The Egyptian Journal of Hospital Medicine Vol., 17 : 20 – 43 Dec.2004
I.S.S.N: 12084
1687 -2002

Ultrastructural study of renal tubular damage induced by captopril in adult and fetal mice

Hamdy H. Swelim and Aleya A.Sakr
Department of Zoology, Faculty of Science, Ain Shams University

Abstract

The present study has been designed to evaluate the possible nephrotoxicity of the angiotensin converting enzyme inhibitor, captopril on renal tubules of adult and maternally treated fetuses of CD-1 mice. The study included the effect of captopril administration for one month up to three months in adults, while in fetuses, they were exposed to the drug through their mothers in two periods. The first was from 6^{th}-12^{th} days of pregnancy, while the second was from 6^{th} -18^{th} day of pregnancy. The dose used in the present study represents the dose equivalent to the therapeutic daily dose taken by human. All the recorded tissue damage was found to be time dependent. The first remarkable feature noticed in all the treated adult animals was the presence of hyaline casts that obstructed most of the renal tubules. The second remarkable feature was the increase of the intertubular space associated with irregularity of the tubules due to the degeneration and vacuolation of the basal regions of the cells. Renal tubule cells showed large blebs, accumulaton of lipids, degeneration and necrosis. In maternally treated fetuses, the proximal convoluted tubule cells displayed moderate vacuolation and marked increase of lysosomes while some of the distal convoluted tubules revealed atrophy and their cells showed loss of mitochondria. In addition, the collecting tubules showed loss of microprojections. Worthy to mention that there was apparent increase of mesenchymal cells as well as fibroblasts in the fetuses maternally treated with captopril. The significance of these changes was discussed and it should be emphasized that captopril must be taken with caution for pregnant women and those who suffer from renal troubles. Moreover, kidney function should be monitored during therapy .

Introduction

Angiotensin converting enzyme (ACE) inhibitors are standard therapy for cardiovascular diseases including congestive heart failure and hypertension. ACE inhibitors have been used worldwide and have been reported to cause relatively few side effects. The antihypertensive effects of these drugs are related to their ability to block the conversion of the decapeptide, angiotensin I, to the potent pressor octapeptide, angiotensin II. Thus cause vasodilation and lowering of blood pressure (Yoshida *et al.*, 1998). .

Captopril (capoten) has been emphasized as the agent with the most renal protective effect (Jovanovic *et al*., 1998 and Gupta *et al*., 1999). It has been used in several clinical trials to slow a progressive decline in glomerular function in patients with diabetic nephropathy (Isogai *et al*., 1998). It also exerts significant functional and structural protection against renal radiation injury in rats (Moulder *et al.*, 1996). In addition, it protects lung from radiation induced pneumonitis and fibrosis (Cohen *et al*., 1996).

Moreover, Cook and Besso (1997) reported improvements in renal function in cases of diabetic nephropathy as well as proteinuric renal disease following capoten treatment. Hii *et al.*, (1998) also found that capoten inhibits tumor growth in human renal cell carcinoma.

On the other hand, it is well known that the treatment with the antihypertensive drugs may be followed by a deterioration in the renal function. Although the renal protective effects of capoten are well recognized, only a few

years after its introduction, there have been several reports of renal failure associated with its use (Smit *et al.,* 1984; Swales, 1995; Rabb *et al.,* 1999 and Ionescu *et al.,* 2002).

The data indicate that although capoten may be the preferred drug for hypertensive neonates, infants, and pregnant women, it exerted undesirable effects in the kidney (Thomas *et al*., 1981). Capoten has been implicated in fetopathies in humans and perinatal mortality in rats, rabbits, sheep and baboons. Human fetopathies were seen when it was given around the 26th week of gestation. The major adverse effects in babies include: oligohydramnios, neonatal anuria, calvarial and pulmonary hypoplasia, mild to severe intrauterine growth retardation and fetal or neonatal death (Buttar, 1998).

The objective of this study is to evaluate the effect of the antihypertensive drug capoten on the kidney tubules of the adult and mice fetuses maternally treated with the drug.

Materials and Methods

A total of 60 pregnant and non pregnant females of CD-1 mice weighing 30 - 35 g were used in this study. They were divided into 4 main groups. The first group is non pregnant females and they were subdivided as follows: A) group recevied capoten for one month, B) group received capoten for two months, C) group received capoten for three months. These animals were sacrificed at the end of the treatment. The second group is pregnant females and they were subdivided as follows: A) group received the drug from 6th to 12th day of gestation, B) group received the drug from 6th to 18th day of gestation and the animals of both groups were sacrificed at the 19th day of gestation. Their uteri were dissected and fetuses were obtained and quickly dissected with the aid of a stereomicroscope to take out the kidneys. Capoten was used in daily oral dose of 0.2 mg which represents the equivalent of the daily human therapeutic dose as calculated by Paget and Barnes (1964). The third and fourth groups were the corresponding control non pregnant and pregnant females that received a similar volume of distilled water. Samples of kidneys were obtained, fixed in 2% glutaraldehyde in 0.1 M phospate buffer, post fixed in 1% osmium tetroxide for 2 hours at 4 ^{o}C, dehydrated and embedded in Epon 812. Semithin sections were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscope.

Results

Control renal tubules

Proximal tubules: The proximal convoluted tubule is lined by a single layer of cuboidal cells (Fig.1) that have an elaborate shape, well developed microvilli (or brush border) along the lumen, an active endocytotic apparatus, many spherical or elongated mitochondria and Golgi apparatus (Fig. 2). The brush border covering the luminal surface of the cells (Fig. 3) consists of long, closely packed microvilli. The nuclei of the cells are relatively large, mostly euchromatic with prominent nucleoli (Figs.2and3a) .

Distal Tubules: The cells lining the tubules appear to be lower and the tubules had larger luminal diameter than the proximal tubules. The cells do not have a brush border (Fig.3b), but a few luminal microvilli are seen (Fig. 4). Golgi bodies are few specially in the fetus and always lie near the nucleus. Nuclei are relatively large and their heterochromatin appear always attached to the nuclear membrane with some dispersed in nucleoplasm (Fig. 5). Adjacent distal tubule cells are adjoined through terminal bars (Fig.4). Mitochondria are not interposed between the nucleus and apical membrane, but do fill the perinuclear region (Fig. 5). They are cylindrical in shape with transverse cristae. The fetal tubules had smaller luminal diameter and exhibit less mitochondria.

Collecting tubules: the collecting tubule cells are low cuboidal with well-defined cell boundaries, spherical or ovoid, centrally located nuclei containing

moderate amount of heterochromatin and prominent nucleoli. They had pale stained cytoplasm and multiple small, randomly oriented mitochondria. In electron microscopical preparations, these cells display few small microvilli and a basal cell region that contained some tortuous infoldings of the basal cell membrane (Fig.6). These infoldings are not clearly seen in the fetal tubules.

Adult treated mice

The most remarkable feature noticed in all treated adult mice was the presence of hyaline casts in the lumen of most of the cortical renal tubules specially the proximal and distal tubules (Fig. 7). The second remarkable feature noticed in light microscopic preparations was the increase of the intertubular space and that increase was directly proportional with the period of administration, reached the maximum in animals administered capoten for 3 months (Fig. 8). Moreover, the tubules showed highly irregular contour and sometimes appeared indented which is due to the vacuolation of the basal regions of the tubule cells (Fig.8). Also, saturated and unsaturated lipids were distinguished as large accumulations of lipid globules between the kidney tubules specially at the juxtamedullary region in animals administered capoten for 3 months (Fig. 9).

In animals administered capoten for one month prominent increase in the amount of lipid granules and droplets was distinguished in proximal tubule cells (Fig. 10). Ultrastructurally, the droplets of saturated lipids were localized as accumulations taking the form of a cap between the nucleus and the brush border of the cells. However, no obvious damage was noticed in the cytoplasmic organelles (Fig.10) .

In animals administered capoten for 2 months, the accumulation of lipids in the apical region of the proximal tubule cells, was still the distinguishing feature (Fig. 11) together with the accumulation of hyaline casts in the lumen of the tubules. The endocytotic apparatus was distorted and transformed into irregular vacuoles . Other cells formed large blebs that extended to the lumen of the tubules (Fig. 12). In light microscopical peparations, some of the cells appeared very lightly stained, lost the characteristic basophilia of proximal tubule cells indicating signs of lysis and degeneration and the margins of all the tubules were irregular and sometimes indented (Fig. 12). The microvilli constituting the brush border were disorganized, hence, the endocytotic apparatus also showed great distortion where the small and large vacuoles fused together and formed irregularly vacuolized apical area (Fig. 11).

After 3 months of administration, proximal tubule cells revealed marked accumulation of hyaline casts which distorted the general architecture of the tubules (Fig. 8). Necrotic cells were frequently observed in many of the proximal tubules (Fig. 13). At the ultrastructural level, the brush borders were found disorganized, sometimes fragmented and the cells contained large amount of lipid droplets. Highly affected nuclei were frequently observed and large vacuoles containing cell debris became a prominent feature for some proximal tubule cells. These vacuoles occupied the basal areas of the cells and were usually found attached to the nuclei (Fig. 14) and the basal membrane infoldings were no more seen.

The distal convoluted tubules after one month of administration of capoten were found filled with hyaline casts (Fig. 7). Their lumena became irregular and the cells formed large blebs that extended deeply into the lumen. Most of the organelles except the mitochondria appeared degenerated leaving large vacuolated areas specially at the basal region. The microvilli became fewer, distorted and sometimes were inflated (Fig. 15). Early signs of pyknosis appeared in the nuclei of distal convoluted tubule cells, as the nuclei became more heteroc-hromatic (Fig. 15). Animals administered capoten for two months showed large apical blebs that obstructed the lumen of the distal convoluted tubules. In most of the cases, these large blebs were found filled with degenerated cytoplasmic organoids and their apical membranes were ruptured leaving the cytoplasm mixed with the lumen of the tubule. The nuclei showed evidence of karyolysis where, margination of the chromatin and depletion of

heterochromatin were the marked features frequently observed (Fig. 16).

Administration of capoten for 3 months caused a great damage to the renal tubules. The distal convoluted tubules displayed irregular contour and hence the basal infoldings of the plasma membrane of the cells became highly irregular and lost a great number of mitochondria, while the remaining mitochondria became opaque and irregular. Intertubular spaces greatly increased and blood capillaries, lying inbetween, were greatly damaged and possessed necrotic endothelial cells (Fig. 17).

