Acknowledgement

- At first and forever, I thank the merciful **God {Y}** for providing me help and strength and blessing for establishing this work.
- My sincere gratitude and appreciation is directed to my supervisor **Prof. Dr. Kawkab S. Najim** for her supervision endless scientific advice and guidance through the year of study. Whom I will never forget and how much I thank her it's not enough.
- My respect and appreciation are gratefully extended to **Dr. Khulud Al-Samaraai** for her help during all years of my study.
- Special respect and gratitude are expressed to **Dr. Mai Al-Habeeb** for her help in providing the research facilities involved in my work.
- I would like to thank **Dr. Ali Hussein Al-Anbaki** for unforgettable support and his help and valuable scientific knowledge which can not be forget.
- Also I gratefully thank **Dalia Abdul Lateef** for her help during this work.
- Also special thanks go to all members of the department of Electron Microscopy/ Nahrain Collage of Medicine for their invaluable help in the period of the research.
- A special thanks go to my faithful best friend **Dr**. **Aseel Basim AL-Saidie** for her moral support during my study.
- At Least I acknowledge with gratitude to light of my life **{my mother}** for her patience, support and continuous encouragement, my God bless her.

Certification

I certify that this thesis titled "**A Study on the Effect of Non-Steroidal Drug (Diclofenac) on the Succinate Dehydrogenase Activity in the Kidney of Albino Mice (An Electron Microscopic Study)"** was prepared under my supervision at the University of Al-Nahrain, Biotechnology Department in Partial Fulfillment of the requirement for the degree of Master of Science in Biotechnology.

Signature:
Supervisor : Prof. Dr. Kawkab S. Najim
Date: / / 2006

In view of the available recommendations, I forward thesis for debate by the examining committee.

Signature:
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Date / / 2006

1.1 INTRODUCTION:

It is well known fact that Kidneys are paired retroperitoneal organs situated in the posterior part of the abdomen on each side of the vertebral column. In the human the upper pole of each kidney lies opposite the 12^{th} thoracic vertebra and the lower pole lies opposite the 3^{rd} lumbar vertbra.The right kidney is usually slightly more caudal in position.

Located on the medial or concave surface of each kidney a region called hilum .through which the renal pelvis, the renal artery and vein.the lamphatics,and a nerve plexus pass into the sinus of the kidney.

The organ is surrounded by a tough fibrous capsule,which is smooth and easily removable under normal conditions.

Two distinct regions can be identified on the cut surface of disected kidney: **Fig. (1.1)** a pale outer region (the cortex) and a darker inner region(the medulla).In humans, the medulla is divided into 8 to 18 striated conical masses,the renal pyramids.The base of each pyramid is positioned on the corticomedullary boundary,and the apex extends toward the renal pelvis to form a papilla.On the tip of each area 10 to 25 small openings that represent the distal ends of the collecting ducts (of Bellini).

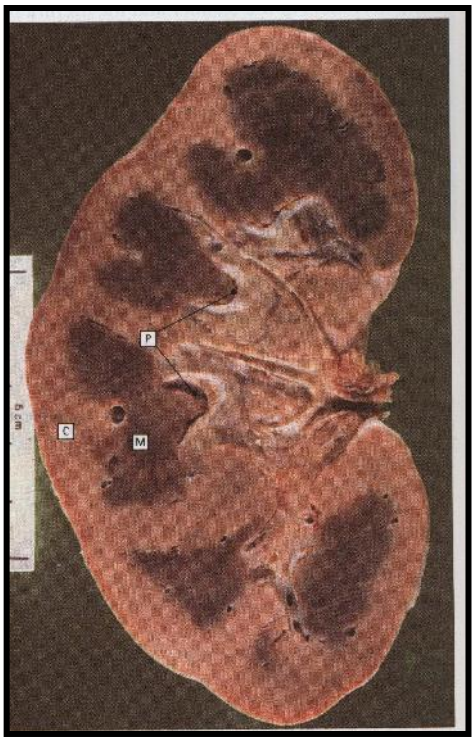


Fig. (1.1): Adult human Kidney. the cortex (C), medulla (M), and papillae (P). (Stevens and Lowe, 1997)

In contrast to the human kidney,the kidney of the rat and of many other laboratory animals has a single renal pyramid and is therefore termed “unipapillate”.Otherwise,these kidneys resemble the humans kidney in their gross appearance **(Brenner, 2004)**.

From the base of the renal pyramid, at the corticomedullary junction, longitudinal elements termed the “medullary rays of Ferrein” extend into the cortex. Despite their name, the medullary rays are actually considered a part of the cortex and are formed by the collecting ducts and the straight segments of the proximal and distal tubules.

The functional and structural unit of kidney, the nephron, consists of a renal corpuscle plus along folded renal tubule. The human kidney contains approximately one million nephrons. Nephrons perform the functions of osmoregulation and excretion by the following processes:

1- Filtration of most small molecules from blood plasma to form an ultra filtrate of plasma.
2- Selective reabsorption of most of the water and some other molecules from the ultra filtrate leaving behind excess and waste materials to be excreted.
3- Secretion of some excretory products directly from blood into the urine.
4- Maintenance of the acid base balance by selective secretion of H^+ ions into the urine **(Young and Health, 2000)**.

1.2 DICLOFENAC:

Diclofenac(Diclo.) which is the brand name is voltaren and cataflam is used in the treatment of mild to moderate pain and inflammation caused by tendonitis, arthritis, soft tissue injuries and other conditions. It's also used in patients with fractured bones for analgetic reasons **(Beck *et al*., 2003)**. Also for the management of rheumatological disorders and as analgesics and antipyretics **(O'Conner *et al*., 2003)**.

Bioavailability studies with single doses of oral and intravenous 14C-labelled diclofenac indicate rat the orally administered drug is almost totally absorbed **(John, 1979; Kendall *et al.*, 1979)**. However, diclofenac undergoes 'first-pass' metabolism, with about 60% of the drug reaching systemic circulation in an unchanged form **(John, 1979)**.

Single doses of commercially available preparations are equally well absorbed whether administered orally as solution or enteric-coated tablets or rectally as suppositories **(Terhaag *et al.*, 1985; Morimoto *et al.*, 1985)**. Diclofenac is also absorbed percutaneously and reaches systemic circulation when administered as an emulsified gel formation **(Riess *et al.*, 1986)**.

1.3 AIM OF THE STUDY:

Toxic doses of diclofenac can cause nephrotoxicity in humans and experimental animals, on the other hand diclofenac has side effects such as shortness of breath, sings of heart failure, peptic ulcer disease with vomiting of blood and decreasing kidney function. However, whether this diclofenac induce histological and ultra structural changes of kidney in addition to the changes in enzyme activity such as succinic dehydrogenase is unknown. The goals of this investigation were to determine the following:

1- Diclofenac effects on the histological structure of the kidney.

2- Diclofenac effects on the ultra structure of the kidney.

3- Diclofenac effects on the activity of succinate dehydrogenase which play an important role in the active transport processes in the kidney tubules.

