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RESEARCH ARTICLE

The protective role of hawthorn in kidneys of the adult albino rat treated with adriamycin: histopathological study

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Abstract

Adriamycin is an anti-neoplastic drug. Its clinical use is restricted due to its tissue toxicity. Hawthorn exhibited good antioxidant activities. We aimed to use a histological approach for evaluating the protective effect of hawthorn against the kidney of rats treated with adriamycin.

MATERIAL AND METHODS: Animals were divided into four groups. Group I was control. Group II was treated with an accumulative dose of 15 mg/kg/day in 6 equal i.p injections (2.5 mg/kg every 3 days). Group III was treated with adriamycin same doses and hawthorn with doses 200 mg/kg for 20 days. Group IV was treated with adriamycin first for 20 days then followed by 20 days hawthorn. Kidneys specimens were examined by light and transmission electron microscopes.

RESULTS: Adriamycin caused degenerative changes in the renal glomeruli and tubules. The capillaries and urinary spaces were dilated. Tubular congestion and presence of tubular cast were visible. Deposition of collagen fibres and fibrosis with interstitial haemorrhage were also noticed. Thickening and irregularity of basement membranes were revealed and the foot processes had disappeared. Vacuoles were observed in the tubular cytoplasm with degeneration and loss of the microvillus. In group III, the cellular structure was better preserved as compared with group II. Improvement is moderate in group IV.

CONCLUSION: Treatment with hawthorn and adriamycin together revealed that the kidney structure was better preserved when compared with group II. Improvement was also noticed in treatment with hawthorn after adriamycin treatment but not the same degree like treatment with adriamycin and hawthorn together.

Introduction

Adriamycin (ADR) is an anti-neoplastic agent used in the treatment of a variety of human neoplasms. However, its clinical use is severely restricted due to its toxicity in various tissues, including the heart, the liver and the kidneys. Experimental studies in animals showed that ADR caused renal toxicity by producing progressive glomerular injuries (Barbey et al., 1989; Manabe et al., 2001; Malarkodi et al., 2003; Deepa and Varalakshmi, 2005).

The exact mechanism of ADR-induced toxicity remains unclear. Some researchers proposed that it is most likely mediated by the formation of an iron–anthracycline complex that generates reactive free radicals (ROS), which in turn, causes diverse oxidative damage on critical cellular components and membrane lipids in the plasma membranes and mitochondria (DeGraff et al., 1994; Sazuka et al., 1989a; Sazuka et al., 1989b). This theory is well

supported by the fact that antioxidants prevent the ADR-induced toxicity in experimental animals as well as in human (Mohamed et al., 2000; Cole et al., 2006).

Hawthorn contains abundant amount of anti-oxidants such as chlorogenic acid, epicatechin, hyperoside and quercetin (Liu et al., 2010). Hawthorn might inhibit the excessive generation of oxidative stress, thus providing an overall protective role in the kidney toxicities induced by ADR administration in rats (Siveski-lliskovic et al., 1995). However until now, the effect of hawthorn on ADR-induced oxidative stress and peroxidative alterations are poorly understood.

The present experimental study is therefore designed to use a histological approach for evaluating the possible protective effect of hawthorn against the kidney of the adult albino rats treated with ADR using light and transmission electron microscopes.

MATERIALS AND METHODS

Forty healthy adult male albino rats (12 weeks old, 160–200 g body weight) were used in this study. The animals were kept in the house animals, King Khaled University, Abha, under standard laboratory conditions (temperature 24±3°C, humidity 40–60%, a 12-h light:12-h dark cycle). A commercial pellet diet and fresh drinking water were given *ad libitum*. The handling of animals was followed the rules for experimental research ethics approved by Research Ethics Committee at King Khaled University.

The rats were randomly divided into four groups; each group containing 10 rats. The first group was normal control non treated rats was receiving 1 ml distilled water intraperitoneal daily. The second group was ADR treated rats administered an accumulative dose of 15 mg/kg/day in 6 equal i.p injections (2.5 mg/kg every 3 days). The third group was treated with ADR same doses and hawthorn with doses 200 mg/kg orally by gavage daily for 20 days. The fourth group was treated with adriamycin first for 20 days then followed by 20 days hawthorn same doses.

The rats were killed under slight ether anaesthesia at the end of treatment period. Kidneys were dissected and removed. Kidneys were fixed in 10% formalin solution for histopathological examinations.

Light microscopy

All specimens for light microscope were cut into small pieces and fixed in a solution of 10% formaldehyde and processed for light microscopic study to get paraffin sections of 5 μm thickness. Sections were stained with Haematoxylin and Eosin (H&E) (Bancroft and Stevens, 1996). Slides were mounted using entellan and covered with cover slips prior to viewing and photography by (Nikon Eclipse E-200) light microscope.

Transmission electron microscopy (TEM)

The kidneys specimens were cut into small pieces of 1mm3 sizes and fixed in 2.5% glutaraldehyde for 24 hours. Specimens were washed in 0.1 M phosphate buffer at 4°c, then post fixed in 1% osmium tetroxide at room temperature. Specimens were dehydrated in ascending grades of ethyl alcohol, and then embedded in Epon resin. Semithin sections (1μm) were stained with toluidine blue in borax and examined with light microscope. Ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate (Bancroft and Stevens, 1996). Specimens were examined and photographed with Jeol 1200 EX transmission electron microscope in College of Medicine, King Khaled University.

RESULTS

The kidney of rats (control group) shows intact glomeruli within Bowman's capsule without evident alterations of the structures. Proximal and distal tubules were normal (Fig. 1). Kidney structure was similar to those described in previous literature regarding mammalian kidney including rat.

Degenerative changes were observed in the renal glomeruli and tubules of group II animals which received only ADR. The flat epithelial cells of the parietal layer of Bowman's membrane were normal in structure. The capillaries and urinary spaces were dilated. Degenerated proximal and distal tubular epithelial cells with interrupted tubular contour, tubular congestion and presence of tubular cast were visible. Interstitial haemorrhage was also noticed (Fig. 2).

Treatment with hawthorn and adriamycin together in group III revealed that the structure of tubules was better preserved when compared with group II. In the glomeruli, the urinary spaces were distinct and the capillaries were normal in structure. The degenerative changes in the glomeruli and tubules were improved as compared with group II (Fig. 3).

Improvement was also noticed in group IV(Fig. 4) in treatment with hawthorn after adriamycin treatment but not the same degree like treatment with adriamycin and hawthorn together in group III.

Light microscopic examination after staining with Masson trichrome revealed marked deposition of collagen fibres and fibrosis with interstitial haemorrhage and marked cells degeneration in the group II (Fig. 5). These changes are markedly improve in group III (Fig. 6) and moderately improved in group IV (Fig. 7)

The ultrastructure study of control group showed normal appearance as described in previous literature. Endothelial cells, basement membrane, visceral epithelial cell with foot processes are all showing normal appearance (Fig. 8). The basement membrane is regular with uniform thickness. Foot processes lie on the regular basement membrane . Adjacent foot processes are separated by filtration slits (Fig. 9).

Thickening and irregularity of glomerular capillary basement membranes were determined in group II. Vacuolation was observed in the capillary lumen. (Fig. 10). Mesangial cells were numerous with increased mesangial matrix and interstitial hemorrhage were noticed (Fig. 10). In the glomerular area, the cytoplasmic foot processes had disappeared (Fig. 11). Thickening of the basement membrane was also noticed in group III but the foot processes were intact (Fig. 12). In group IV irregularity and variable thickness of the basement membrane were revealed but the foot processes were moderately intact (Fig. 13). Condensation of the peripheral chromatin were noticed in podocyte cells and endothelial cells. Urinary space was wider in group IV than group III (Figs. 12 &13).

The proximal tubule in control rat is lined by cells with apical microvillus. The nucleus is large and oval. Basal mitochondria are located between basal enfolding. The basement membrane is regular and of normal thickness (Fig. 14).

In the cytoplasm of proximal tubular epithelial cells in group II, numerous vacuoles were observed (Fig. 15). The cells were small with irregular and atrophic nuclei. Many lysosomes of various sizes were observed in the cytoplasm. The mitochondria were dilated and dispersed throughout the cytoplasm. Destruction, degeneration and loss of the microvillus were observed (Fig. 15). The intertubular deposition of collagen fibres had increased considerably (Fig. 15).