In all treated groups whether for 1 , 2 or 3 months, the collecting tubules were obstructed by the dense hyaline matrix or the giant blebs formed by the principal cells or the dark cells (intercalated cells). This was more evident in individuals treated with capoten for 3 months (Fig. 18). Administration of capoten for one month specifically, was associated with the degeneration of the basal regions of collecting tubule cells. Prolonged administration of capoten for two and three months caused various forms of drastic changes. Obvious increase in the lipid content and lysosomes became the characteristic feature of the individuals given capoten for two months (Fig. 19). Moreover, nuclear damage in the form of vacuolization was evident and the basal cell membrane became extensively tortuous (Fig. 19). Electron microscopical preparations revealed abnormal blebing of the apical regions of the principal cells and the dark cells, in individuals administered capoten for 3 months (Fig. 20). The blebs contained granulated matrix with no definite organelles.

Maternally-treated fetuses

Light microscopic examination of the kidney cortex of fetuses maternally treated with capoten either from 6^{th} -12^{th} day or from 6^{th}-18^{th} day of gestation revealed apparent accumulation of mesenchymal cells. These cells were spindle-shaped with well defined cytoplasmic margins and ovoid or elongated nuclei (Fig. 21). The other characteristic feature was the presence of numerous fibroblasts inbetween the renal tubules and specially around blood vessels. Intertubular edema was also observed (Fig. 21).

Ultrastructural examination of the proximal convoluted tubule cells of the fetuses maternally treated with capoten from 6^{th} -12^{th} day of gestation revealed marked cytoplasmic vacuolation. The vacuoles were of different sizes, filled large cytoplasmic areas and sometimes contained cell debris (Fig. 22). The cells showed nuclei with irregular contour and the brush border microvilli were intact. In fetuses maternally-treated with capoten from 6^{th}-18^{th} day of pregnancy, cytoplasmic vacuolation was also represented and the vacuoles occupied nearly the apical area of the cells. Moreover, there was a marked increase in lysosomes which were found at the basal region of the cells (Fig. 23).

The distal convoluted tubules of the fetuses maternally treated with capoten from 6^{th} -12^{th} day of gestation showed no apparent change as compared with the control fetuses. However, in fetuses maternally treated from 6^{th} -18^{th} day of gestation, most of the distal convoluted tubule cells showed atrophy, their apical surface membranes were degenerated with marked loss of their surface mircroprojections. The tubules were surrounded by fibrillary networks and the cells also showed marked loss of their mitochondria (Fig. 24).

The collecting tubule cells of the fetuses maternally treated with capoten from 6^{th} -12^{th} day of gestation showed mild loss of their apical microprojections and few small vacuoles were seen in the apical regions of the cells (Fig. 25). In the fetuses maternally treated with capoten from 6^{th} -18^{th} day of gestation, there was a marked loss of the cell microprojections while the cytoplasmic organelles were intact (Fig. 26).

Ultrastructural investigations confirmed the same feature observed at the light microscopical level which was the presence of a fibrillary network and numerous fibroblasts inbetween and surrounding the renal tubules. In addition to edema in the kidney of nearly all fetuses (Figs.24 and 25) .

Explanation of figures

Figures from 1 to 6 are for control kidney of mice

Fig. 1 Semithin section of kidney cortex, showing several profiles of the proximal convoluted tubules with their characteristic brush borders (arrows) and centrally located nuclei (arrow heads) with prominent nucleoli Notice that the intertubular space is narrow. (Toluidine blue X 400)

Fig. 2 Normal structure of proximal convoluted tubule cell characterized by numerous mitochondria (m) and the apical microvilli (arrow) constituting a brush border, below which lie the endocytotic apparatus (arrow head). Golgi apparatus takes the C shape (g). On the lower right hand corner appear small part of proximal tubule cell where, the basal striations of the basal plasma membrane takes the palisade appearance and contain elongated mitochondria. (X 8300)

Figs. 3a & b Semithin sections, showing the difference between A) the proximal tubule (arrows) which possess brush border (b) and B) distal convoluted tubule profiles (arrow heads). (Basic fuchsin-methylene blue X 1000)

Fig. 4 Distal convoluted tubule cells with their characteristic apical microvilli (arrow), which do not take the brush border form and extensive amplification of the basolateral membranes (bm) by forming numerous invaginations. Numerous mitochondria (m)are present and vacuoles of endocytotic apparatus appear at the apical region (nda). Terminal bars (tb) are the sites of joining between the adjacent cells . (X11300)

Fig. 5 Distal convoluted tubule cell with its basal plasma membrane forming extensive basal striations (arrow) containing elongated mitochondria (m) with transverse cristae while Golgi apparatus lie near the nucleus (g). Nucleus is large containing enormous heterochromatin. (X13700)

Fig. 6 Two collecting tubule cells possessing ovoid centrally located nuclei with few relatively heterochromatin. Mitochondria (m) are small randomly oriented and the cell surface carry few small microvilli or microprojections (arrow head). Basal plasma membrane contains some tortuous infoldings (arrows). (X7000)

Fig. 7 Semithin section of kidney cortex from a mouse administered capoten for 1 month. Intertubular space is slightly dilated, proximal and distal tubules are filled extensively with hyaline casts (arrows) and possessed blebs (arrow heads). (Toluidine blue X 400)

Fig. 8 Semithin section of kidney cortex from a mouse administered capoten for 3 months. Intertubular space became very wide and the contours of the kidney tubules became highly irregular due to the vacuolation of the basal areas (arrow heads), while their lumena are obstructed by thickened hyaline casts. One of the blood capillaries carries abnormal endothelium (arrow).The intertubular spaces are greatly widened. (Toluidine blue X 400)

Fig. 9 Semithin section of kidney at the juxtamedullary region from a mouse administered capoten for 3 months. Lipid globules accumulate at the region between cortex and medulla (arrows). (Toluidine blue X 320)

Fig. 10 Proximal tubule cells from the kidney of a mouse administered capoten for 1 month. Lipid droplets accumulate like a cap above the nucleus (arrows) otherwise the cells are intact. (X4800)

Fig. 11 Proximal tubule cell from the kidney of a mouse administered capoten for 2 months. Lipid droplets (arrow head) are numerous and mostly apical in position. Vacuolization of the apical region (arrow) distorted the endocytotic apparatus. (X8000)

Fig. 12 Semithin section of kidney cortex from a mouse administered capoten for 2 months. One of the tubules

(arrow) is characterized by a necrotic lightly stained cell and a large bleb (arrow head). Most of the tubules have irregular architecture and the intertubular space is widened . (Toluidine blue X 400)

Fig. 13 Semithin section of kidney cortex from a mouse administered capoten for 3 months. Obvious damage of the tubule cells (arrows) characterize the 3 months treatment.(Toluidine blue X 650)

Fig. 14 Proximal tubule cells from the kidney of a mouse administered capoten for 3 months. Microvilli are disorganized (arrows), while the lumen contains cell debris from ruptured blebs (arrow head). Large vacuoles containing cell debris (thick arrow) are seen. (X 3200)

Fig. 15 Distal convoluted tubule cells from the kidney of a mouse administered capoten for 1 month. Microvilli are distorted (arrows), basal cytoplasmic areas are degenerated (arrow heads) and nuclei show early signs of pyknosis. (X 7000)

Fig. 16 Distal convoluted tubule from the kidney of a mouse administered capoten for 2 months. Apical blebs (arrows) obstruct the lumen and their membranes are ruptured and their degenerated apical cytoplasm is mixed with the lumen (arrow head). (X 3900)

Fig. 17 Distal convoluted tubule (arrow) from the kidney of a mouse administered capoten for 3 months. Basal membrane infoldings are disorganized (arrow head), mitochondria became opaque and irregular (arrow) and the blood capillary possessed damaged endothelium (asterisk). (X 7000)

Fig. 18 Semithin section showing a collecting tubule from the kidney of a mouse administered capoten for 3 months. The dark cell (arrow) and principal cell (arrow head) possess giant blebs. The dark cell is highly infiltrated with lipids and its nucleus (n) appeared pyknotic. The lumen of the tubule is filled with hyaline matrix (asterisk). (Toluidine blue X 1000)

Fig. 19 Collecting tubule cells from the kidney of a mouse administered capoten for two months. The cells show remarkable increase in lipids and lysosomes (arrows), while the nuclei (n) show vacuolization and the basal membrane became extensively tortuous (arrow head). (X 7000)

Fig. 20 Collecting tubule cell from the kidney of a mouse administered capoten for 3 months showing the blebing of the apical plasma membrane (arrow). (X 8000)

Fig. 21 Semithin section of kidney cortex of a fetus maternally treated with capoten from 6^{th}-12^{th} day of gestation showing apparent accumulation of mesenchymal cells (short arrows). Fibroblasts are seen between the tubules and around the blood vessel (arrow head). Notice the presence of edema (long arrow). (Toluidine blue X 150)

Fig. 22 Electron micrograph of a proximal convoluted tubule cell of a fetus maternally treated with capoten from 6^{th}-12^{th} day of gestation. Numerous vacuoles (v) are seen in the cytoplasm, some of them contained cell debris), while the brush border is intact. (X 6000)