2.1 OVERVIEW OF KIDNEY STRUCTURE:

2.1.1 The Nephron:

The functional unit of the kidney is the nephron, an expression first coined by **Braus (1924).** Each human kidney contains about 0.4×10^6 to 1.2×10^6 nephron, which contrasts with approximately 30,000 to 34,000 nephron in each rat kidney **(Oliver, 1968; Nyengard and Bendtsen, 1992)**. The essential components of the nephron include the renal or malpighian corpuscle (glomerulus and Bowmans capsule), the proximal tubule, the Henle tubules , the distal tubule, and the connecting segment or connecting tubule **(Osathanondh and Potter, 1963; Neiss, 1982)**.

Two main populations of the nephrons are recognizable in the kidney: those possessing a short loop of Henle and those with a long loop of Henle **Fig.(2.1).** The loop of Henle is composed of the straight portion of the proximal tubule (pars recta), the thin limb segment, and the straight portion of the distal tubule (thick asending limb, or pars recta). The length of the loop of Henle is generally related to the position of its parent glomerulus in the cortex. Most nephrons originating from superficial and midcortical locations have short loops of Henle that bend within the inner stripe of the outer medulla close to the inner medulla. Nephrons originating from the juxtamedullary region near the corticomedullary boundary have long loops of Henle with descending and ascending thin limb segments that enter the inner medulla. Many variations exist, however,

between the two basic types of nephrons, depending on their relative position in the cortex. In the human kidney, there are approximately seven times more short-than long-looped nephrons **(Oliver, 1968)**. In the rat kidney, approximately 28 % of the nephrons have long loops **(Schmidt-Nielsen and O'Dell, 1960)**.

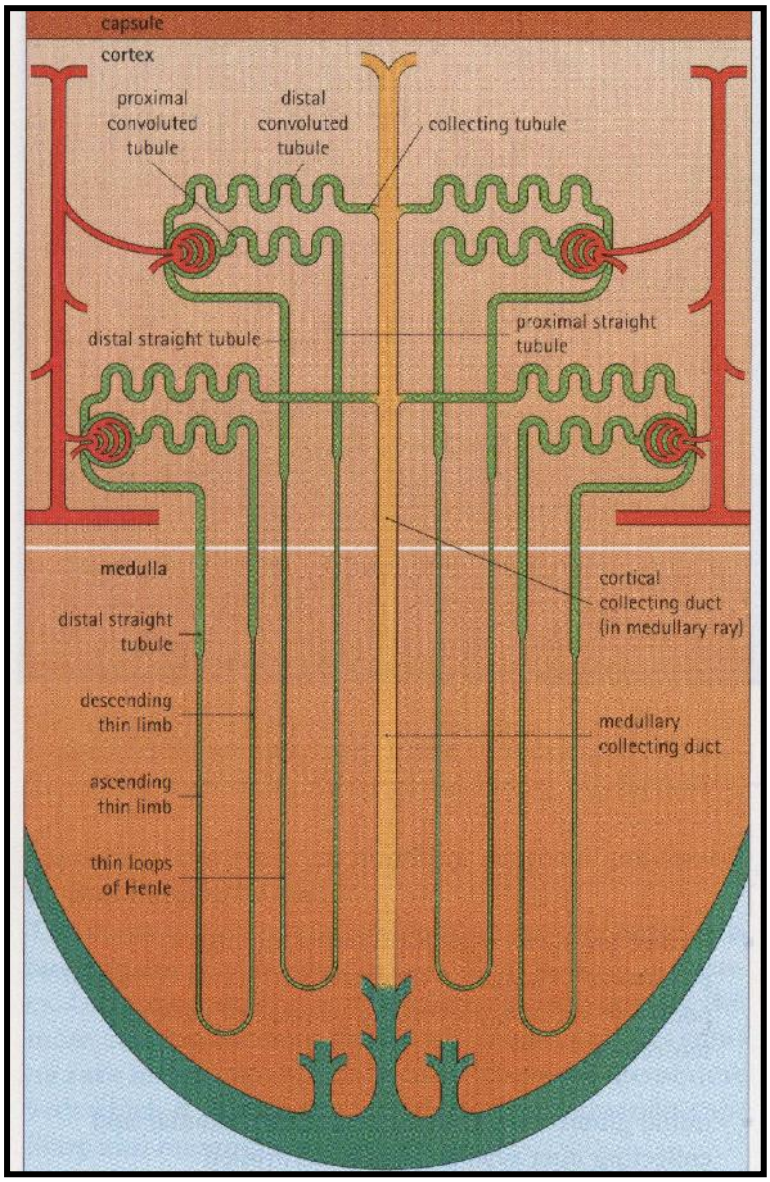


Fig. (2.1): Tubular and collecting system of the nephron. (Stevens and Lowe, 1997)

On the basis of the specific segments of the renal tubule located at various levels in the medulla, it is possible to divide the medulla into an inner and an outer zone, with the outer zone further subdivided into an inner and an outer stripe **(Peter, 1909)**. The inner medulla contains both descending and ascending thin limbs and large collecting ducts, including the ducts of Bellini. In the inner stripe of the outer medulla, descending thin limbs and thick ascending limbs are present in addition to the collecting ducts. The outer stripe of the outer medulla contains the terminal segments of the pars recta of the proximal tubule, the thick ascending limbs (partes rectae of the distal tubule), and collecting ducts. The division of the kidney into cortical and medullary zones and the further subdivision of the medulla into inner and outer zones are of considerable importance in relating renal structure to the ability of an animal to form a maximally concentrated urine. According to the countercurrent hypothesis for urine concentration as originally proposed **(Wirz *et al*., 1951; Wriz, 1954)**.

2.1.2 Endothelial Cell:

The glomelular capillaries are lined by thin fenestrated endothelium. The endothelial cell nucleus usually lies adjacent to the mesangium, away from the urinary space, and the remainder of the cell is irregularly attenuated around the capillary lumen. The endothelium is perforated by pores or fenestrate, which in the human kidney range from 70 to 100 nm in diameter **(Jorgensen, 1966)**. Thin diaphragms have been observed extending across these

fenestrate. When present, however, these diaphragms are not believed to represent a significant barrier to the passage of macromolecules. **Bulger and co-workers (1983)** found that in the rat, the fenestrated regions of the endothelium represented approximately 54% of the total surface area, whereas the fenestrate accounted for 13% of the capillary surface. Non fenestrated, ridge-like structures termed cytofolds are found near the cell borders. In both the human and rat kidney **(Vasmant *et al*., 1984)**.

Ballerman and Marsden (1991) suggested that the glomerular endothelial cells synthesize both nitric oxide (NO), previously called endothelium-derived relaxing factor, and endothelin-1, a vasoconstrictor.

Simon and His Collagens (1995) suggested that the receptors for vascular endothelial growth factor (VEGF) are expressed on the surface of the glomerular endothelial cells. (VEGF) is an important regulator of microvascular permeability that is produced by the glomerular visceral cells **(Brown *et al*., 1992; Simon *et al*., 1995)**.

In vitro studies on endothelial cells of different origins demonstrated that VEGF increases endothelial cell permeability and induces the formation of the endothelial fenestrations **(Roberts and Palade, 1995; Esser *et al*.,1998)**. An increase in the number of fused caveolae was also reported **(Esser *et al*., 1998)**. Studies in renal microvascular endothelial cells have demonstrated VEGF-induced mobilization of caveolae and formation of fenestrae **(Chen *et al*., 2002)**, and there is evidence that VEGF is important for

endothelial cell survival and repair in glomerular diseases characterized by endothelial cell damage **(Ostendorf *et al.*, 1999)**.