The microvillus is intact in group III with improved in pathological features (Fig. 16). In group IV, the lumen of the proximal tubules occasionally contained a homogeneous, dense substance. Vacuoles and spaces were observed in the cytoplasm, forming wide, vacant regions between the nuclear and cytoplasmic membranes (Fig. 17).

Distal tubule in the control kidney showing a normal basement membrane, short microvilli, round nucleus and lumen (Fig.18).

In the II group, most of the distal tubule cells were degenerated, and their length had decreased. In the cytoplasm of tubular epithelial cells, numerous vacuoles and many mitochondria were small in size (Fig. 19). In some tubules “empty” epithelial cells, with “washed out” cytoplasm and nuclei with circumferential chromatin condensation were observed (Fig. 19). In group III, the cellular structure was better preserved as compared with group II (Fig. 20). Improvement is moderate in group IV with some mitochondria ring shaped (Fig. 21).

Fig. 1:- A photomicrograph of a section in the control kidney showing renal glomeruli (G) with their tuft of capillaries and normal Bowman’s capsule with the presence of urinary space (arrow). Notice the contour of the proximal (P) and distal (D) tubules is intact and regular. (H&E X 400)

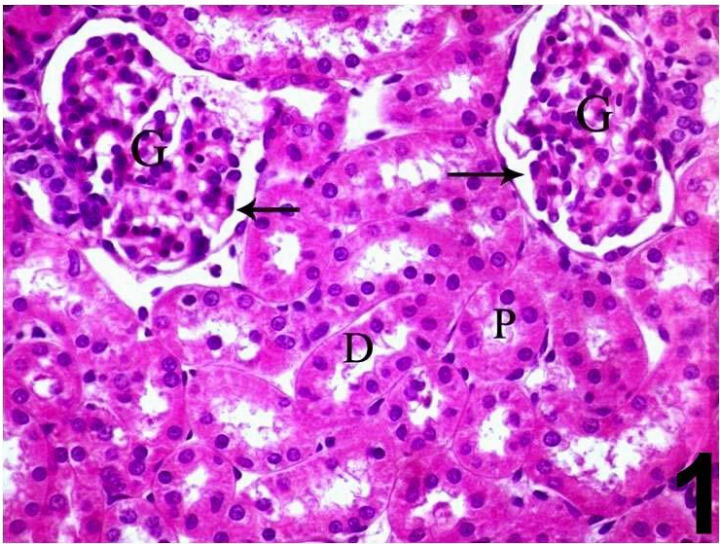


Fig. 2:- A photomicrograph of a section in the kidney group II showing renal glomeruli (G) with wide urinary space (arrow). Notice the degeneration (d) of some cells of the proximal (P) and distal (D) tubules with disrupted contour and interstitial area of hemorrhage (rb). (H&E X 400)

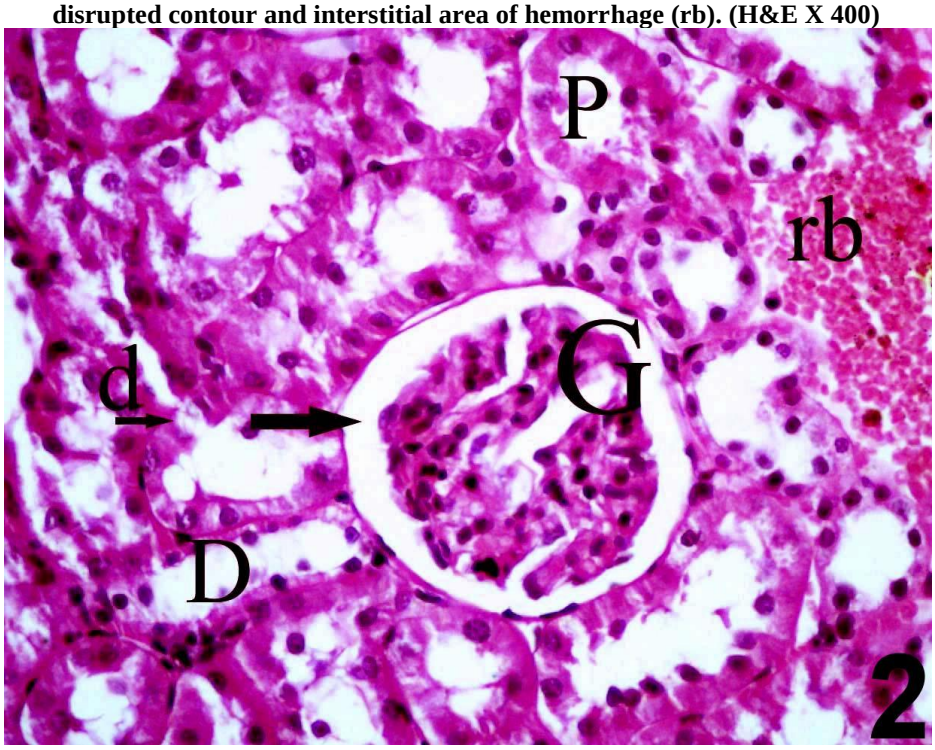


Fig. 3:- A photomicrograph of a section in the kidney group III showing renal glomeruli (G) with their tuft of capillaries with the presence of urinary space (arrow). Notice that the proximal (P) and distal (D) tubules are mild disrupted with some degenerated cells. (H&E X 400)

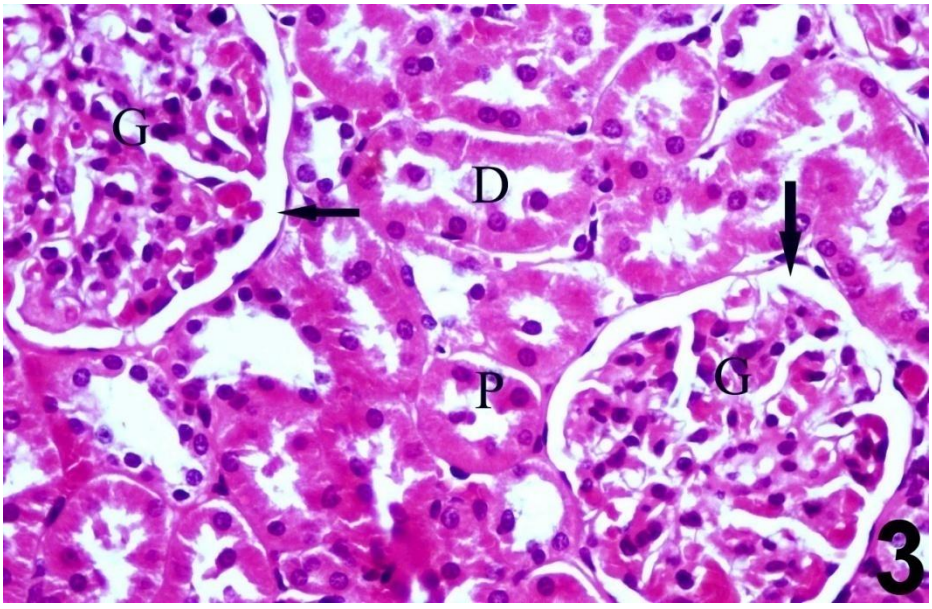


Fig. 4:- A photomicrograph of a section in the kidney group IV showing renal glomeruli (G) with their tuft of capillaries with the presence of urinary space (arrow). Notice that the proximal (P) and distal (D) tubules are moderate disrupted with some degenerated cells and interstitial hemorrhage (rb). (Hx&E X 400)