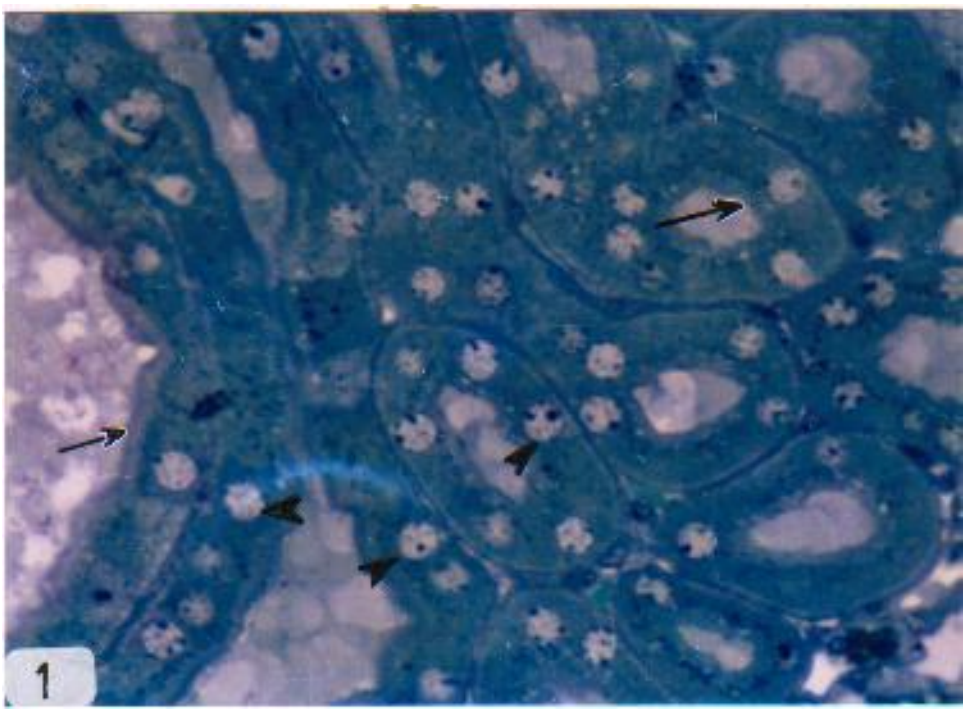
Fig. 23 Electron micrograph of a proximal convoluted tubule of a fetus maternally treated with capoten from 6^{th}-18^{th} day of gestation. Numerous lysosomes (L) and vacuoles (v) are seen in the cytoplasm. A fibrillary network is seen surrounding the tubule (arrow). (X 4000)

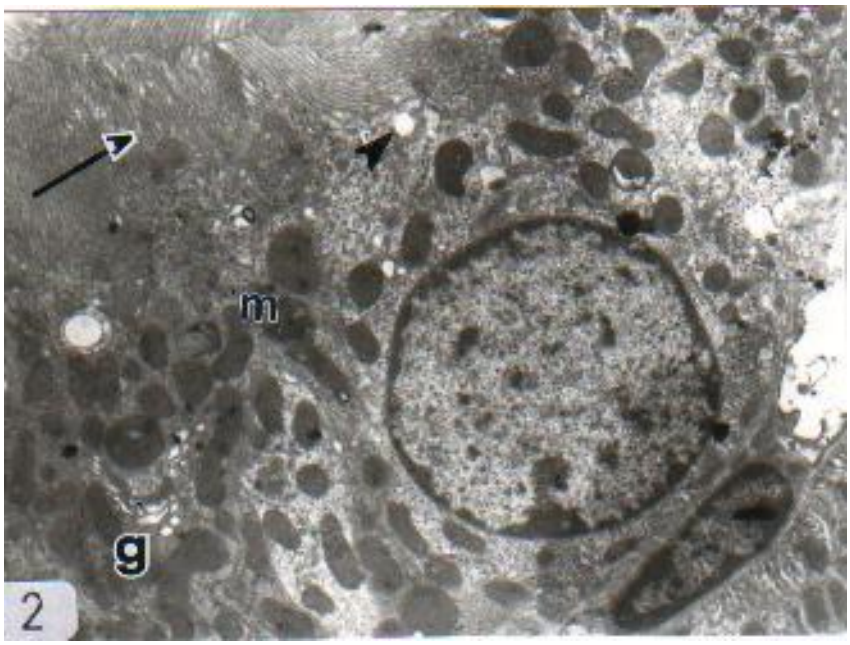
Fig. 24 Electron micrograph of a distal convoluted tubule of a fetus maternally treated with capoten from 6^{th} - 18^{th} day of gestation. The cells lost their microprojections and their apical surfaces are degenerated (arrow head). Fibrillary network is seen surrounding the tubule (arrows). (X 3000)

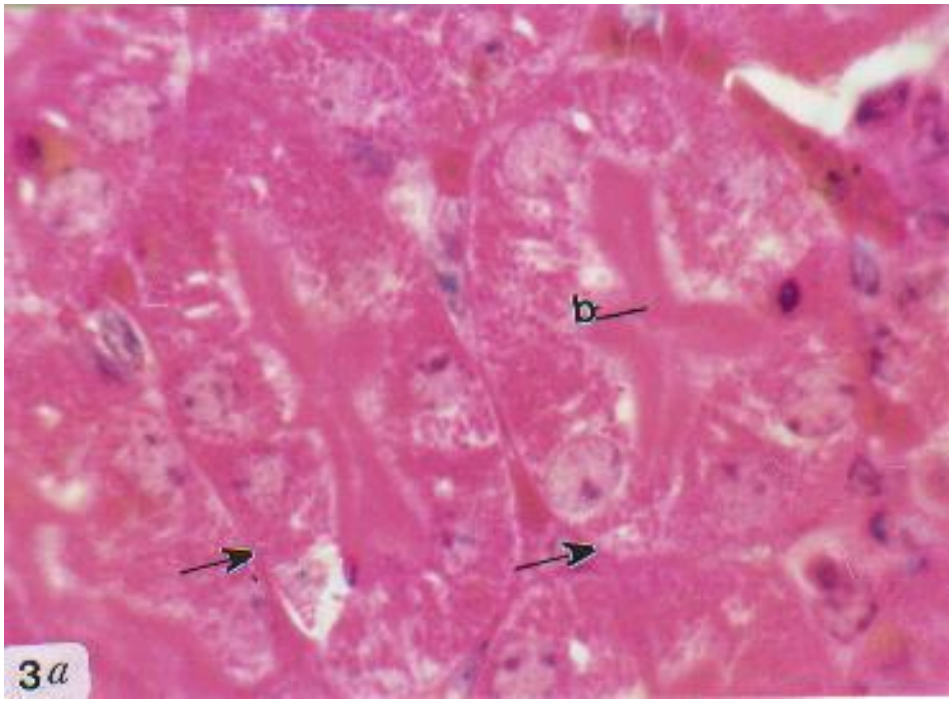
Fig. 25 Electron micrograph of a collecting tubule of a fetus maternally treated with capoten from 6^{th} -18^{th} day of gestation showing mild loss of surface microprojections (arrow). Few vacuoles (v) are seen in the

apical regions of the cells. Intertubular edema (E) and fibrous network (arrow heads) are seen. (X 3000)

Fig.26 Electron micrograph of a collecting tubule of a fetus maternally treated with capoten from 6^{th}-18^{th} day of gestation showing nearly complete loss of surface microprojections (arrows) The nuclei showed fragmented chromatin. (X 4300)