2.1.3 Visceral Epithelial Cells:

The visceral epithelial cells, also called podocytes, are the largest cells in the glomerulus. They have long cytoplasmic processes, or trabeculae, that extend from the main cell body and divide into individual foot processes, or pedicels, that come into direct contact with lamina rarae externa of the glomerular basement membrane. By scanning electron microscopy, it is apparent that adjacent foot processes are derived from different podocytes **(Arakawa, 1970; 1971)**.

In the normal glomerulus, the distance between adjacent foot processes near the basement membrane varies from 25 to 60 nm. This gap, referred to as the filtration slit or slit pore, is bridged by a thin membrane called the filtration slit membrane **(Yamada, 1955; Farquhar *et al*., 1961; Latta, 1970)**, or slit diaphragm **(Rodewald and Karnovsky, 1974)**, which is located approximately 60 nm from the basement membrane. A continuous central filament with a diameter of approximately 11 nm can be seen in the filtration slit diaphragm **(Farquhar *et al*., 1961)**. Detailed studies of the slit diaphragm in the rat, mouse, and human glomerulus have revealed that the 11-nm-wide central filament is connected to the cell membrane of the adjacent foot processes by regularly spaced cross-bridges approximately 7 nm in diameter and 14 nm in length, giving

the slit diaphragm a zipper-like configuration **(Rodewald and Karnovsky, 1974; Schneeberger *et al*., 1975)**.

The visceral epithelial cells are capable of endocytosis, and the heterogeneous content of their lysosomes most likely reflects the uptake of proteins and other components from the ultra filtrate **(Rollason and Brewer, 1984)**. There is evidence that the visceral epithelial cells are responsible, at least in part, for the synthesis and maintenance of the glomerular basement membrane **(Striker and Striker, 1985)**.

2.1.4 Mesangial Cells:

The mesangial cells and their surrounding matrix constitute the mesangium, which is separated from the capillary lumen by the endothelium. The mesangial cell is irregular in shape, with a dense nucleus and elongated cytoplasmic processes that can extend around the capillary lumen and insinuate themselves between the basement membrane and the overlying endothelium. In addition to the usual component of subcellular organelles, the mesangium possesses an extensive array of microfilaments **(Mundel *et al*., 1988)**, composed at least in part of actin, α-actinin, and myosin **(Drenckhahn *et al*., 1990)**. The contractile mesangial cell processes appear to bridge the gap in the glomerular basement membrane encircling the capillary, and bundles of microfilaments interconnect opposing parts of the glomerular basement membrane, an arrangement that is believed to prevent capillary wall distention secondary to elevation of the

intracapillary hydraulic pressure **(Drenckhahn *et al*., 1990; Kris *et al*., 1995)**.

A matrix material that is similar to but not identical with peripheral glomerular basement menbrane surrounds the mesangial cell; the mesangial matrix is more coarsely fibrillar and slightly less electron dense. The mesangial matrix contains sulfated glycosaminoglycans **(Farquhar, 1981)**, as well as large amounts of fibronectin, laminin, and various collagens **(Mardi *et al*., 1988; Scheinman *et al*., 1980; Courtoy *et al*., 1980; 1982)**.

Several cell surface receptors of the β-integrin family have been identified on the mesangial cells, including α1β1, α3β1, and the fibronectin receptor, α 5 α 1 **(Kerjaschki *et al*., 1989; Gosio *et al*., 1990; Petermann *et al*., 1993)**. An additional α-chain, α8, has been identified on mesangial cells in human as well as rat and mouse kidney **(Hartner *et al*., 1999)**. The α 8 β 1 intergrin receptor can also serve as a receptor for fibronectin. The intergin receptors mediate attachment of the mesangial cells to specific molecules in the extracellular mesangial matrix and link the matrix to the cytoskeleton. The attachment to the mesangial matrix is important for cell anchorage, contraction, and migration, and ligand-integrin binding also serves as a signal transduction mechanism that regulates the production of extracellular matrix as well as the synthesis of various vasoactive mediators, growth factors, and cytokines **(Rupprecht *et al*., 1996)**.

There is evidence that the mesangial cell is also capable of phagocytosis. **Baud and colleagues (1983)** reported phagocytosis of

opsonized zymosan by cultured mesangial cells in association with production of prostaglandins, reactive oxygen species, and lipoxygenase products.

2.1.5 Glomerular Basement Membrane:

The glomerular basement membrane is composed of a central dense layer, the lamina densa, and two thinner, more electron-lucent layers, the lamina rarae externa and the lamina rarae interna. The latter two layers measure approximately 20 to 40 nm in thickness **(Jorgensen, 1966)**.

Studies employing chemical, enzymatic, and physical methods have provided insight into the biochemical composition of the glomerular basement membrane. The adjacent endothelial and epithelial cells secrete glycoproteins including type IV collagen **(Dean *et al*., 1983)**, laminin **(Mardi *et al*., 1988; Scheinman *et al*., 1980; Courtoy *et al*., 1982)**, Fibronectin **(Courtoy *et al*.,1980; 1982)**, and various heparan sulfate proteoglycans **(Farquhar, 1981)** including perlecan and agrin **(Groffen *et al*., 1997; 1998)** that together constitute the glomerular basement membrane **(Hudson *et al*., 2003)**. Collagen IV is the major consitiuent of the glomerular basement membrane **(Kashtan, 1998)**.

Most investigators believe that the basement membrane is the principal structure responsible for the charge-selective permeability properties of the glomerulus. Ultrastructural tracer studies have provided evidence that the glomerular basement membrane constitutes both a size-selective and a charge-selective barrier.

Caulfield and Farquhar (1974) infused dextrans of different molecular weights into rats and demonstrated that filtration depended on the size of the molecule and that the basement membrane was the main barrier to filtration.

2.1.6 Parietal Epithelial Cells:

The parietal epithelium, which forms the outer wall of Bowman's capsule, is continuous with the visceral epithelium at the vascular pole, The parietal epithelial cells are squamous in character, but at the urinary pole there is an abrupt transition to the taller cuboid cells of the proximal tubule, which have a well-developed brush border. The cells are 0.1 to 0.3 μm in height, except at the nucleus, where they increase to 2.0 to 3.5 μm. Each cell has a long cilium and occasional microvilli up to 600 nm in length **(Jorgensen, 1966)**.

Cell organelles are generally sparse and include small mitochondria, numerous vesicles that are 40 to 90 nm in diameter, and the Golgi apparatus. Large vacuoles and multivesicular bodies are rarely, if ever, seen. The thickness of the basement membrane of Bowman's capsule is variable but has been found to range from 1200 to 1500 nm **(Jorgensen, 1966)**. The basement membrane is composed of multiple layers, or lamellae, that increase in thickness with many disease processes.

2.1.7 Juxtaglomerular Apparatus:

The juxtaglomerular apparatus is located at the vascular pole of the glomerulus. Where a portion of the distal nephron comes into contact with its parent glomerulus. It has a vascular and a tubule component; the vascular component is composed of the terminal portion of the afferent arteriole, the initial portion of the efferent arteriole, and the extraglomerular mesangial region. The tubule component is the macula densa, which is that portion of the thick ascending limb that is in contact with the vascular component **(Barajas, 1970; 1979; Barajas and Salido, 1994)**.

