/day has side effects on kidney cortex after four weeks, discovered by histological and ultrastructure changes like: congestion, hypercellularity and hypertrophied of glomeruli capillaries and diminished urinary space of Bowmen's capsule. In the proximal convoluted tubules, vacuoles in their epithelium lining, and accumulation of few cell debris in their lumen were also detected. Ultrastructural changes included focal fusion of podocytes, effacement of processes of podocytes in few places, and disorganization of microvilli of renal tubules. Mitochondria appeared opaque and irregular in shape.

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