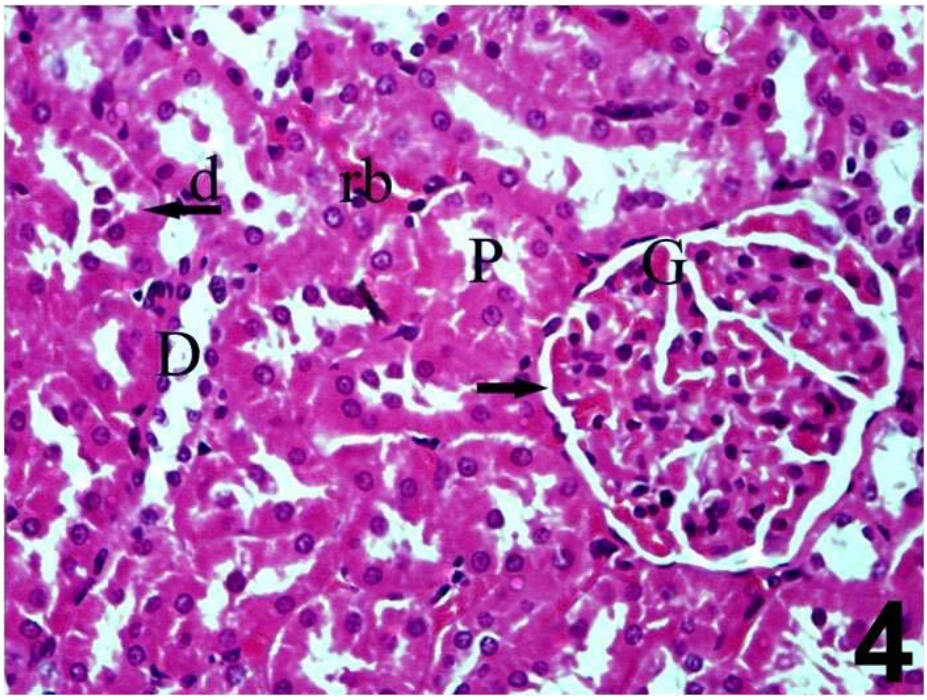


Fig. 5:- A photomicrograph of a section in the kidney group II showing renal glomeruli (G) with wide urinary space (arrow). Notice the degeneration of the tubules with interstitial area of hemorrhage (rb) and marked deposition of collagen fibres (blue area). (Masson trichrome X 400)

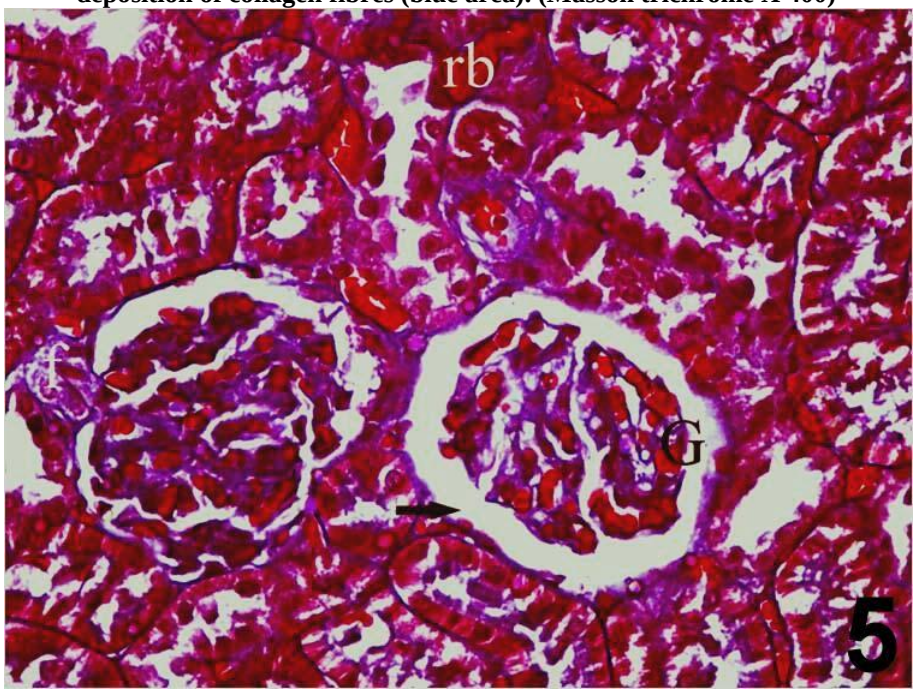


Fig. 6:- A photomicrograph of a section in the kidney group III showing more or less normal renal glomeruli (G) with urinary space (arrow). Notice the mild degeneration of some of the proximal (P) and distal (D) tubules with mild deposition of collagen fibres (blue area). (Masson trichrome X 400)

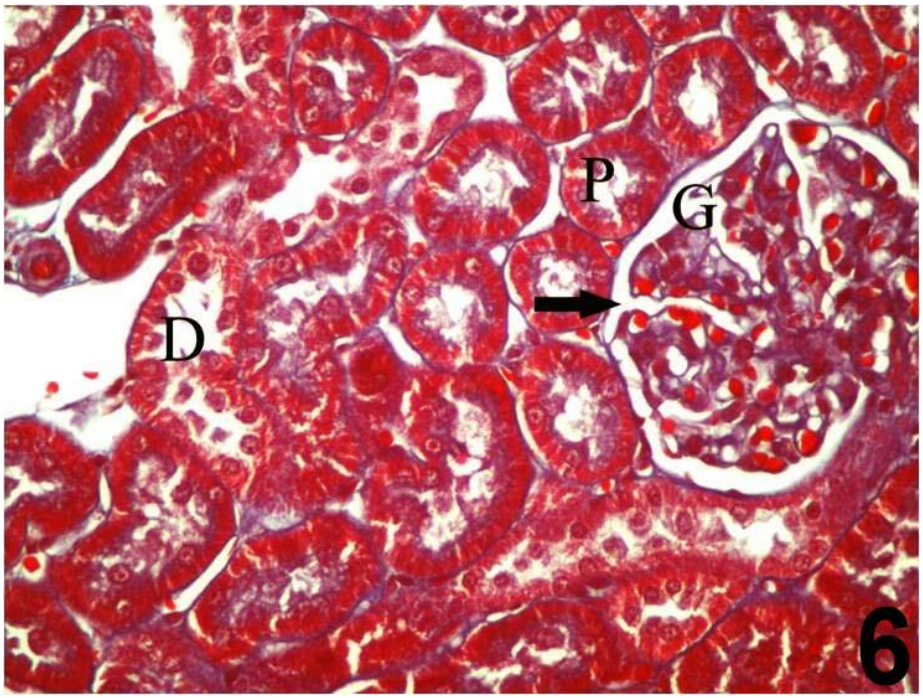


Fig. 7:- A photomicrograph of a section in the kidney group IV showing nearly normal renal glomeruli (G) with urinary space (arrow). Notice the moderate degeneration of some of the proximal (P) and distal (D) tubules with some vacuoles (v), mild interstitial hemorrhage (rb) and moderate deposition of collagen fibres (blue area). (Masson trichrome X 400)

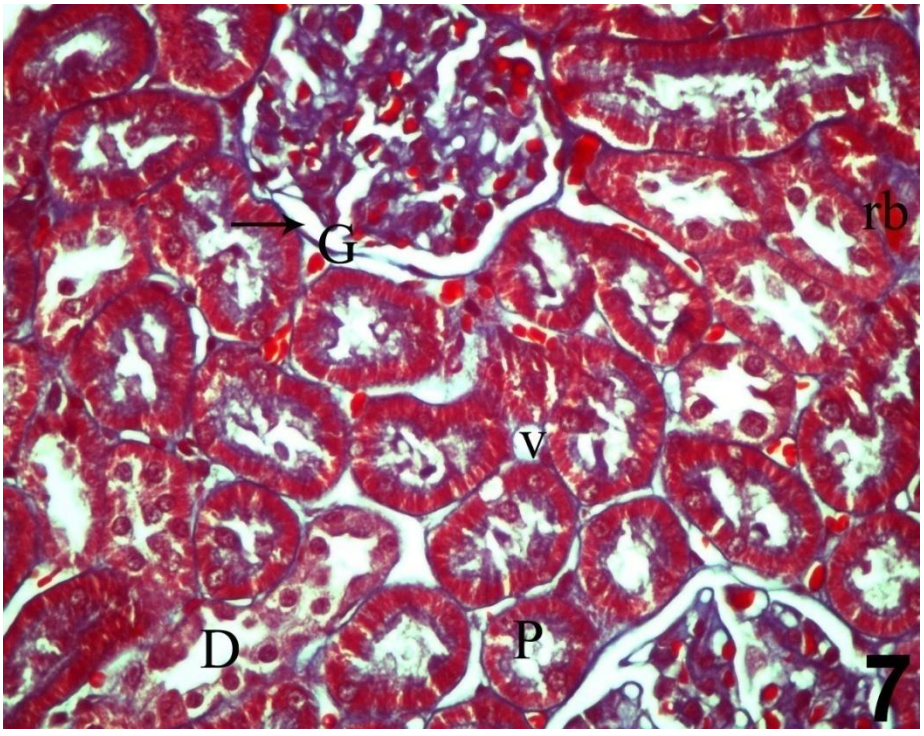


Fig. 8 :- Transmission electron micrograph of kidney sections of rats of the group I showing regular basement membrane (BM) with uniform thickness. Foot processes (F) from podocyte cell (P). Red blood corpuscle (rb), urinary space (U) and endothelial cell (E) are seen (bar = 2 µm X8000)