2.1.7.1 Juxtaglomerular Granular Cells:

The granular cells are located primarily in the walls of the afferent and efferent arterioles, but they are also present in the extraglomerular mesangial region **(Latta and Maunsbach, 1962; Barajas and Latta, 1963; Tisher *et al*.,1968; Barajas, 1979; Barajas and Salido,1994)**. They exhibit features of both smooth muscle cells and secretory epithelial cells **(Barajas, 1979)**.

Biava and West, 1966, and **Barajas, 1979** believed that juxtaglomerular granular cells are modified smooth muscle cells, they contain myofilaments in the cytoplasm and, except for the presence of granules, are indistinguishable from the neighboring arteriolar smooth muscle cells, they also exhibit features suggestive of secretory activity, including a well-developed endoplasmic

reticulum and a Golgi complex containing small granules with a crystalline substructure.

2.1.7.2 Extraglomerular Mesangium:

The exraglomerular mesangium is also called the lacis or the cells of Goomaghtigh. It is located between the afferent and efferent arterioles in close contact with the mucula densa **(Elger *et al*.,1998)**.

The extraglomerular mesangium is continuous with the intraglomerular mesangium and is composed of cells that are similar in ultrastructure to the mesangial cells **(Barajas, 1979; Barajas and Salido, 1994)**.

The extraglomerular mesangial cells is contact with the arterioles and the macula densa, and gap junctions are commonly observed between the various cells of the vascular portion of the juxtaglomerular apparatus **(Pricam *et al*., 1974; Taugner *et al*., 1978)**.

2.1.7.3 Macula Densa:

The macula densa is lining directly adjacent to the afferent and efferent of arteriols and adjacent to some extraglomerular mesangial cells at the vascular pole of the renal corpuscles.Only those cells immediately adjacent to the hilus are morphologically distinctive and form the macula densa, they are low columnar cells and exhibit an apically placed nucleus.With electron microscopy**(Tisher *et al*.,1968)**,the cell base is seen to interdigitate with the adjacent

extraglomerular mesangial cells to form a complex relationship,The position of the Golgi apparatus is located to and beneath the cell nucleus, in addition, other cell organelles, including lysosomes, autophagic vacuoles, ribosomes, and profiles of smooth and granular endoplasmic reticulum, are located principally beneath the cell nucleus.

Schnermann, (1998) and **Schnermann and Briggs, (1992)** demonstrated that the juxtaglomerular apparatus represents a major structural component of the renin-angiotensin system, the role of the juxtaglomerular apparatus is to regulate glomerular arteriolar resistance and glomerular filtration and to control the synthesis and secretion of renin.

2.1.8 Proximal Tubule:

The proximal tubule begins abruptly at the urinary pole of the glomerulus, it consists of an initial convoluted portion, the pars convoluta, which is a direct continuation of the parietal epithelium of Bowman's capsule, and a straight portion, the pars recta, which is located in the medullary ray, in the rabbit, a well-defined neck segment has been described between the parietal epithelium of the glomerulus and the proximal tubule **(Schonheyder and Maunsbash, 1975)**.

The outside diameter of the proximal tubule is about 40 μm, in several animals, including the rat **(Maunsbach, 1966)**, the rabbit **(Woodhall *et al*.,1978; Kaissling and Kriz, 1979)**, the mouse

(**Rhodin, 1958)**, and the rhesus monkey **(Tisher *et al*., 1969)**, three morphologically distinct segments S1, S2, and S3 can be distinguished in the proximal tubule, the S1 segment is the initial portion of the proximal tubule, it begins at the glomerulus and constitutes approximately two thirds of the pars convoluta. The S2 segment consists of the remainder of the pars convoluta and the initial portion of the pars recta. The S3 segment represents the remainder of the proximal tubule; it is located in the deep inner cortex and the outer stripe of the outer medulla **(Tisher *et al*., 1966)**.

In the rat, the structural features that distinguish the three segments of the proximal tubule have been described in detail by **Maunsbach (1966; 1973)**. The S1 segment has a tall brush border and a well-developed vacuolar-lysosomal system, the basolateral plasma mambrane forms extensive lateral invaginations, and lateral cell processes extending from the apical to the basal surface interdigitate with similar processes from adjacent cells, elongated mitochondria are located in the lateral cell processes in proximity to the plasma membrane, an arrangement that is characteristic of epithelia involved in active ion transport **(Maunsbach, 1966)**.

The ultrastructure of the S2 segment is similar to that of the S1 segment; however, the brush border is shorter, the basolateral invaginations are less prominent, and the mitochondria are smaller. Numerous small lateral processes are located close to the base of the cell, the endocytic compartment is less prominent than in the S1 segment.

In the S3 segment, lateral cell processes and invaginations are essentially absent, and mitochondria are small and randomly distributed within the cell, the length of the brush border in the S3 segment varies among species, it is tall in the rat, fairly short in the rabbit, and intermediate in length in the human kidney **(Tisher *et al.*, 1966).**

2.1.9 Pars Recta:

The pars recta of the proximal tubule consists of the terminal portion of the S2 segment and the entire S3 segment, the epithelium of the S3 segment is simpler than that of the S1 and S2 segments **(Maunsbach, 1966; 1973; Tisher *et al.*, 1966)**. Basolateral invaginations of the plasma membrane are virtually absent, mitochondria are small and randomly scattered throughout the cytoplasm, and the intercellular spaces are smaller and less complex, these morphologic characteristics suggest that the S3 segment may be less involved in the reabsorption of solute and water **(Kats *et al.*, 1979)**.

In addition, studies examining transport parameters in individual segments of the proximal tubule have demonstrated that fluid reabsorption in the S3 segment is significantly less than in the S1 and S2 segments **(Clapp *et al.*,1988)**.

The morphologic appearance of the pars recta varies considerably among species, in the rat, the microvilli of the brush border measure up to 4 μm in length, whereas in the rabbit and human kidney they are much shorter, the vacuolar-lysosomal system

is less prominent in the S3 segment of the proximal tubule, in the rabbit, however, large vacuoles are often observed **(Maunsbach, 1973)**, and in both rabbit and human, many small lysosomes with electron-dense membane-like material are common in the S3 segment **(Tisher *et al*.,1966; Madsen and Park, 1987)**.

2.1.10 Thin Limbs of the Loop of Henle:

The transition from the proximal tubule to the descending thin limb of the loop of Henle is abrupt, and marks the boundary between the outer and inner stripes of the outer medulla. The transition from the thin to the thick ascending limb forms the border between the outer and inner medulla, nephrons arising in the extreme outer cortex may possess short cortical loops that do not extend into the medulla, the thin limb segments of both short-and long-looped nephrons are located outside the vascular bundles **(Jamison and Kriz, 1982)**.

In contrast, most animals with a high urine concentrating ability, such as the rat **(Dieterich, 1968; Kriz *et al*.,1972; Schwartz and Venkatachalam, 1974)**, mouse **(Kriz and Koepsell, 1974)**, and desert sand rat **(Barrett *et al*., 1978)**, have a complex medulla in which the descending thin limbs of the short-looped nephrons are incorporated into the vascular bundles in the outer medulla together with the vasa recta.