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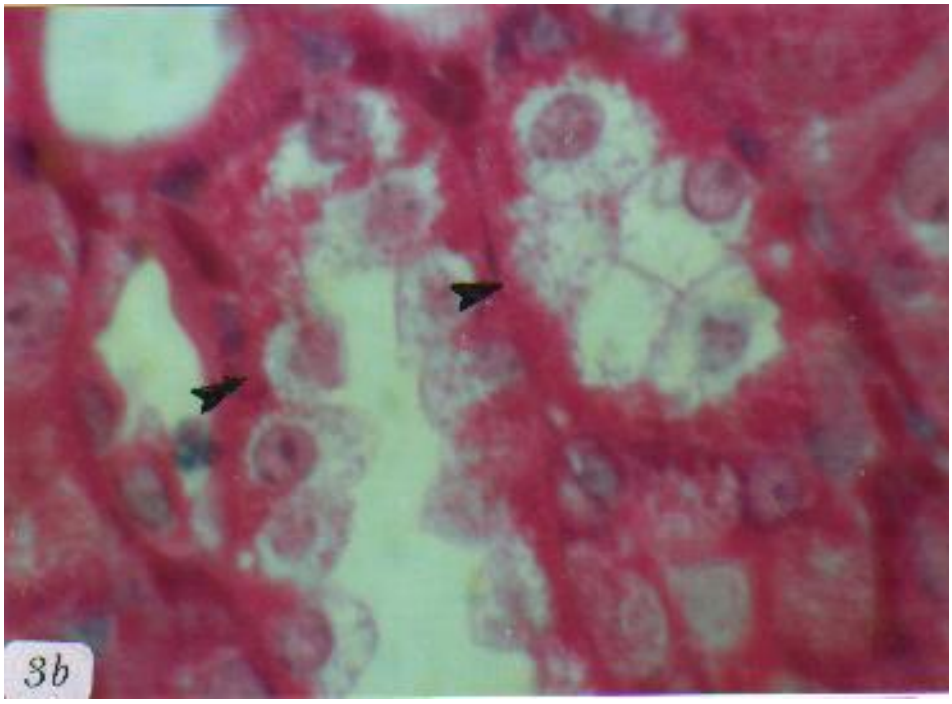
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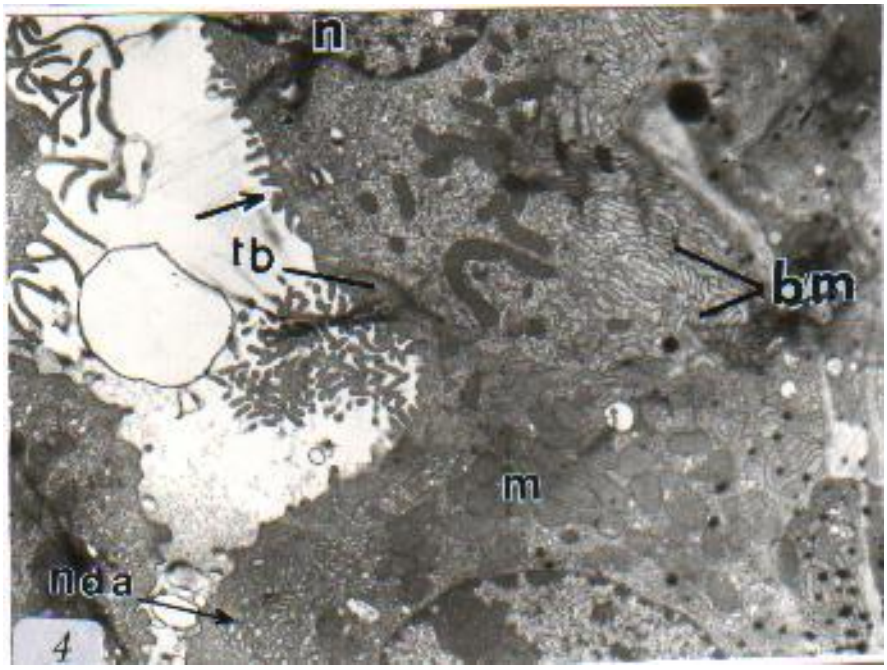
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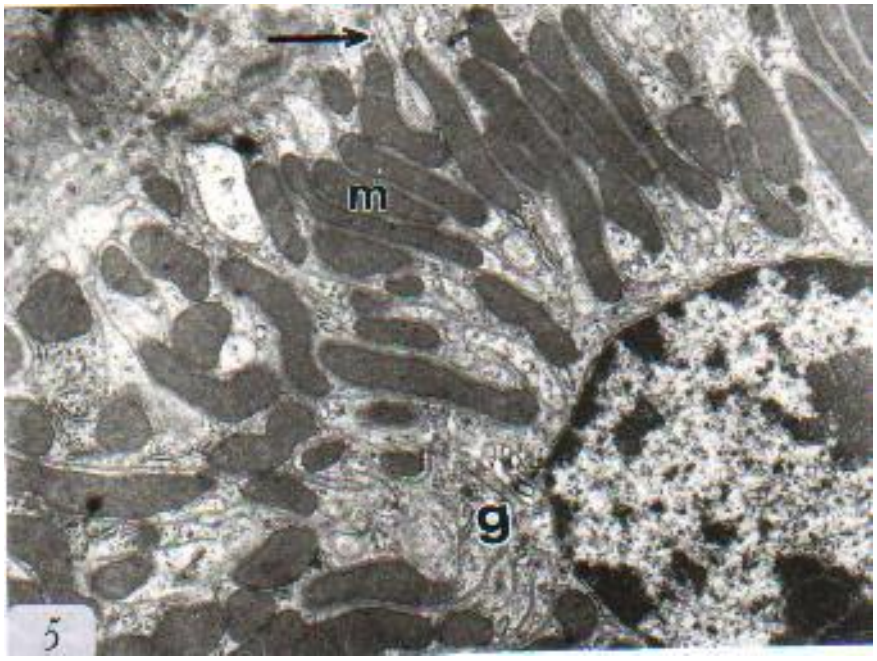
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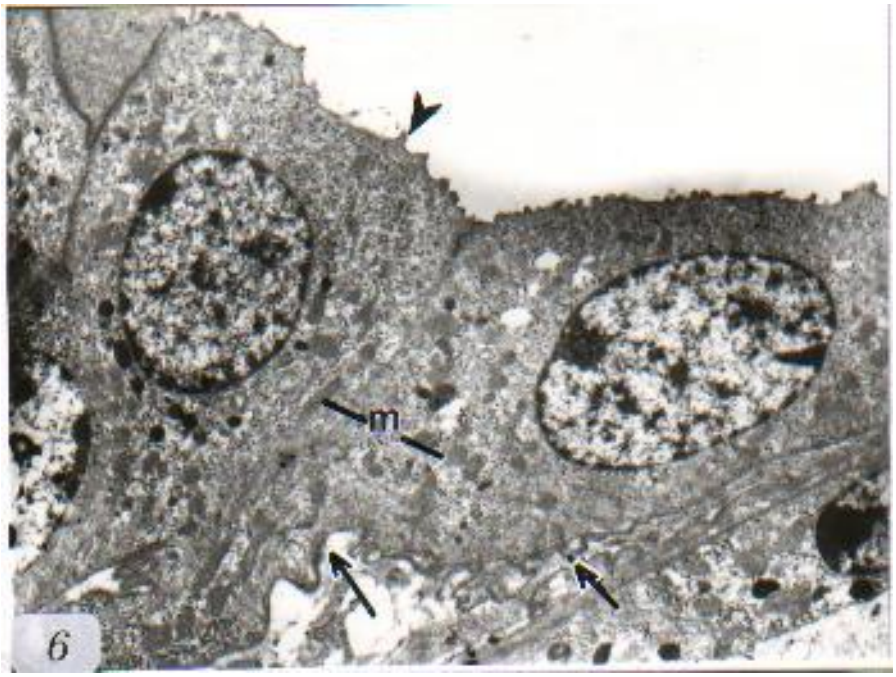
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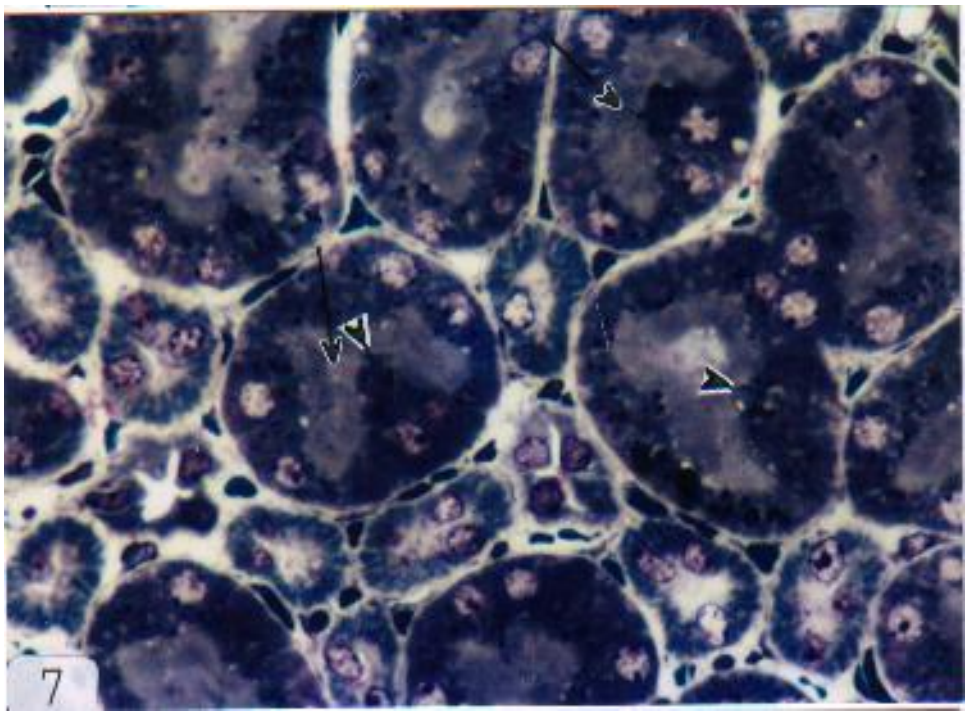
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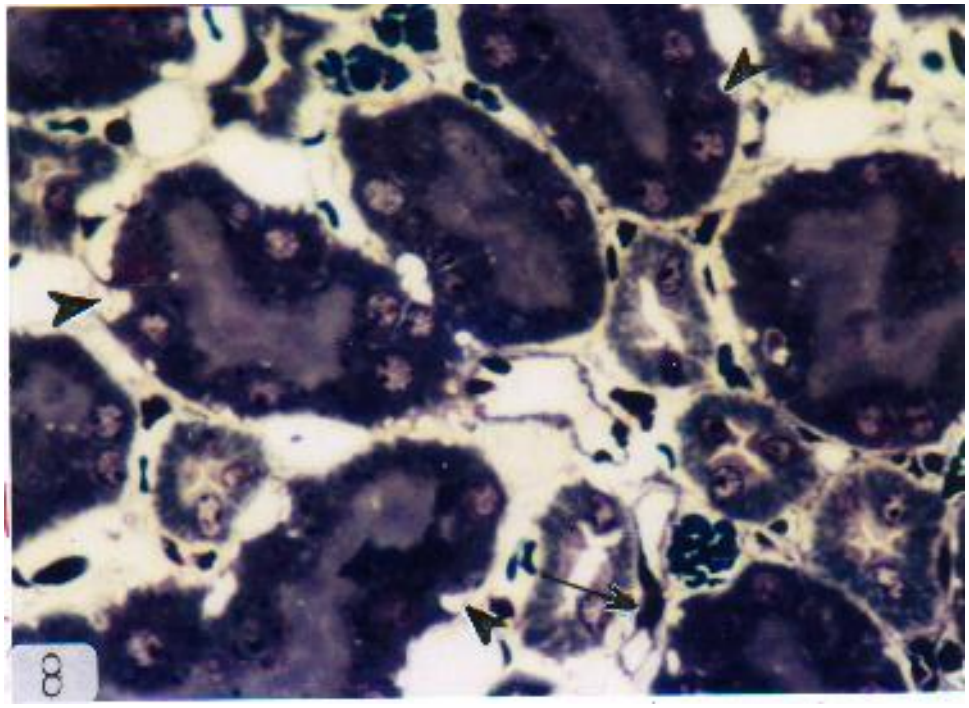