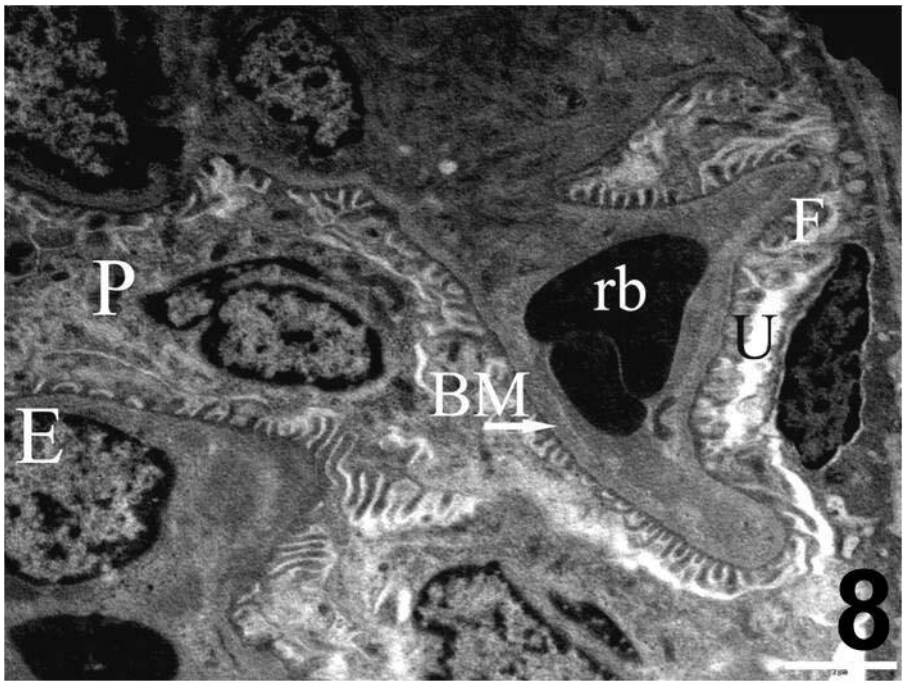


Fig. 9 :- Transmission electron micrograph of kidney sections of rats of the group I showing regular basement membrane (BM) with uniform thickness. Foot processes (F) of podocyte cell (P) with its nucleus (N). Notice the filtration site (fs). Red blood corpuscle (rb). (bar = 500 nm X40000)

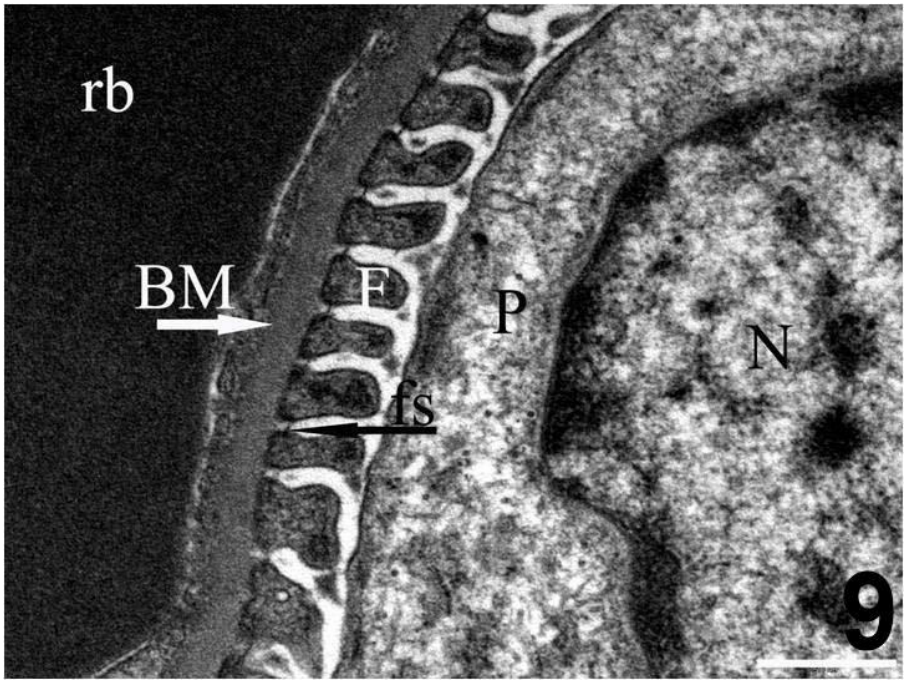


Fig. 10 :- Transmission electron micrograph of kidney sections of rats of the group II showing irregular basement membrane (BM) with variable thickness. Foot processes (F) destructed, many mesangial cells (M) are seen surrounded by mesangial matrix. Some red blood corpuscle (rb) present inside the capillary lumen with the presence of vacuoles (v). Interstitial hemorrhage with red blood corpuscles (rb) are also present . (bar = 5 µm X5000)

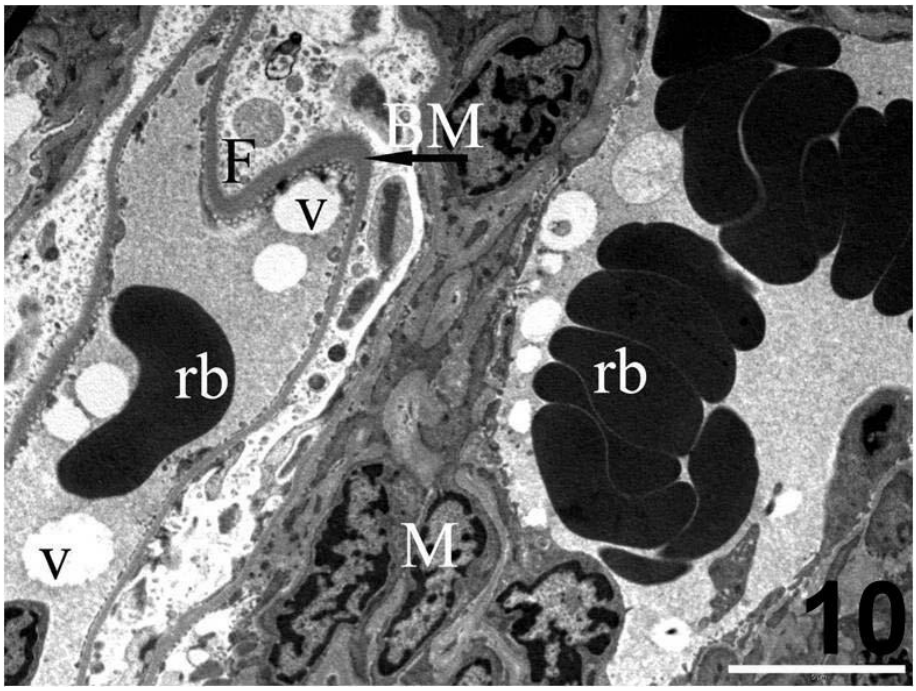


Fig. 11 :- Transmission electron micrograph of kidney sections of rats of the group II showing irregular basement membrane (BM) with variable thickness. Most of the foot processes (F) disappear, podocyte branches (P), endothelial cells (E). Red blood corpuscle (rb). (bar = 1 µm X20000)