2.1.11 Thick Ascending Limb:

The thick ascending limb, or pars recta, represents the initial portion of the distal tubule and can be divided into a medullary and a cortical segment. In long-looped nephrons, there is an abrupt transition from the thin ascending limb to the thick ascending limb, which marks the boundary between the inner medulla and the inner stripe of the outer medulla. In short-looped nephrons, the transition to the thick ascending limb occurs shortly before the hairpin turn. The transition to distal convoluted tubule occurs shortly after the macula densa. The cells forming the medullary segment in the inner stripe of the outer medulla measure approximately 7 to 8 μm in height **(Kaissling and Kriz, 1979; Kone *et al*., 1984)**.

2.1.12 Distal Convoluted Tubule:

The distal convoluted tubule or pars convoluta, measures approximately 1 mm in length **(Crayen and Thoenes, 1978; Kaissling and Kriz, 1979)**. Its a part of macula densa variable and extends to the connecting tubule that connects the nephron with the collecting duct. The cells of the distal convoluted tubule resemble those of the thick ascending limb but are considerably taller, the cells appear tall and cuboid and they contain numerous mitochondria, the cell nuclei occupy a middle to apical position, the distal convoluted tubule lacks the well-developed brush border and the extensive endocytic apparatus that are characteristic of the pars convoluta of the proximal tubule **(Brenner, 2004)**.

Transmission electron microscopy reveals numerous elongated mitochondria that are located in lateral cell processes and are closely aligned with the plasma membrane, they are oriented perpendicular to the basement membrane and often extend from the basal to the apical cell surface. Lysosomes and multivesicular bodies are common in the cells of the distal convoluted tubule, but microbodies are absent **(Brenner, 2004)**.

The distal convoluted tubule is also involved in the reabsorption of Ca^{2+} and has a higher Ca^{2+} , Mg^{2+} -ATPase activity than any other segment of the nephron **(Doucet and Katz, 1982)**.

2.1.13 Collecting Tubules and Collecting Duct:

The collecting tubules begin in the cortical labyrinth, as either connecting tubules or arched collecting tubules, and proceed to the medullary ray where they continue as straight collecting tubules. Straight collecting tubules, in turn, merge in the medullary ray to form cortical collecting ducts. These collecting ducts continue into the pyramids where they continue to merge, ultimately forming large ducts (up to 200 μm), the papillary ducts (ducts of Bellini) **(Ross *et al.*, 1995).**

Both the collecting tubules and ducts are composed of a simple epithelium, the arched and cortical collecting tubules have flattened cells, somewhat squamous to cuboid in shape, the medullary collecting ducts have cuboidal cells, with a transition to columnar cells as the ducts increase in size, the collecting tubules and ducts are readily distinguished from proximal and distal tubules

by virtue of the cell boundaries that can be seen in the light microscope **(Ross *et al.*, 1995).**

2.1.14 Interstitium:

The renal interstitium is composed of interstitial cells and a loose, flocculent extracellular matrix material consisting of sulfated and nonsulfated glucosaminoglycans **(Bohman, 1980; Lemley and Kriz, 1991)**.

The amount of interstitial tissue in the cortex is limited, and the tubules and capillaries are often directly opposed to each other, the interstitium constitutes 7% to 9% of the cortical volume in the rat **(Pedersen *et al*., 1980; Pfaller, 1982)**.

Three percent of the 7% represents the interstitial cells, and the remaining 4% represents the extracellular space **(Bohman; 1980)**.

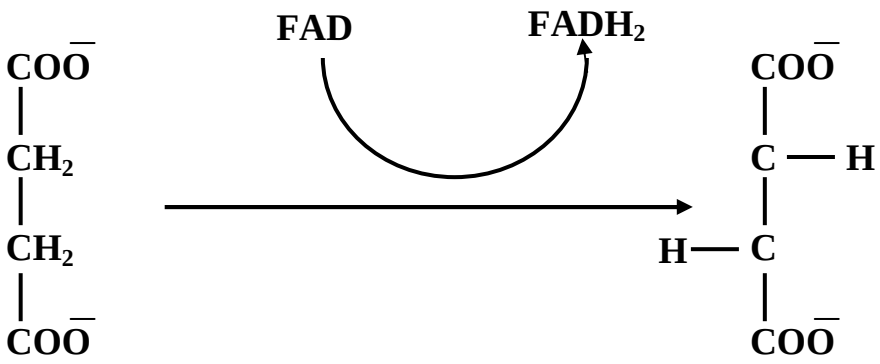
In the medulla, a gradual increase occurs in interstitial volume, from 10% to 20% in the outer medulla to approximately 30 to 40% at the papillary tip in both the rat and the rabbit **(Knepper *et al*., 1977; Pfaller, 1982)**.

2.2 SUCCINATE DEHYDROGENASE (SDH):

Succinate dehydrogenase is a mitochondrial-bound, flavoprotein enzyme, and the most venerable of all the dehydrogenases studied in histochemistry **(Stoward *et al.*,1991)**. It

is one of the series of tricarboxylic acid cycle (kreb's cycle), which occupies a key position in this cycle **(Payne, 1979; Stoward *et al.*,1991)**. It catalysis hydrogen (electron) transfer to flavoprotein in the later stages of the krebs.

Citric acid cycle, for which its often used as a marker **(Bernath and Singer,1962 ; Hiatt,1976 ; Stoward *et al.*,1991 ;Stryer,1995)**.



Succinate **Fumarate**

This enzyme is related to a dehydrogenasis group, which is belonged to a large and important class of enzymes which catalyzes biological oxidations, these enzymes, the oxidoreductases **(Borger and Verheyen, 1985)**. Dehydrogenases as a class of enzymes accomplishs the removel of hydrogen from the substrate undergoing oxidation "hydrogen donor", to another substance "hydrogen accepter". The dehydrogenase which catalyze reduction of this kind are not only specific for the hydrogen donor but also for hydrogen accepter **(Hayhoe and Quaglino, 1988)**.

This serves as the basis of the histochemical reaction used in demostrating this enzyme. The present in the tissue acts on the substrate (usually sodium succinate), causing the removal of hydrogen, which is picked up by a synthetic acceptor (a tetrazolium compound) present in the incubating medium. The reduced accepter substance called formazan, appears as a colored insoluble reaction product **(Kuhn and Abood,1949; Hiatt,1976; Payne,1979; Stoward *et al*.,1991; Kapour,1997)**.

The SDH system form an exception since it contains its own "bulitin" flavoprotein and does not require the intervention of a diaphorase in transferring electron from succinate to the electron transport system **(Payne, 1979; Hayhoe and Quaglino,1988; Stoward *et al*.,1991)**.

SDH is tightly associated with the inner mitochondrial membrane **(Singer and Kaerney, 1963; Lehniger, 1965; Mahler and Cordes, 1968; Stryer, 1995; Davidson and Sittman, 1999)**.There is general agreement that mitochondria succinate dehydrogenase is an intregral part of the membrane in close relationship with the other enzymes of the respiratory chain **(Payne, 1979)**. However, **Sedar and Rosa, (1961)**, **Mcewen *et al*.,(1963)** have presented some evidence for its presence in the nucleus which remained without satisfactory explanation.

Succinate dehydrogenase, has been considered as a reliable marker of the general functional state of a cell as an indicator of energy prduction by the tricarboxylic acid cycles and for which a specific and reliable histochemical technique exists **(Baker and**

Santer, 1990). So it was studied extensively to determine its precise interplay in biological oxidations, since its presence indicates an active metabolic state **(Hiatt, 1976)**.