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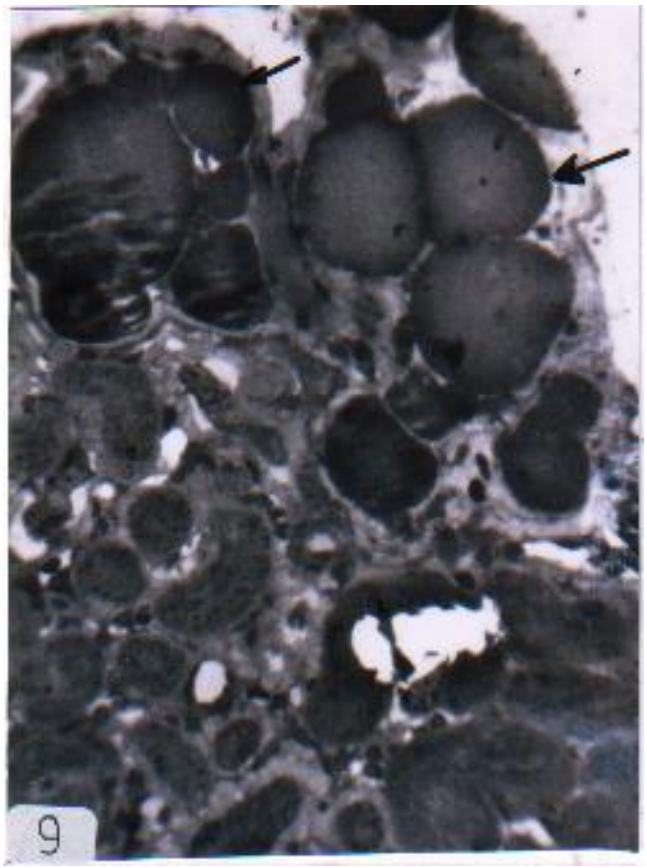
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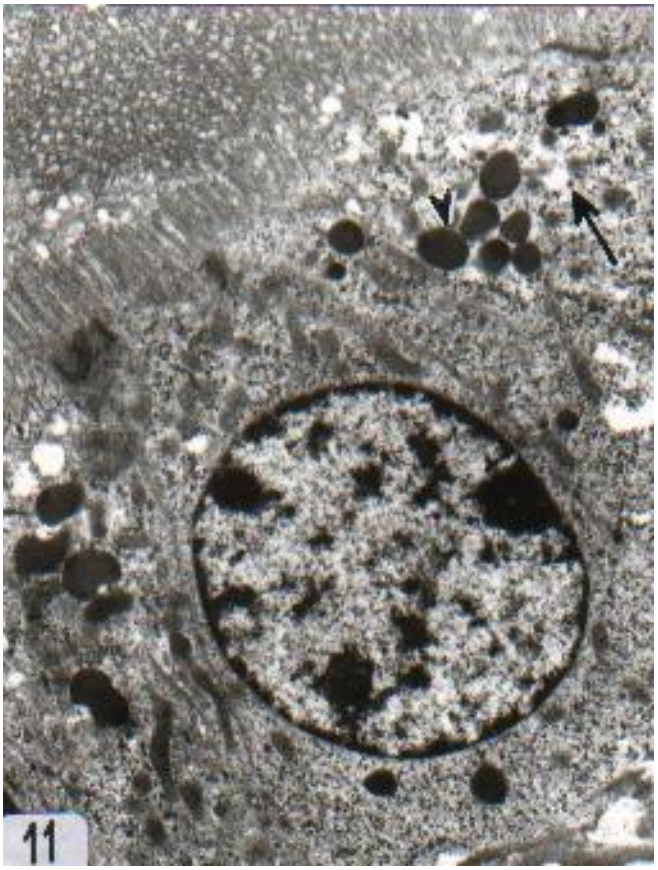
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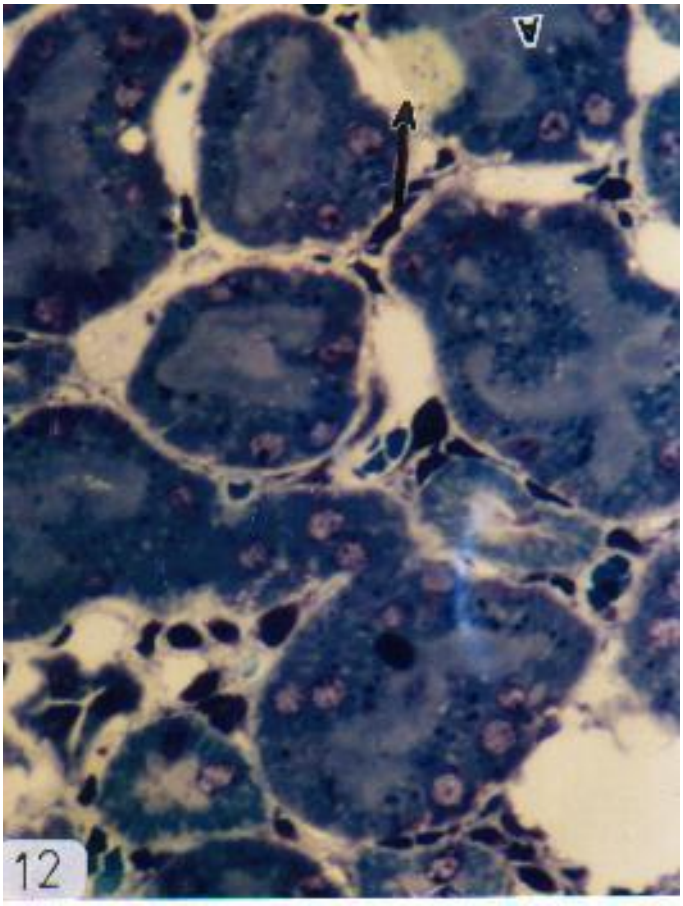
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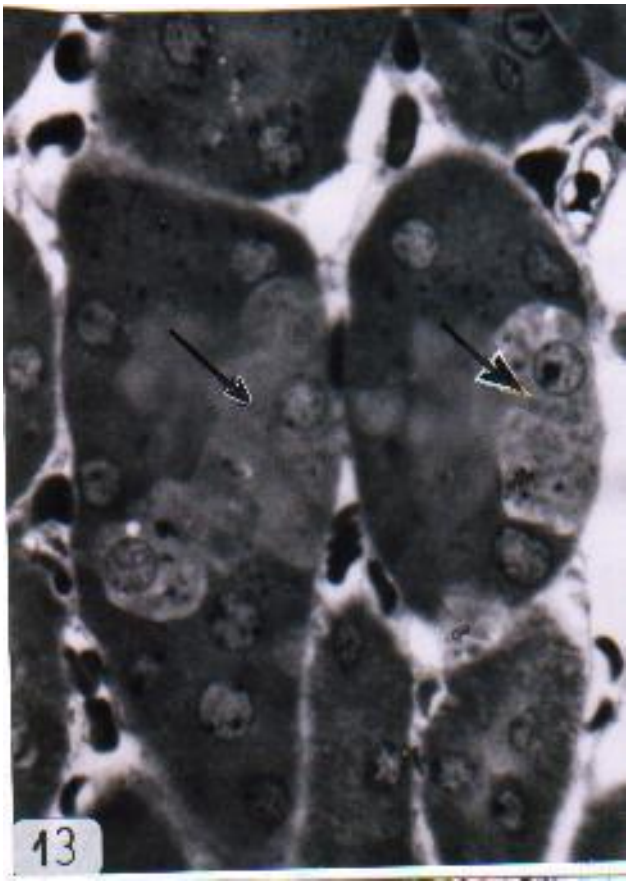
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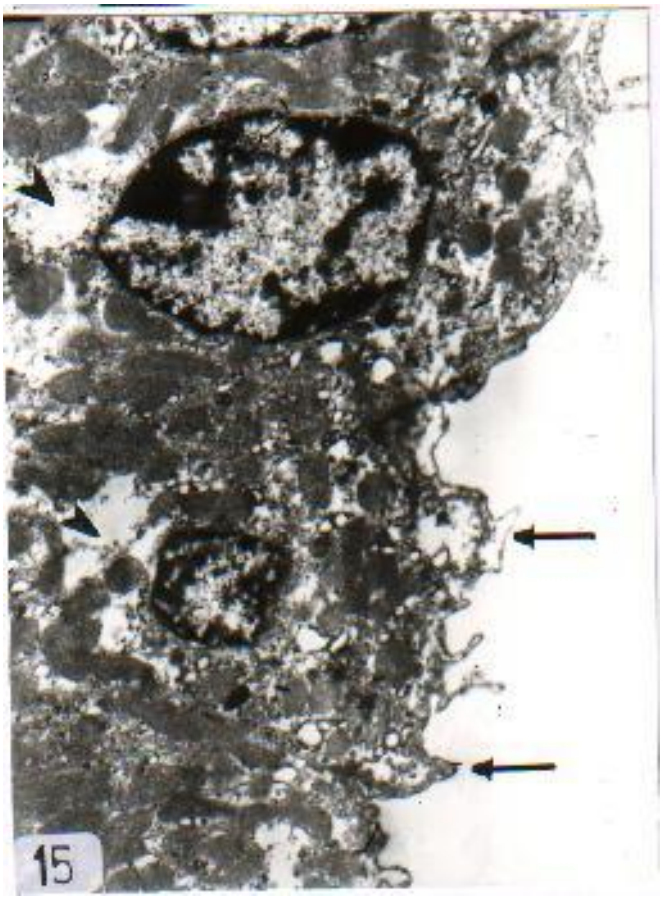