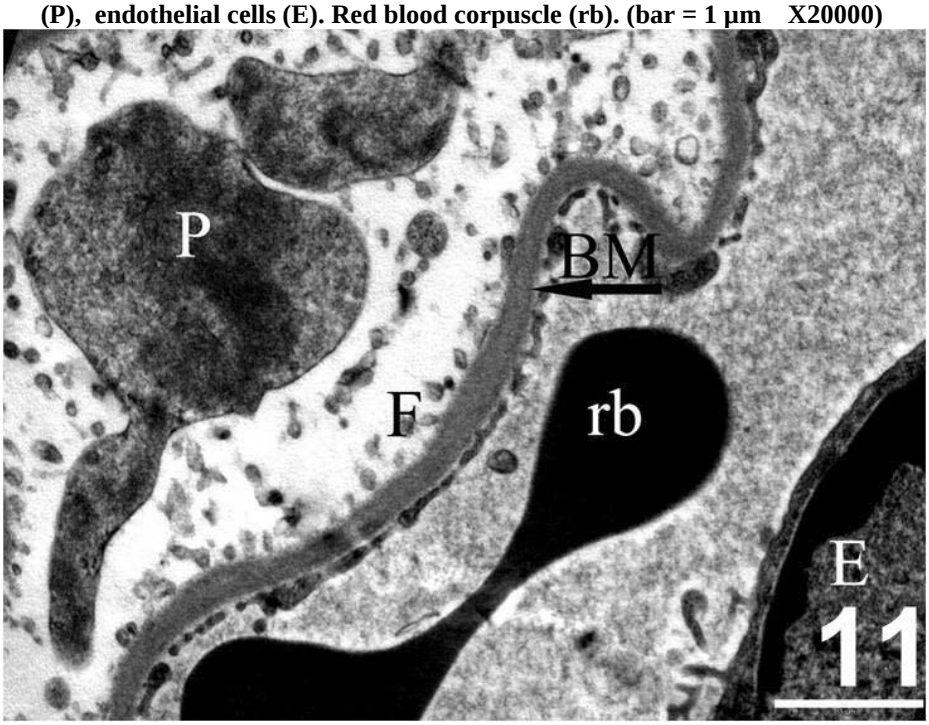


Fig. 12 :- Transmission electron micrograph of kidney sections of rats of the group III showing irregular basement membrane (BM) with variable thickness. Foot processes (F) are intact. Capillary lumen (Lu), red blood corpuscle (rb) and narrow urinary space (U) are present. (bar = 2 µm X10000)

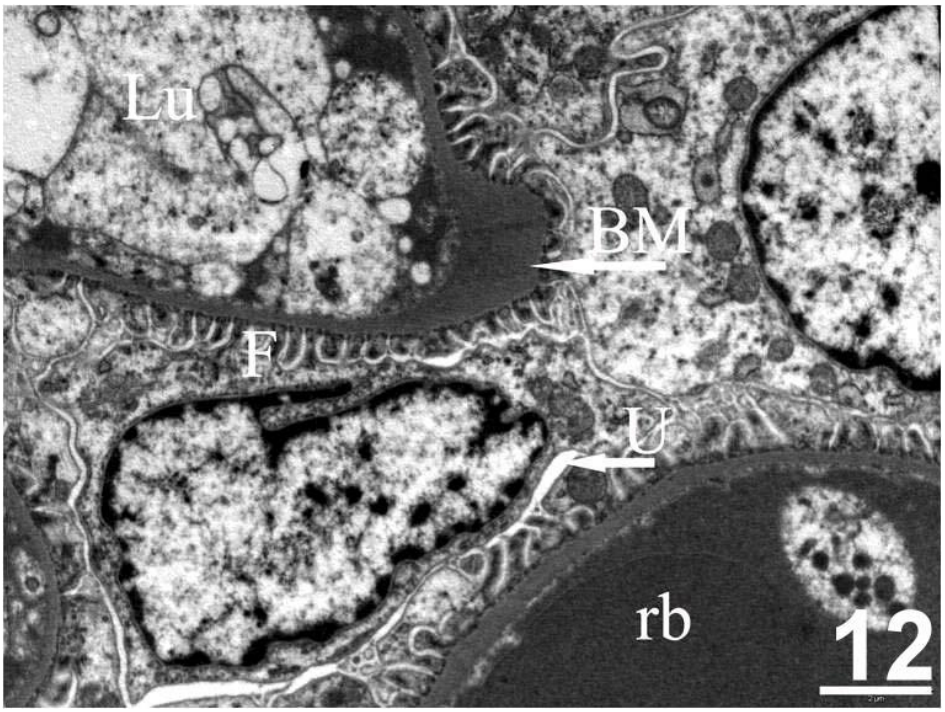


Fig. 13 :- Transmission electron micrograph of kidney sections of rats of the group IV showing irregular basement membrane (BM) with variable thickness. Foot processes (F), epithelial cell (EP) with irregular nucleus (N) and endothelial cell (E) with condensed peripheral nuclear chromatin (N). Red blood corpuscle (rb) and narrow urinary space (U) (bar = 2 µm X10000)

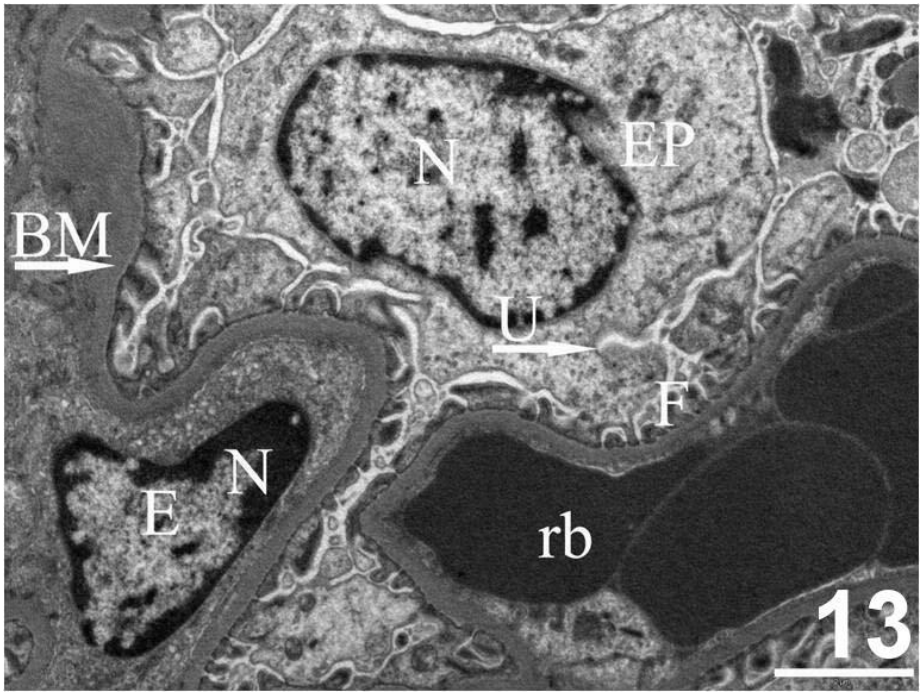


Fig. (14):Transmission electron micrograph of proximal tubule of kidney sections of control rats showing oval nucleus (N), multiple mitochondria (m) and apical microvilli (mv). (bar = 2 µm X8000)