The intracellular distribution of oxidative enzyme such as SDH enzyme has been studied cytochemically at the level of the electron microscope by various authors **(Sedar *et al*., 1962; Avers and Tkal, 1963; Sabatini *et al*.,1963)**. Some of the srudies **(Von Schulze and Butschak, 1962; Avers and Tkal, 1963)** suggested that only a part of the mitochondrial population of a given cell was enzymatically active and there seemed to be a heterogeneity among mitochondria in term of oxidative enzymatic activity.

Sedar and Rosa, (1961), during their cytochemical demonstration of the SDH system with electron microscope, found that there were extramitochondrial formazan deposits encountered in some micrographs.

2.3 DICLOFENAC:

Diclofenac is a non-steroidal anti-inflammatory drug with analgesic and antipyretic activity, and is common with other aspirin-like anti-inflammatory drugs, it is a potent inhibiter of prostaglandin (PG) synthesis. It is a phenylacetic acid derivative, and its chemical structure was designed based on information gained about the structure-activity relationships of other anti-inflammatory drugs **(Sallmann, 1986).**

Diclofenac is extensively metabolised, but none of its metabolites possess significant pharmacological activity compared with the parent drug as assessed from *in vitro* ability to inhibit prostaglandin synthesis and from *in vivo* anti-inflammatory or analgesic activity in animal models **(Menasse *et al.*, 1978; Maier *et al.*, 1979).**

Diclofenac, as the sodium salt, is a benzeneacetic acid derivative, designated chemically as 2-[(2,6- dichlorophenyl)amino] benzeneacetic acid, monosodium or monopotassium salt. The structural formula is shown in figure 2 (http//www, 2001).

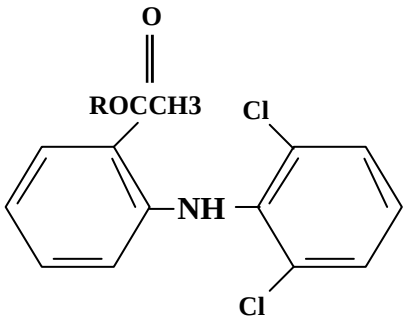


Fig. (2.2): Structure of Diclofenac

R = K: Cataflam® , diclofenac potassium

R = Na: Voltaren® or Voltaren® -XR, diclofenac sodium

Diclofenac, as the sodium or potassium salt, is a faintly yellowish white to light beige, virtually odorless, slightly hygroscopic crystalline powder. Molecular weights of the sodium and potassium salts are 318.14 and 334.25, respectively. It is freely soluble in methanol, soluble in ethanol, and practically insoluble in

chloroform and in dilute acid. Diclofenac sodium is sparingly soluble in water while diclofenac potassium is soluble in water. Diclofenac potassium is available as cataflam immediate release tablets of 50 mg for oral administration. While diclofenac sodium is available as vlotaren delayed-release (enteric-coated) tablets of 25 mg, 50 mg, and 75 mg for oral administration, and voltaren-xr extended- release tablets of 100 mg **(http/www, 2001).**

Diclofenac has several properties:

1-Advantages properties:

1.1 Anti-Inflammatory Activity

Diclofenac is active in suppressing inflammation in animal models including: oedema induced by carrageenan **(Krupp *et al*., 1975; Menasse *et al*., 1978; Noguchi *et al*., 1984)**, in addition, the drug also suppresses cotton pellet granuloma formation **(Dorietto de Menezes and Catanzaro-Guimaraes, 1985; Tsurumi *et al*., 1973a)** and vascular permeability induced by human plaque in rats **(Dorietto de Menezes and Catanzaro-Guimaraes, 1985)**, and ultraviolet-induced erythema in guinea-pigs **(Tsurumi *et al*., 1973a; Peters *et al*., 1977; Menasse *et al*., 1978).**

Diclofenac is also effective in reducing primary and secondary inflammation in adjuvant arthritis in rats **(Tsurumi *et al*.,1973a; Menasse *et al*., 1978; Noguchi *et al*., 1984)**. The anti-inflammatory activity of diclofenac is not caused by stimulation of the hypothalamo-pituitary adrenocortical axis, as the effect is

maintained in adrenalectomised rats **(Tsurumi *et al*., 1973b; Krupp *et al*., 1975).**

1.2 Analgesic Activity

Diclofenac is an effective analgesic in rats and mice, in which it inhibits writhing induced by ethacryic acid **(Menasse *et al*., 1978)**, acetic acid **(Takashima *et al*., 1972, Tsurumi *et al*., 1973a, Menasse *et al*., 1978, Noguchi *et al*., 1984)**, phenyibenzoquinone **(Menasse *et al*., 1978), and yeast (Noguchi *et al*., 1984)**.

In a placebo-controlled double-blind study by **Stacher *et al*., 1986**, the analgesic activity of single oral doses of diclofenac 75 and 150mg was compared with codeine 60mg in relieving experimental pain induced by electrical and thermal stimulation of skin in 48 healthy human subjects. Pain threshold values increased with all active treatments compared with placebo: diclofenac 150mg was more potent than codeine 60mg, which was in turn more potent than diclofenac 75mg. Codeine produced more side effects than placebo and diclofenac, while diclofenac and placebo were similarly well tolerated.

1.3 Effects on Arachidonic Acid Metabolism

Many of the pharmacological effects of diclofenac, as with other NASID2 , are believed to be mediated by inhibition of prostaglandin synthesis **(Ku *et al*., 1986).**

Diclofenac is a potent inhibitor of cyclooxygenase (prostaglandin synthetase) *in vitro*, as measured by the marked reduction in synthesis of prostaglandin, prostacyclin and thromboxane products in sheep seminal vesicles **(KU *et al*, 1975;**

1985), bovine seminal vesicles, guinea-pig gut and bovine cerebral cortex **(Krupp *et al*., 1976)**, bovine seminal vesicles **(Taylor and Salata, 1976)**, and rat polymor-phonuclear cells and macrophages **(Ku *et al*., 1985)**.

At high concentrations *in vitro*, diclofenac did not inhibit phospholipase A2, which controls arachidonic acid formation from phospholipids, and had negligible effects on the 5- and 15-lipoxygenase enzymes **(Ku *et al*., 1985 ; 1986)** . However, these authors showed that the formation of products from the lipoxygenase pathway (Leukotrienes and 5-hydroxyeicosatraenoic acid) is reduced by high concentrations of diclofenac *in vitro* and *in vivo* in rat and human leucocytes. This seems to be caused by the decreased availability of intracellular arachidonic acid, which results from enhanced reincorporation of this substrate into the triglyceride pool, this effect on lipoxygenase inflammatoty product may contribute to the anti-inflammatory effect of diclofenac *in vivo*, but the formation of cyclooxygenase is propably the primary site of action.

In vivo, diclofenac decreased urinary PGF2& and PGE2 in rabbit renal medulla **(Oliw *et al*., 1978),** and PGE2, 6-keto-PGF1& and PGI2 in the gastric mucosa of rats and pigs **(Kobayashi *et al*., 1985; Rainsford and Willis, 1982)**. Arachidonic acid-induced mortality in rabbits (death resulted from platelet aggregated in vessels of the microcirculation of the lungs) was also inhibited by low diclofenac concentrations **(DiPasquale and Mellace, 1977).**

2- Disadvantages properties:

1.2 Gastrointestinal Effects

The ulcerogenic activity of diclofenac compared with other non-steriod-anti inflammatory drugs (NSAIDs) has varied somewhat according to the experimental method used.