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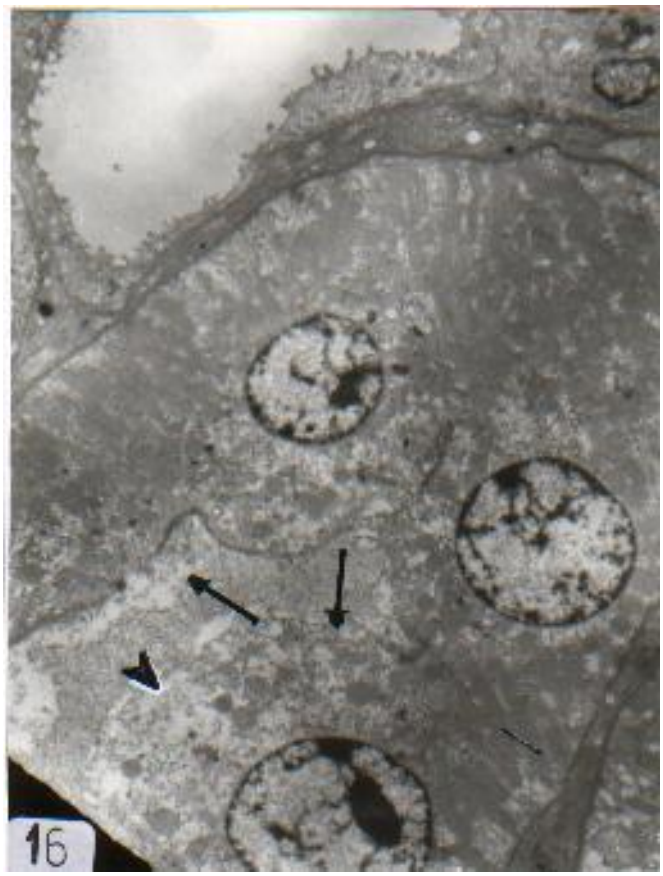
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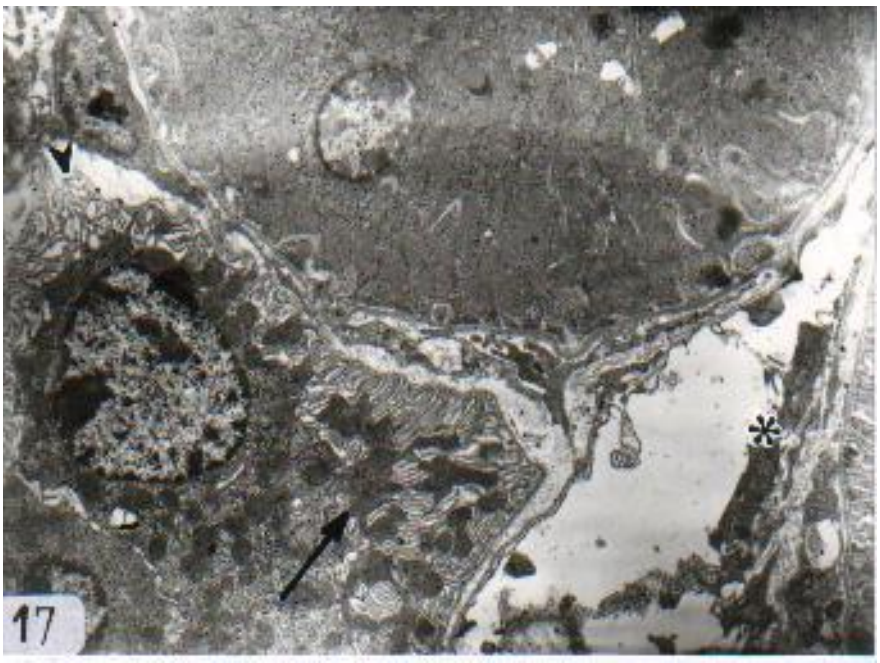
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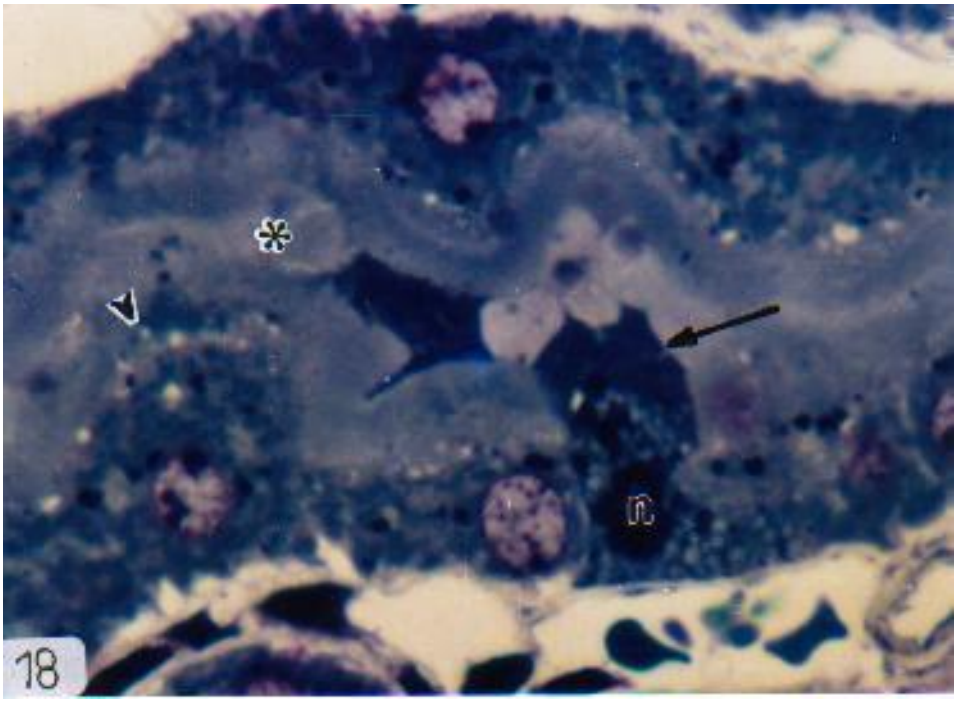
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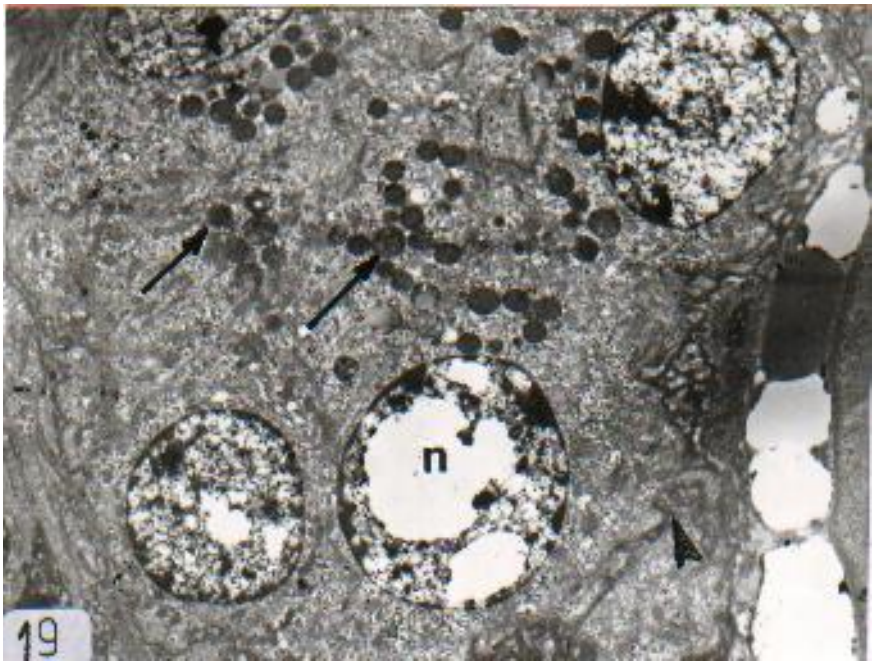
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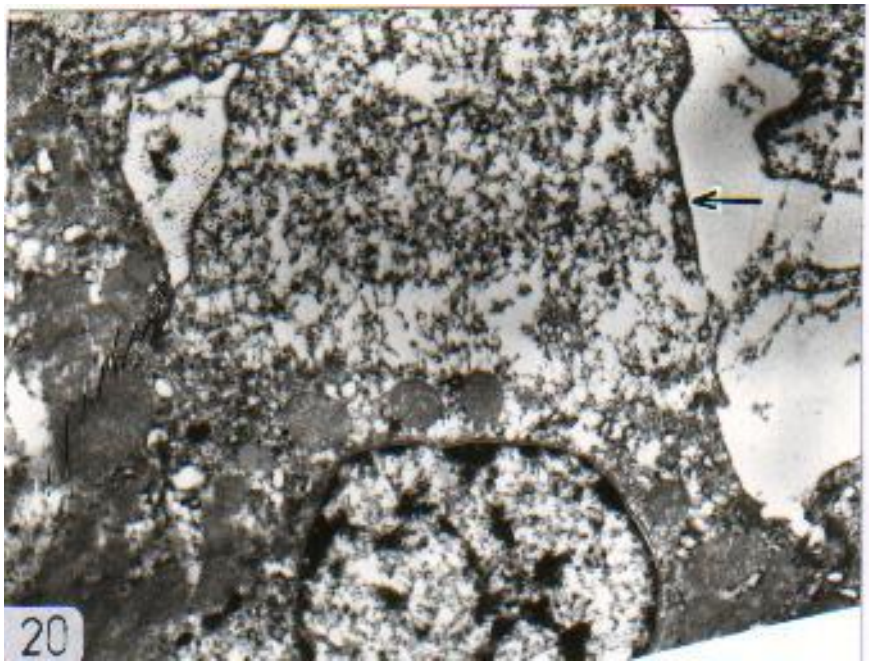
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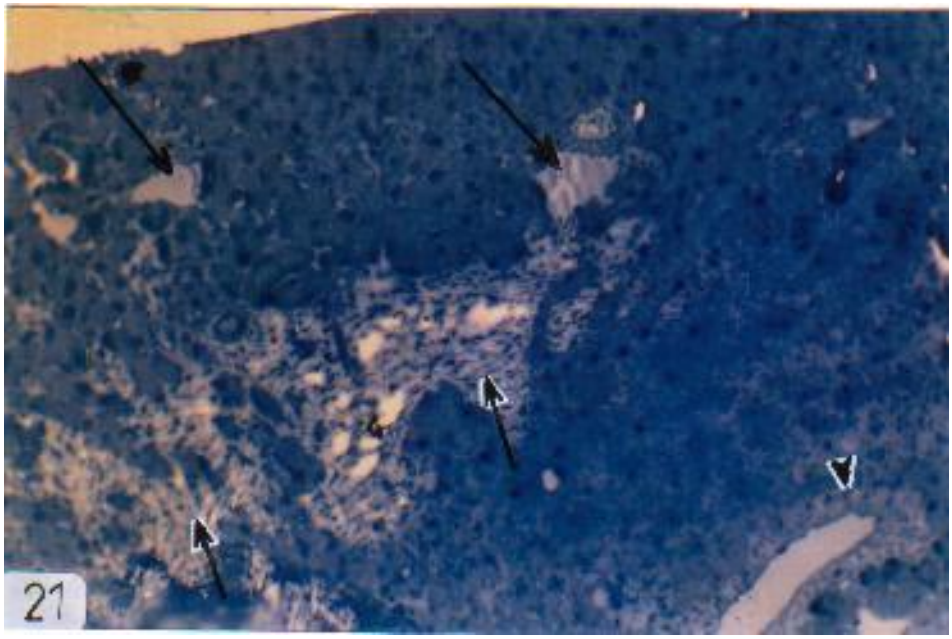