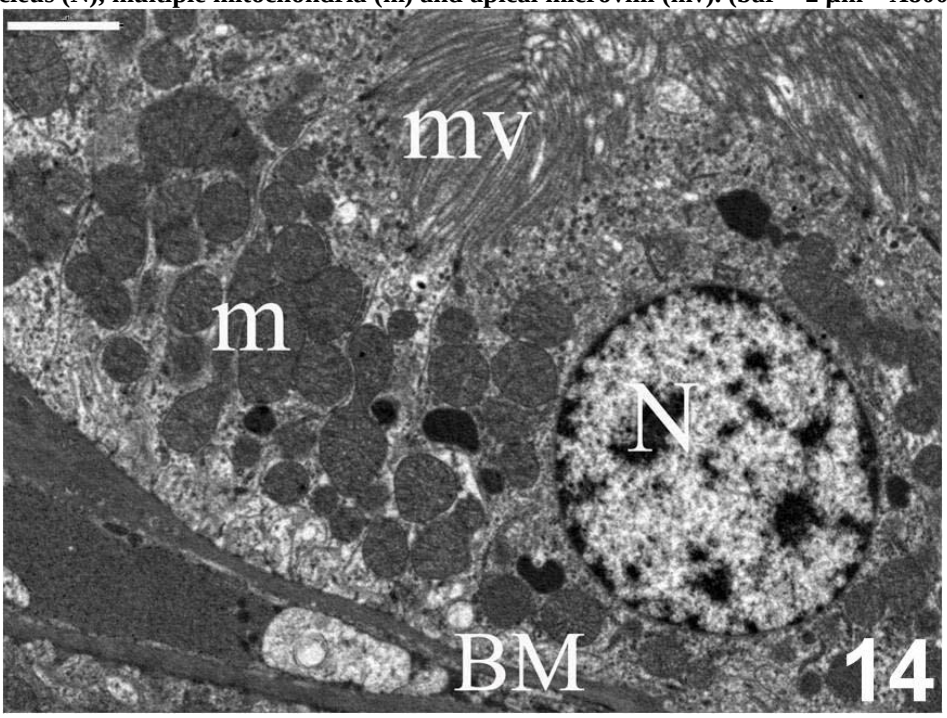


Fig. (15):Transmission electron micrograph of proximal tubule of kidney sections of group II rats showing irregular basal membrane (BM), atrophic irregular nucleus (N), multiple basal mitochondria (m). Notice the presence of vacuoles (v) and many electron dense granules (L) and destruction of microvillus (mv). Notice also the presence of casts (c) in the lumen and deposition of collagen fibres (f). (bar = 5 µm X4000)

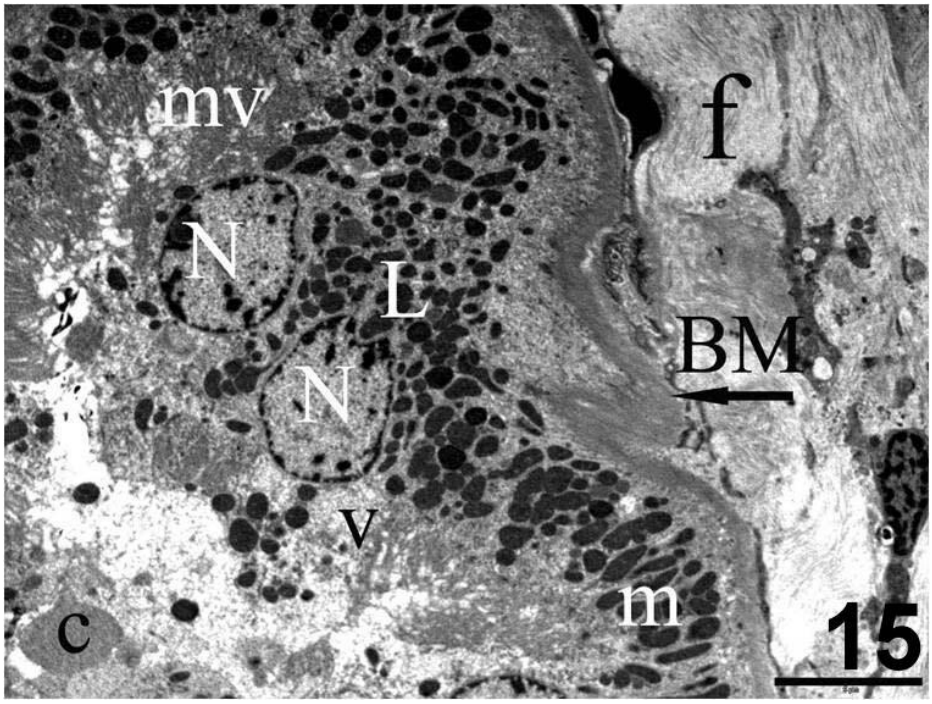


Fig. (16):Transmission electron micrograph of proximal tubule of kidney sections of group III rats showing round nucleus (N), multiple mitochondria (m). Irregular thickness of basal membrane, lysosomes (L) and intact apical microvilli (mv) are present (bar = 2 µm X8000)

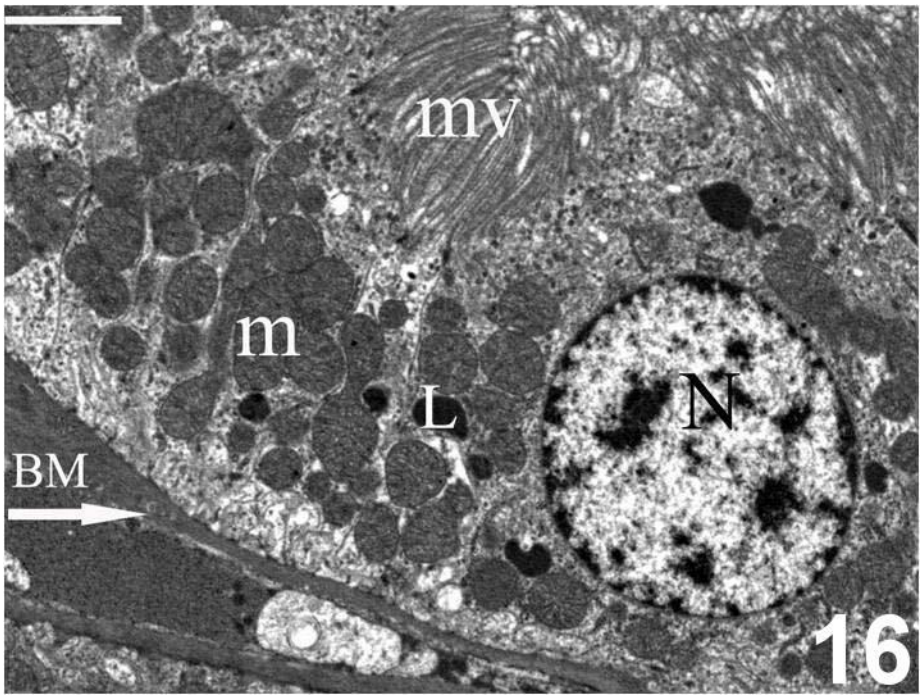


Fig. (17):Transmission electron micrograph of proximal tubule of kidney sections of group IV rats showing round nucleus (N), multiple mitochondria (m). Notice the presence of vacuoles (v) and many electron dense granules (L) and closed to the lumen of the proximal tubules there is a homogeneous, dense substance (D*) and mild disrupted microvilli (mv). (bar = 5 µm X5000)

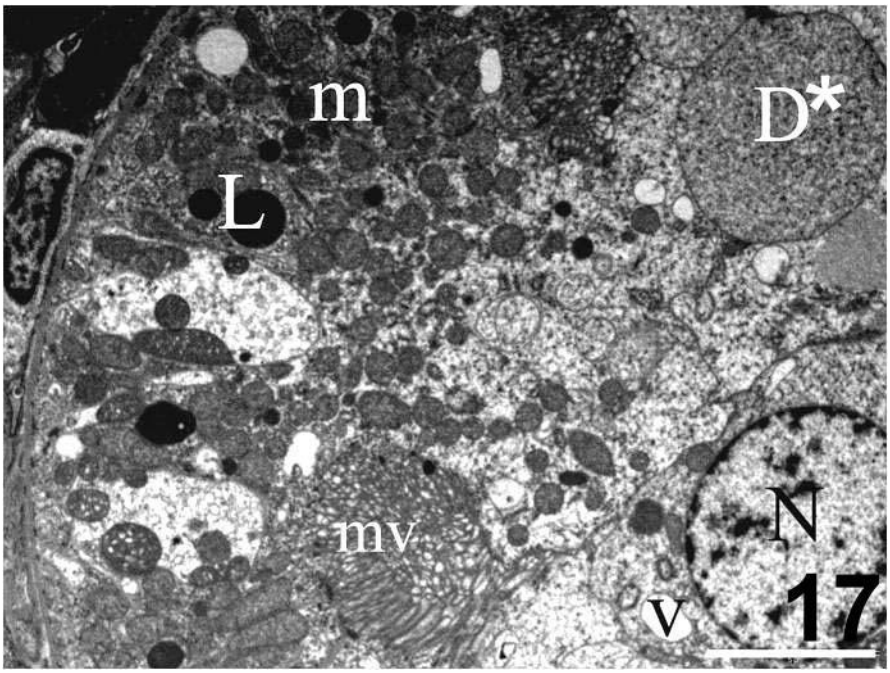


Fig. (18):Transmission electron micrograph of distal tubule of kidney sections of control rats showing round nucleus (N), multiple basal mitochondria (m) vertically oriented in-between basal enfolding of the plasma membrane (f). (bar = 500 nm X6000)