Takashima *et al.*, (1972) found a greater number of rats developed stomach ulcers after administration of diclofenac 2 and 8 mg/kg than after the same dose of indomethacin, but the severity of lesions tended to be greater after indomethacin 32 mg/kg than after the same dose of diclofenac.

Kobayashi *et al.*, (1985) also investigated the relationship between gastric mucosal damage and gastric prostaglandin concentrations in rats. At approximately equivalent anti-inflammatory dosages, single oral doses of diclofenac 20 mg/kg and tiaprofenic acid 30 mg/kg gave similar ulcer indices of 10 and 8, respectively, while indomethacin 20 mg/kg was more ulcerogenic, with an index of 32. Mucosal damage was not related to PGE2 levels as the degree of inhibition was similar for all 3 drugs, however, indomethacin was significantly more potent in reducing mucosal PGI2 , suggesting a relationship to ulcerogenicity.

2.2 Effect on Renal Function

The main mechanism of action of diclofenac involves the inhibition of cyclooygenase (COX) **(Vane and Botting, 1998),** the enzyme that mediates prostaglandin (PG) synthesis from arachidonic acid. NSAID nephrotoxicity is linked to this mechanism, since PGs not only act in response to inflammatory stimuli, but also play a role

as modulators of physiological functions in different organs, so inhibition of PG synthesis results in alterations of homeostasis **(Galli and Panzetta, 2002).**

PGs exert physiological action where they are synthesized; Thus, they act as autocoids rather than hormones. Renal PGs include prostacyclin (PGI2), synthesis mainly by arterioles and glomeruli, thromboxane (TXA2), produced by the glomeruli, and (PGE2), primary synthesized by interstitial cells **(Henrich, 1992).**

Under euvolemic conditions renal PG synthesis is low, making it difficult to demonstrate an important role in maintaining renal function. In contrast, in circumstances in which the systemic circulation is destabilized and blood volume or effective arterial blood volume are compromised , PGs exert a compensating influence on renal function **(Galli and Panzetta, 2002).**

PGs have a number of important roles in the renal circulation, including vasodilatation, renin secretion, and sodium and water excretion. PG induced renal vasodilation attenuates vasoconstrictor responses to angiotensin II or norepincphrine **(Henrich, 1992)**, and opposes the vascconstrictive effects of the renal sympathetic nervous system **(Susic and Malik, 1981)**, thus contributing to the balance between vaso-dilators and vasocontrictor forces that regulate renal circulation. PGs affect sodium excretion both indirectly and directly. As a consequence of renal vasodilatation they increase the filtered load of sodium **(Dunn and Zambrask, 1980)**.

In a non-blind study investigated by **Vandenbrug and his worker (1984),** 62 elderly patients with osteoarthritis received

diclofenac 75 mg/day or sulindac 400 mg/day for 12 weeks. Mean blood urea increased ($p<0.05$) from 7.63 to 9.17 mmol/l on diclofenac but was unchanged on sulindac, clinically significant increases in blood urea nitrogen have been rarely reported during treatment with diclofenac.

Laurent *et al.*, (1987) have reported that treatments 29 patients with membranoproliferative or IgA glomerulonephritis with diclofenac 100 mg/day or placebo in a randomised double-blind study, diclofenac produced a significantly greater median decrease in protein-urea after 2 months' treatment compared with placebo (-70% vs –6% $p < 0.01$). Thus, while diclofenac exerted a short term antiproteinuric effect, it remains to be determined whether it has any therapeutic value in affecting the final outcome of glomerulonephritis.

Power *et al.*, (1992) examined the effect of diclofenac on renal function after major surgery in a randomized, double- blind, controlled study of 20 patients undergoing oesophagogastrectomy. Diclofenac 75 mg or placebo was given i.m. 12-hour for 2 days. On the first day after surgery, use of diclofenac was associated with a decreased urine flow rate, decreased urinary sodium and potassium excretion and a tendency to hyperkalaemia.

3.1 MATERIALS

3.1.1 Equipments and Apparatus:

The following equipments and apparatus were used in this study:

Equipments and Supplies	Company
- Light Microscope	Olympus
- Hot air oven	Memmert, West Germany
- Ultramicrotome	Reichert- Jung
- Transmission electron microscope	Philips CM10
- Electron balance	Metler (Switzerland)
- Knife maker	Reichert – Jung
- E.M. grid	Emscape
- Rotary microtome	Reichert- Jung

3.1.2 Materials:

Materials	Company
- Formaldehyde	BDH
- Gluteraldehyde	BDH
- Ethanol alcohol	BDH
- Paraffin wax	BDH
- Haematoxyline crystals	Fluka
- Eosin stain	Fluka
- Disodium succinate	BDH
- Triphenyl tetrazolium chloride	BDH
- Nitro blue tetrazolium	Fluka AG
- Osimium tetroxide	Sigma
- Uranyl acetate	BDH
- Propylene oxide	BDH
- Araldite	Emscope
- Lead citrate	Fisher Scientific Copmany
-Sodium phosphate diabasic, anhydrous	Fluka
- Potassium phosphate monobasic	Fluka

3.2 PREPARATION OF SOLUTION USED

3.2.1 Preparation of Harris Heamatoxylin Stain:

Dissolved 1.25 gm haematoxylin in 12.5 ml absolute ethanol and 25 gm of potassium alum dissolved in 250 ml of distilled water was added to mixture, boiled and 0.625 gm of mercuric oxide was added to mixture then cool under tap water **(Bancroft and Stevens, 1982)**.

3.2.2 Preparation of Eosin Stain:

Dissolved 0.5 gm of eosin in 50 ml of 70% ethanol alcohol with drops of glacial acetic acid **(Bancroft and Stevens, 1982)**.

3.2.3 Preparation of Acid Alcohol:

Mix 1 ml of HCL with 99 ml of 70% ethyl alcohol **(Bancroft and Stevens, 1982)**.

3.2.4 Gluteraldehyde:

Mix 2.5 ml of 25% gluteraldehyde with 22.5 ml of phosphate buffer solution (pH 7.4) (Hayat, 1986).

3.2.5 Osmium Tetroxide (O_sO_4):

Dissolved 1 gm of O_sO_4 in 100 ml distilled water in dark bottles (Hayat, 1986).

3.2.6 Incubation Media:

The incubation media composed from 0.1 ml of substrate solution in 0.9ml tetrazolium solution. The substrate solution prepared by adding 0.675 gm disodium succinate in 1 ml of distilled water, while the tetrazolium solution which was 2,3,5-triphenyl tetrazolium chloride, C19H15CIN4 (TTC) was prepared as follows:

TTC (2 mg /1 ml distilled water)	2.5 ml
0.2 M Tris buffer (pH 7.4)	2.5 ml
0.5 M cobalt chloride	0.5 ml
0.05 M magnesium chloride	1.0 ml
Distilled water	2.5 ml

The pH of this solution is adjusted to 7.0 **(Al-Kasiy, 2003)**.