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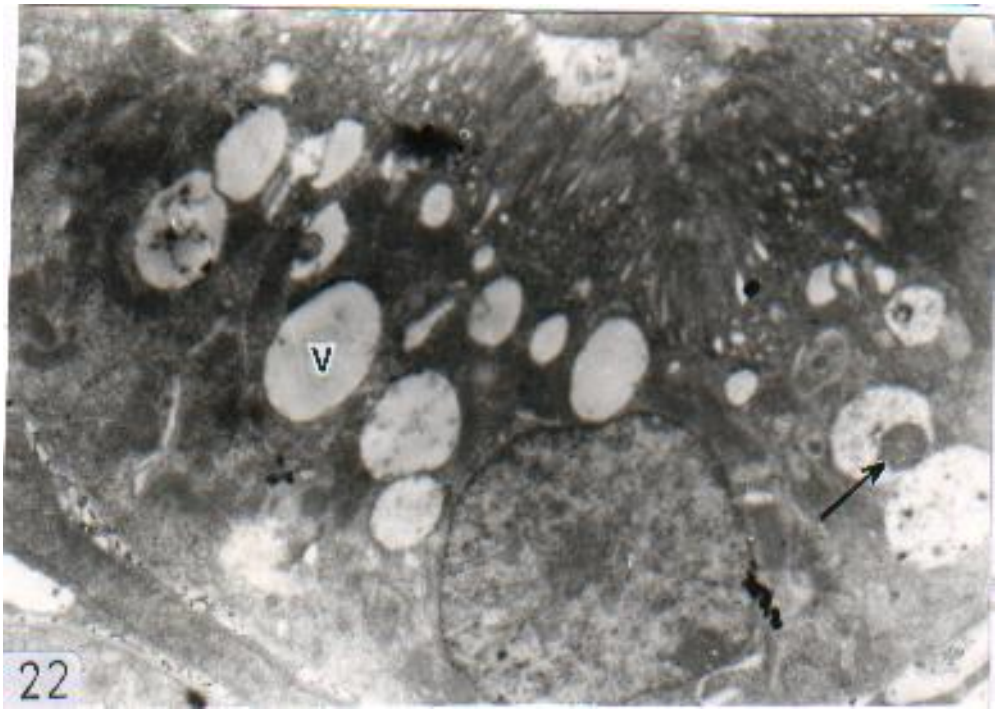
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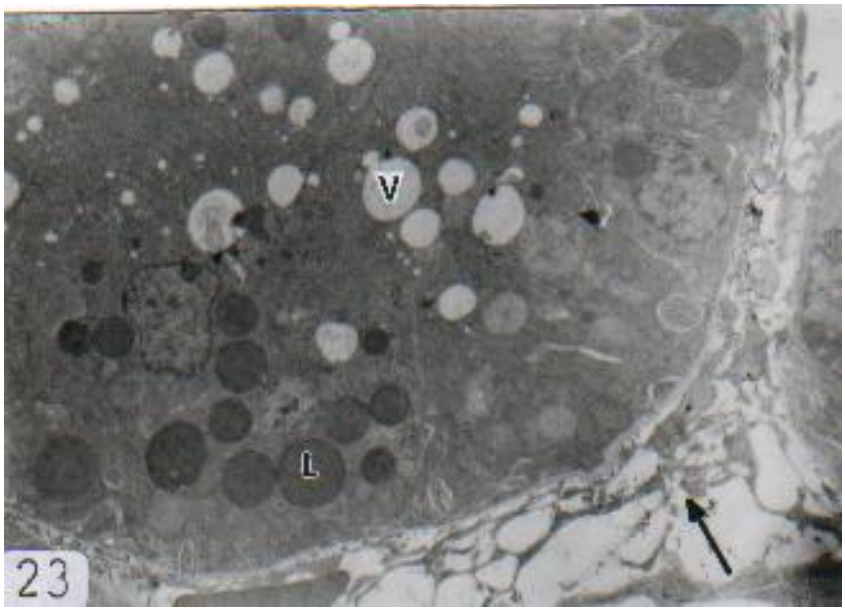
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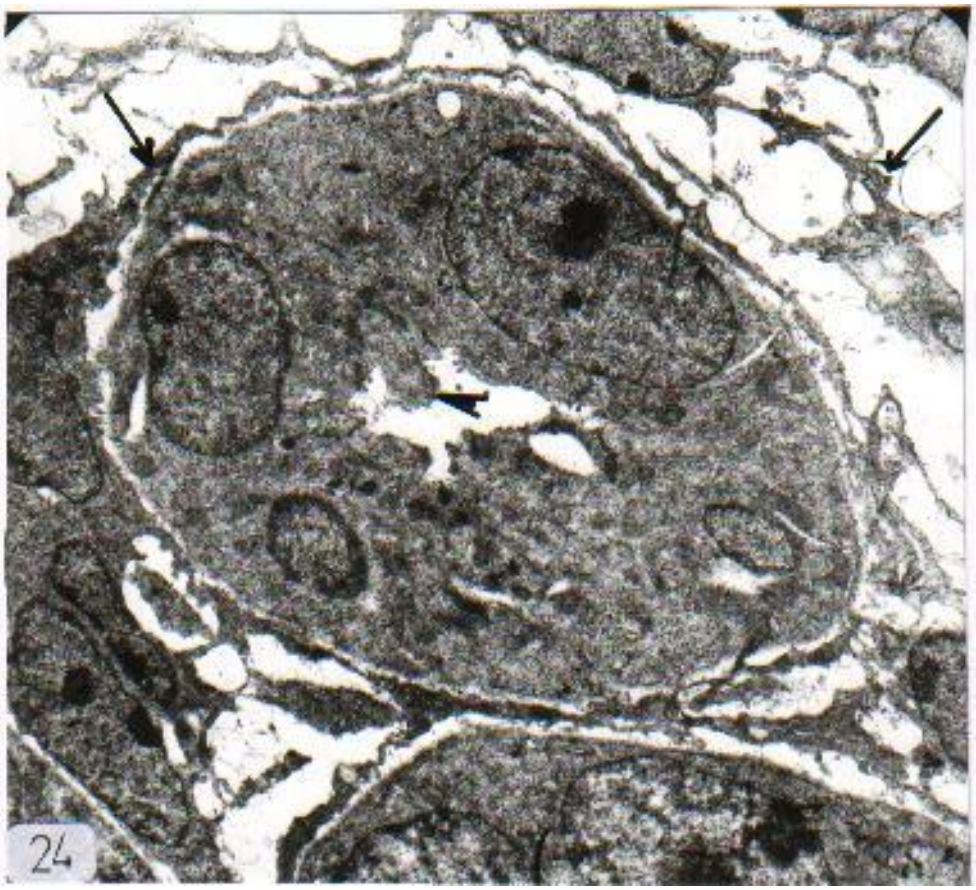
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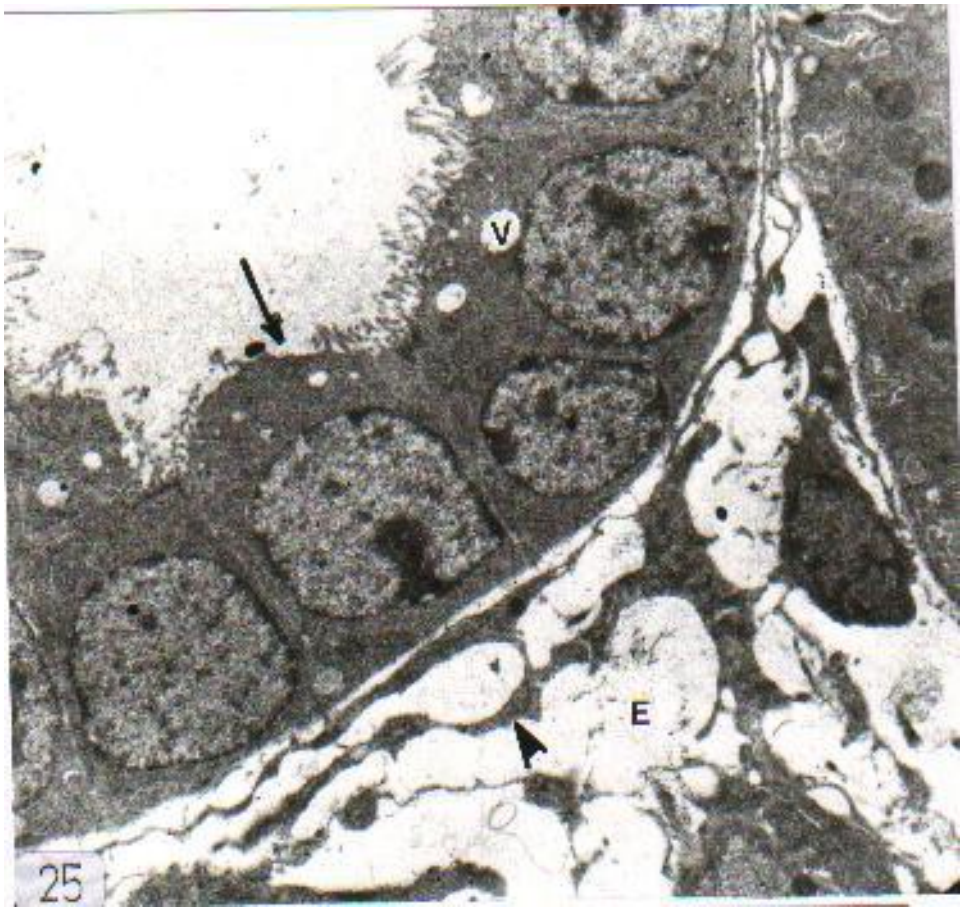
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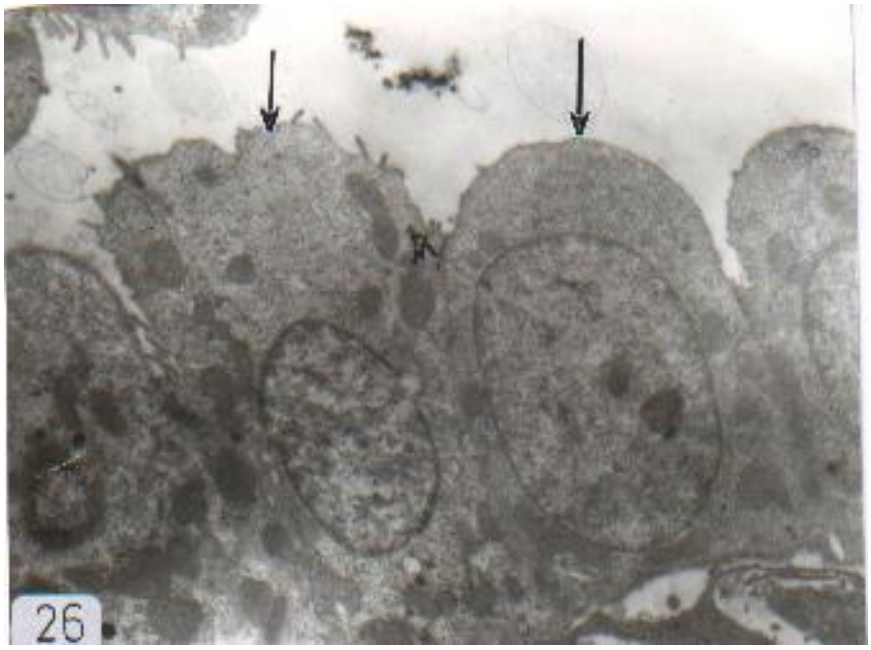