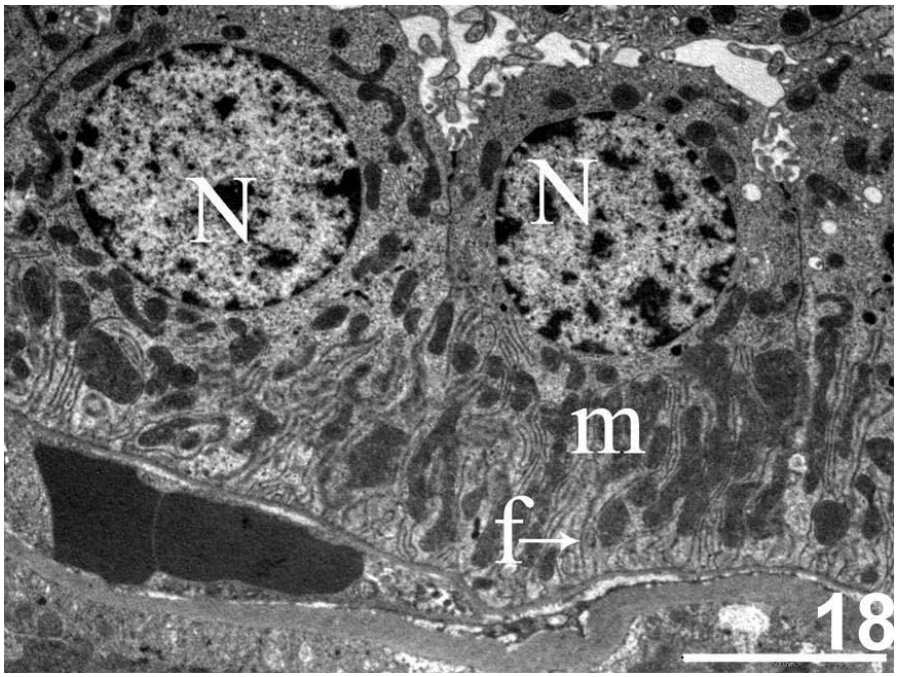


Fig. (19):Transmission electron micrograph of distal tubule of kidney sections of rats group II showing round nucleus (N), many vacuoles (v), multiple basal mitochondria (m) vertically oriented inbetween basal infoldings of the plasma membrane (f) and destruction of the microvillus (mv). (bar = 5 µm X5000)

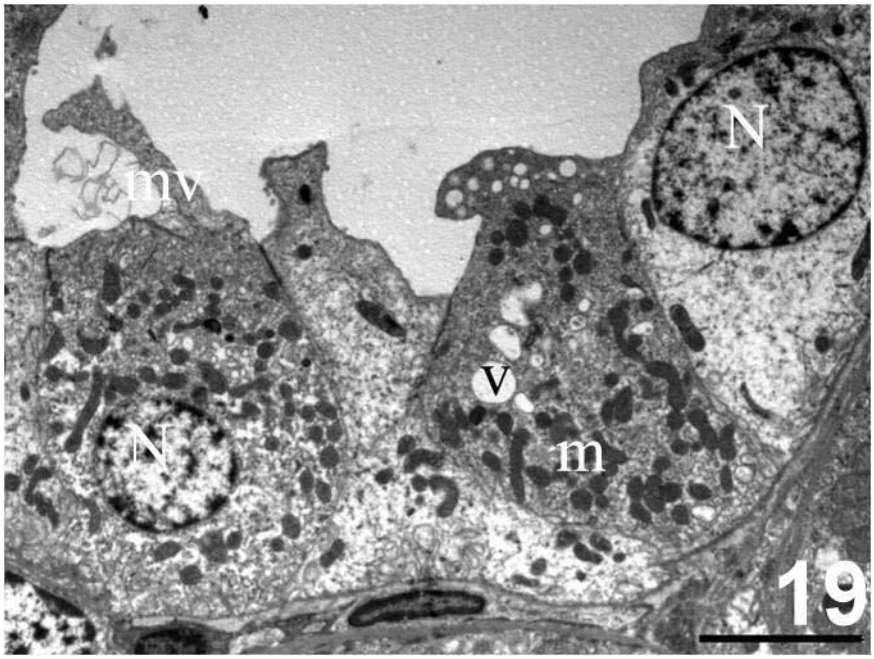


Fig. (20):Transmission electron micrograph of distal tubule of kidney sections of rats group III showing round nucleus (N), multiple basal mitochondria (m) vertically oriented in-between basal enfolding of the plasma membrane (f). Notice the destructed microvillus (mv) (bar = 200 nm X8000)

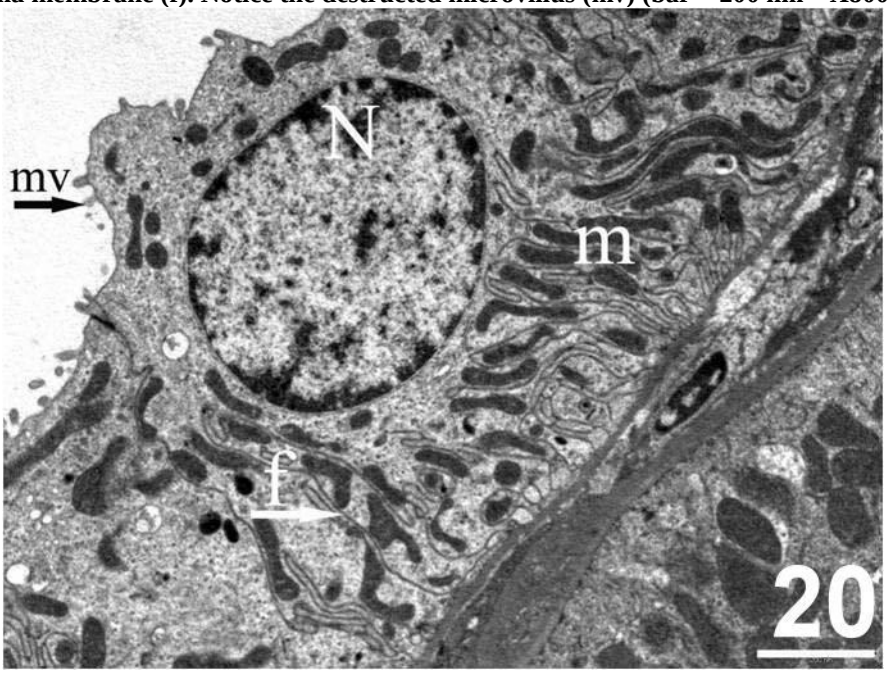
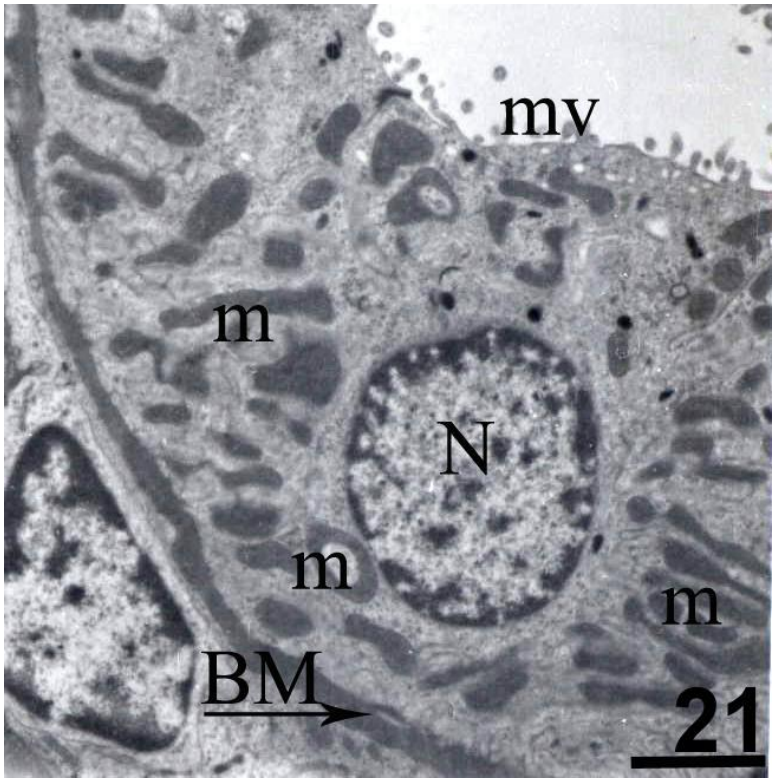


Fig. (21):Transmission electron micrograph of distal tubule of kidney sections of rats group IV showing round nucleus (N), multiple elongated mitochondria (m) some of them are ring shaped. Noticed the irregular thickness of basal membrane (BM) and the destructed microvillus (mv). (bar = 5 µm X5000)



DISCUSSION

Adriamycin is known to generate superoxide radicals. The formation of free radicals, as well as accumulation of lipid peroxides in response to treatment with adriamycin, has been documented. Lipid peroxidation is recognized as one of the possible biochemical mechanisms for adriamycin associated side effects (Doroshow, 1983; Mimnaugh et al., 1986). Adriamycin form the semiquinone which is a toxic metabolite that interacts with molecular oxygen and initiates a cascade of reactions, producing reactive oxygen species (ROS) (Davies and Doroshow, 1986). ROS generation and lipid peroxidation may be responsible for ADR-induced cardiotoxicity and nephrotoxicity (Mimnaugh et al., 1986; Milner et al., 1991; Chularojmontri et al., 2005).

Histological changes observed in the present study revealed that the glomerules in rat from the 2nd experimental group revealed glomerular congestion, enlarged with wide urinary spaces. Urinary space dilation may be the result of the high pressure across glomerular capillaries (Chagnac et al., 2000). Glomerular enlarged and hypertrophy may be responsible for the podocytes abnormalities (Chen et al., 2006).

Renal glomerular filtration barrier is one of the most important structures, and the integrity of barrier determines the permeability characteristics for the protein. Glomerular filtration barrier is divided into 3 layers: fenestrated endothelial cell layer, glomerular basement membrane, podocyte, and slit diaphragm.

The basement membrane had irregular in thickness with destruction of the foot processes that affect the glomerular filtration. Previous studies carried out by Fajardo et al. (1980) and Strenberg et al. (1972) revealed that cytoplasmic foot processes of podocytes were destructed by the effect of adriamycin and also that irregularities of the glomerular basal membrane and dense agglomerations in certain regions of the basal membrane arose as a consequence of its use. Identical result were found in our study.

Certain stimuli act on podocytes to induce shape changes. Toxic reactions, such as adriamycin, decrease in podocyte surface charge by neuraminidase digestion (Gelber et al., 1996). Flattening or destruction of podocytes could be the cause of increased glomerular permeability.

Damage to the structure of the glomerular filtration barrier can lead to protein leakage (Niels et al., 2005). Proteinuria is risk factor of podocyte injury owing to glomerular disease, such as minimal change nephropathy syndrome, focal segmental glomerulosclerosis, membranous glomerulopathy, diabetes mellitus, and lupus nephritis (Reiser et al., 2002). Studies from some nephrotic syndrome implied that distribution abnormality of podocyte molecules could lead to the abnormalities of the foot process structures and proteinuria (Koop et al., 2003; Zhang et al., 2004). It is recognized that the foot processes of podocytes interlinked by ultra-thin slit is likely the centre structure to be the barrier in the glomerular capillary wall to retain proteins and cells from leaking to urine (Kawachi et al., 2006). In the present study, the foot processes were severely damaged in adriamycin-treated rat that resembling human minimal change nephropathy syndrome (Korzets et al., 1997). The present study showed that foot process became destructed in adriamycin treated rats, which were diminished by hawthorn treatment. These results suggest that hawthorn improves the podocyte injury in adriamycin-treated rats. Literature provides results that ADR also induced nephritic syndrome in pregnant rat females (Pedrycz-Wieczorska, 2002).

Bertani et al., (1986) reported that ADR has the potential to induce renal damage with glomerulosclerosis. Similar pictures of glomerules were described in literature in rats suffering from nephrotic syndrome induced by ADR (Rangan et al., 2001). Minimal dose of ADR like 5 mg/kg of body weight induced nephrotic syndrome (Song et al., 2002) and in these cases, the fusion of foot processes was described (Raats et al., 2000).

Degenerated tubular epithelial cells with interrupted tubular contour were visible. Most of the distal tubule cells were degenerated, with tubular congestion and presence of tubular cast. Tulio et al. (1982) reported that kidneys from ADR-treated animals showed glomerular necrosis, hemorrhage and tubular degeneration. Endotoxin can induce renal damage, particularly in the renal tubule cells that leads to cell degeneration, necrosis and apoptosis (Yang et al., 2009).

In the cytoplasm of tubular epithelial cells, numerous vacuoles were observed, which indicated microvacuolar degeneration. Asakura et al. (2001) reported that adriamycin induce apoptotic DNA fragmentation via caspase-3 activation. Apoptotic signal which could activate caspase-3 was induced 6 hours after drug administration (Asakura et al., 1999; Asakura et al., 2001).

Degeneration of tubular cells described in the present study may be secondary to lesions in glomeruleus or it is due to cytotoxic effect of adriamycin.

In the present study, evident changes in mitochondria in tubular cells were described. It was proved that ADR influences mitochondria function. Adriamycin decreases mitochondrial ATP-ase and oxidative phosphorilation ADP. It results in the inhibitions of cell perspiration process (Wallace and Starkov, 2000). Mitochondria pass into the orthodox form due to adriamycin activity. It contains a big amount of ATP, which could not process the energy.

Vacuoles were observed in the cytoplasm between the nuclei and cellular membranes of the tubules. Basal invaginations and microvilli in some tubules had disappeared while the mesangial cells and matrix increased in the

present study. Strenberg et al. (1972) showed the disappearance of basal invaginations and microvilli in the tubules. Identical result were found in our study.

Bertani et al. (1986) established that fibrosis occurred in the interstitial zone of kidneys after administration of adriamycin. We also noted a significant increase in the collagen fibres between tubules. Okasora et al. (1992) reported that lumina of tubules in the kidney tissues of subjects given adriamycin were filled with an amorphous material which was observed in some tubules in our study also.

Interstitial haemorrhage was also noticed. Haemolysis of interstitial haemorrhage releases haemoglobin and leads to tissue accumulation of ferric ions. Free haemoglobin promotes the production of inflammation and tissue injury (Martín Cleary et al., 2010).

Adriamycin has widely been used for hematological malignancies. However, the toxic effect of adriamycin on the kidneys is a limiting factor of its usefulness. Therefore, novel therapeutic agents with improved efficacy seem to be considerable for clinical approach.

Several drugs have been investigated in an attempt to reduce adriamycin toxicity. Vitamin E is among these (Wang et al., 1980; Görgün et al., 1999) which protects the glomeruli and tubules to some extent from adriamycin-induced damage.

Since oxidative stress is implicated in adriamycin toxicity. We have investigated the protective potential of hawthorn as a powerful antioxidant. Hawthorn contain a high proportion of polyphenolic compounds and exhibited good antioxidant activities. Hawthom is of significant biological importance for its antioxidant properties (Kostić et al., 2012). The present results confirmed that hawthorn improved kidney structure in male rats treated with adriamycin. Also it revealed for the first time that administration of hawthorn in combined with adriamycin attenuated the kidney pathology than giving hawthorn after treatment with adriamycin. In the present study, we have elaborated the protective effects of hawthorn against adriamycin-induced damages of the kidney structure of male rats.

In conclusion, this research demonstrated that adriamycin caused damage in the kidney structures and hawthorn administration improves these structural deficits.

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