Discussion

Capoten recently has attracted much attention as an oral antihypertensive agent. It causes long-term inhibition of the converting enzyme which converts angiotensin I to the vasoconstrictor angiotensin II. Thus it causes a decrease of angiotensin II in plasma which by its turn lowers the blood pressure (Rang *et al*., 2003). The angiotensin converting enzyme (ACE) inhibitors seem to be the most suitable antihypertensive drugs for diabetic patients as well as pregnant women (Schersten, 1988) and animal studies have demonstrated a renal protective effect of angiotensin converting enzyme inhibition therapy in chronic renal failure (Savage and Schrier, 1992). In the study of Gupta *et al*. (1999), it was found that administration of capoten offered protection against the development of nephrotoxicity in diabetic patients. They concluded that, the abnormalities of renal perfusion which lead to nephrotoxicity might be mediated by renin angiotensin system, hence capoten is indicated for the treatment of diabetic nephropathy. Capoten is also reported to decrease the rate of progression of renal insufficiency and development of serious adverse clinical outcomes (Rang *et al*., 2003).

In spite of the above mentioned positive effect on diabetic patients, Isogai *et al*. (1998) reported that doses of capoten at 25 mg/k body weight didn't exert any antihypertensive or antidiabetic effect on diabetic spontaneously hypertensive rats. Moreover, Herings *et al.* (1995) reported that hypoglycaemia is associated with the use of ACE inhibitors in diabetic patients.

Clinically, Swales (1995) reported that there is no reasonable doubt that in severe hypertension or in hypertensive patients with primary renal disease, control of blood pressure by using ACE inhibitors has a beneficial effect on renal function. In agreement with this assumption, capoten treated rats after subtotal (5/6) nephrectomy showed conspicuously better function of kidney tissue remnants than untreated animals (Jelinek *et al*., 1995). Also, in the study of Brooks *et al.* (1995) they found that capoten significantly attenuated hypertension in rats with chronic renal failure induced by 5/6 nephrectomy and also attenuated the pressor activity of exogenous angiotensin II and angiotensin I in addition to attenuation of renal hypertrophy. As a renal protective, nephrologists suggested that capoten may be beneficial as a protective agent against nephrotoxicity (Mansour *et al*., 1999).

It is known that the reactive oxygen has been proposed as a key mediator of the progression of renal injury. Among the defense systems operating against the

reactive oxygen, superoxide dismutase, glutathione peroxidase and catalase are the most important antioxidant enzymes and it was found that capoten increases the activities of these enzymes, so it has the ability to improve renal injury (Rang *et al*., 2003).

On the contrary to the above mentioned reports several authors reported that capoten might had side effects on the kidney, where Ganapathy *et al*. (1985) found that capoten induced inhibition of renal and hepatic prolidases in rat and human. Also Reznik *et al*. (1989) emphasized the need to be aware of the possibility of renal injury in premature infant patients receiving capoten therapy. High doses of ACE inhibitors were found to be toxic at the doses of 500 and 1000 mg/k body weight in rats and that this toxicity included, kidney weight increase, proximal tubular degeneration and juxtaglomerular hyperplasia (Takase *et al*., 1995).

Swelim and Sakr (1996) found that capoten had induced ultrastructural changes in renal corpuscles of CD-1 adult mice and these changes were time dependent and suggested that ultrafiltration process might be affected. The present study is complementary to that study and the present results assured that long term administration of capoten could affect also the kidney tubules to a great extent. This effect included the formation of hyaline casts which blocked the tubular lumena, increased lysosomes and lipid content, necrosis and tubular degeneration. This tubular damage has features in common with a pattern produced by hypoxia, which can be produced from ACE inhibitors treatment (Rubin, 1995). The latter author attributed this tubular damage to: (1) Impaired mitochondrial oxygen uptake. (2) Increased calcium influx from the extracellular to intracellular compartment, with activation of calcium-dependent phospholipases, resulting in breakdown of cell membranes with production of toxic free fatty acids and lysophospholipids. (3) Release of cytotoxic lysosomal enzymes into the cytosol. Other authors (De-Azevedo *et al*., 1997 and Lin *et al.,* 1997) reported that capoten failed to show any effect on the glomerular filtration rate and urinary albumin excretion or to improve a case that had congenital nephrotic syndrome respectively.

Recently, it was reported in a medical study on patients receiving capoten therapy that although it is an antihypertensive drug, 23.6% had increasing blood pressure proportional with ACE inhibitor dose, 14.4% had renal failure, and 1.2% had both increasing blood pressure and renal failure (Ionescu *et al*., 2002) .

The present results confirm the previous mentioned reports which suggested that capoten might had nephrotoxic effect. These effects might be due to the rapid fall of the blood pressure, followed by development of hypotension that can lead to symptomatic renal hypoperfusion (Al Shohaib and Raweily, 2000). This might lead to disturbance of the secretory and reabsorption processes performed by renal tubules. Therefore, tubular necrosis and high creatinine levels were recorded in patients receiving capoten (Al Shohaib and Raweily, 2000 and kutyrina *et al*., 2002). Also, hypotension that might be induced due to capoten therapy is likely to be the reason for the histologic and ultrastructural changes recorded in the present study. This is in agreement with Kreisburg *et al*. (1976) and Kumagai *et al*.(1999) who found that hypotension caused cellular changes and cell death in proximal tubules, pars convoluta and pars recta. Also, one of the remarkable features noticed in the present study in adults treated with capoten was the formation of tubular casts in the lumena of most of the tubules . This finding is similar to that reported by Rabb *et al*. (1999), as they reported that some patients had developed intratubular cast nephropathy after the use of angiotensin converting enzyme inhibitors .

Concerning the effect of capoten on pregnant females, August *et al*. (1995) reported that the role of the renin-angiotensin system in pregnant females is different from that in non pregnant females. They reported blood pressure decrease and renin activity increase in both pregnant and non pregnant women following captopril treatment. However, in

pregnant females, the plasma renin activity increased four times higher than normal. They suggested that this rise in renin activity during pregnancy is partly due to estrogen- mediated stimulation of angiotensinogen production and partly to the extrarenal renin, derived from the placenta or uterus. This increase in renal renin release increased the generation of angiotensin II which by its turn resulted in increase of blood pressure to equalize the lowered blood pressure (August *et al*., 1995) and accordingly the fetal abnormalities did not result from mother hypotension. On the contrary, Buttar (1998) attributed the fetal and neonatal abnormalities following capoten treatment to the maternal hypotension followed by decrease in fetal placental blood flow and lower oxygen nutrient delivery to the fetus. He also attributed these abnormalities to the direct action of ACE inhibitors on the fetal renin-angiotensin system.

In the present work, marked increase in the mesenchymal cells and fibroblasts were observed in all fetuses maternally-treated with capoten. Mesenchymal cells are formed during early embryonic development from mesoderm and neuroectoderm. These cells are the ancestors of most of the connective tissue cells, including fibroblasts (Weiss, 1988). This might explain the present recorded increase in the number of fibroblasts which found distributed elsewhere between the renal tubules. Generally, tissue injury is followed by a complex set of interrelated cellular and humoral reactions that remove or neutralize injurious agents, eliminate the damaged tissue, and promote healing. Most of these reactions occur in the fibroblasts (Rubin, 1995). These fibroblasts might be responsible for secretion of the fibrillary network which were observed surrounding nearly all the cortical tubules in the current experiment.

Interstitial edema was also observed in the kidney of the fetuses maternally treated with capoten. Rubin (1995) attributed renal edema caused by ACE inhibitors to the massive loss of protein to the urine, the magnitude of which exceeds the rate of replacement by the liver. The resulting decline in the concentration of plasma proteins, particularly albumin, reduces the oncotic pressure of the plasma and promotes edema. Conclusively it can be suggested that capoten-induced damage in non pregnant females might be caused by supposed hypotension, while in fetuses it might be due to direct toxic effect of the drug or its interference with the renin angiotensin system. So it is recommended that caution should be exerted during capoten treatment, and biochemical monitoring of kidney function before and after therapy is a must .

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دراسة تركيبية دقيقة عن الضرر المستحدث بواسطة عقار الكابتوبريل فى الأنيبيبات الكلوية للفئران البالغة والأجنة

حمدى حامد سويلم وعليه احمد محمد صقر
جامعة عين شمس – كلية العلوم – قسم علم الحيوان

أجري هذا البحث لدراسة التغيرات المرضية المحتملة فى كلية فئران التجارب نتيجة للمعالجة بعقار الكابتوبريل الخافض للضغط (مثبط لأنزيم أنجيوتنسين). وقد أجرى هذا البحث على إناث الفئران البيضاء من نوع س دى-1 لمعرفة تأثير هذا العقار على الأنيبيبات الكلوية لكل من الفئران البالغة وأجنة الفئران الحوامل. ولقد عوملت الفئران البالغة لمدة شهر وشهرين وثلاثة اشهر على التوالى أما الفئران الحوامل فتمت المعاملة لمدة أسبوع (من اليوم السادس الى اليوم الثانى عشر) وأسبوعين (من اليوم السادس الى اليوم الثامن عشر) أثناء الحمل. وتمثل الجرعة التى استخدمت فى هذا البحث المكافىء للجرعة العلاجية التى يستخدمها الانسان . وقد أظهرت النتائج وجود قوالب زجاجية والتى ظهرت فى صورة أجسام سادة لمعظم الأنيبيبات الكلوية , و أظهرت الدراسة أيضا زيادة واضحة فى حجم الفراغات الموجودة بين الأنيبيبات الكلوية وكذلك تعرج واضح فى شكل الأنيبيبات (عدم انتظام الشكل) وذلك نتيجة تحلل وتراكم الفجوات فى الأجزاء القاعدية من الخلاياالمبطنة للأنيبيبات. وقد تسبب الدواء فى ظهور زوائد كبيرة فى الخلايا المبطنة للأنيبيبات وزيادة فى الأجسام الدهنية بالأضافة الى التحلل الواضح لمجموعة كبيرة من الخلايا.
كما أظهرت الدراسة أن لهذا الدواء تأثير على الانيبيبات الكلوية لأجنة الفئران المعاملة عن طريق الأم أثناء الحمل ، تمثلت هذه التأثيرات فى زيادة الأجسام الدهنية والليسوسومات فى الأنيبيبات الملتوية القريبة وتحلل وضمور فى الانيبيبات الملتوية البعيدة خاصة فى المجموعة المعاملة لمدة أسبوعين وبالاضافة الى ذلك,فقد كان هناك فقد واضح للنتوءات الدقيقة فى الأنيبيبات الجامعة . وجدير بالذكر أنه قد ظهرت زيادة كبيرة فى عدد الخلايا الميز نشيمية وكذلك الليفية كما ظهرت شبكة دقيقة من الالياف حول الانيبيبات.
وقد تمت مناقشة هذه التغيرات ويوصى البحث بالحرص فى إستخدام هذا العقار خاصة فى الحوامل وكذلك المرضى الذين يعانون من خلل فى الكلى , كما يجب مراقبة وظائف الكلى أثناء العلاج.