3.3 LABROTARY ANIMALS:

Healthy adult and male albino mice weighted about (25-28) gm were obtained from drug control laboratory. They were reared in plastic cages at room temperature about (20-25)C° and kept under 14:10 h light dark cycle (illumination onset at 8 a.m.).They were fed with pellet and tap water was offered *ad libitium*. These animals were left about (7) days for adaptation before beginning experiment.

3.3.1 Experimental Design:

In this study (12) animals were used which were divided into two groups:

1- Group I

(6) mice were injected intramuscularly (i.m) with (0.5)ml/ sterilized normal saline 0.9% for 7 days and used as control group.

2-Group II

(6) mice were injected i.m with 0.5 ml diclofenac sodium (voltarine) 0.5 mg/Kg one time in the day for 7 days **(Turan *et al.*, 1998)**.

After one week, all animals groups (control and experimental) were sacrified by spinal dislocation. Samples from right kidney were obtained and divided to two sets, first prepared for light microscopic examination. The second set was cut into small pieces and prepared for electron microscopic examination.

3.4 PREPARATION FOR LIGHT MICROSCOPIC STUDIES:

1- Fixation:

Kidney samples were fixed in 10% buffered formaline for 24 hours (**Bancroft and Stevens, 1982).**

2- Dehydration:

Samples were dehydrated by ascending grades of ethanol alcohol concentration 70%, 80%, 90%, 100% for two hours and 100% overnight.

3- Clearing:

Samples were cleared with xylene for 1-1 1/2 hours.

4- Impregnation and Wax Embedding

Samples were infiltrated with melted paraffin for 3 hours in the 58C° oven. Then samples were embedded in paraffin wax with melting point of 60 C°.

5- Blocking and Cutting:

The obtained blocks were sectioned serially (5 µm thickness) by rotary microtome and floated in water bath at (40-45C°) **(Bancroft and Stevens, 1982).**

6- Staining:

Sections were stained with Harris hematoxyline and eosin (H and E).

3.4.1 H and E Procedure

1- Sections were deparafinized in xylene for 10 minutes.
2- Sections were hydrated by descending grades of ethanol alcohol concentration, 100%, 90%, 80%, 70% for two minutes for each.
3- Sections were stained with Harris haematoxyline for 5 minutes.
4- Sections were rinsed. Wash well in tap water.
5- Sections were differentiated in acid alcohol for 5 seconds.
6- Sections were blued with running tap water for 10 minutes.
7- Sections were stained with alcoholic eosin for 1 minute.
8- Sections were dehydrated by ascending concentration of alcohol 70%, 80%, 90% for 2 minute and 100% for 5 minutes.
9- Sections were cleared in xylene for 5-10 minutes.
10- Sections were mounted in D.P.X and covered by cover slide **(Bancroft and Stevens, 1982).**

3.5 EXAMINATION BY ELECTRON MICROSCOPE:

3.5.1 Ultrahistochemical Localization of SDH:

The standard dehydrogenase technique as prescribed by **Bancroft and Stevens** (**1982)**, with certain modification was employed, that unfixed tissue blocks of size (2 × 1 ×1mm) which represent both treated kidneys (right) and control, put immediately

after dissection in the freshly prepared incubation media for 30 minutes at 37 C°.

As control incubation, many tissue blocks of kidney region were incubated in media with the absence of substrate as a negative control, in addition to using the heart tissue block of the same animals as a positive control (incubated in media with presence of substrate) **(Al-Kaisy, 2003)**.

Preparation of transmission electron microscope were taken place that the steps of fixation, dehydration and embedding were performed by using the method of **Glauert, 1975 and AL-Kaisy, 1988**, In which samples transfers to 2.5% gluteraldhyde as a fixation solution for 4 hours, washed with 0.5 M phosphate buffer solution (BPS) pH (7.4), 3 times, for 1 hour each time, then the specimen left in the solution overnight. The specimens were post-fixed in 1% osmium tetraoxide for one hour, then washed twicely with distilled water for 5 minutes.

Clearing of the specimens take place by propylene oxide for 15 minutes (two times). Then they were placed in mixture of propylene oxide and embedding materials (araldite), we used 1:1, v/v mixture, they were put in shaker for 1-1 ½ hour, then left in pure araldite overnight at room temperature.

Each specimen was cleaned from adherent araldite by filter papers, then the specimens properly oriented in special type of plastic model and filled with araldite and left in oven for 48 hours at 60 C°, left at room temperature for sectioning and staining **(Al-Kaisy, 2003)**.

3.5.2 Sectioning and Staining:

The specimen blocks were cut by ultramicrotome, semithin sections (0.5-1 µm) were prepared and stained with methylene blue for identification, localization and orientation of the section.

Ultrathin sections that have gold/sliver (60-80 nm thickness) were picked up and mounted on the copper grids, then the sections were stained with saturated uranyl acetate in 70% ethanol for 1 hour, and washed well with 70% ethanol, then stained with lead citrate for 20 minutes and washed again in distilled water, dried by filter paper before examination **(Al-Kaisy, 2003)**.

All sections were examined by electron microscope, using special films which photographed and printed in electron microscope unit / Collage of Medicine / Al-Nahrain University.

3.6 STATISTICAL ANALYSIS:

Results were analyzed statistically, using T-test to compare the significances between the study groups at P-value less than 0.05 were considered to be significant **(Dancan *et al* ., 1983).**

4.1 CHANGES IN BODY WEIGHT:

Marked significant decrease (p >0.05) in the body weight was observed in animals treated with diclofenac as compared with the body weight of control animals, since the mean body weight was decreased from (28.62±1.70) to (24.75±1.74) **tab (1).**

These changes related to the side effects of diclofenac, which was necrosis of the leg, nausea, tarry, and sleeping **(Whole healthmd Comp, 2004)**. All these symptoms change the appetite and effect the food nutrition.

As well as a significant (p>0.05)decrease in organ/ body weight ratio was observed in animals treated with diclofenac as compared with organ body weight ratio of control group, **tab (1).** These change due to the effects of diclofenac on the structure of the kidney such as atrophy of some renal corpuscles with tubular necrosis.

Tab.(1): The effect of Diclofenac on Body Weight and on Organ / Body ratio of Albino mice

Group	Body weight (gm) Mean ± SE		Organ weight (gm) Mean ± SE	Organ weight / Body weight ratio
	Before	After		
Control	28.75 ± 1.68	29.45 ± 1.74	0.178 ± 2.217	0.00420798
Treated	28.62 ± 1.97	24.75 ± 1.70	0.123 ± 1.707	0.005007071

4.2 LIGHT MICROSCOPIC EXAMINATION:

4.2.1 Control group

The examination of H and E stained sections of kidney in this animal group have shown cortical tissue **(Fig.4.1).** Renal corpuscles appear as dense, rounded structures (the glomeruli), surrounded by narrow spaces (Bowman's spaces). In this fig. it is evident that the tubules comprising the tissue between the renal corpuscle differ from one another in diameter, staining intensity and shape. The mass of cortical tubules seen in section mainly consist of proximal convoluted tubules with smaller number of distal convoluted tubules and a lesser number of other segments of renal tubules.

In semi-thick sections **(Fig.4.2)** which permit much greater resolution at high magnification. In this preparation, the glomerular capillaries was observed which contain erythrocytes, with a prominent basement membrane. Also a capillary endothelial cell nucleus was seen bulging into the capillary lumina. Besides these component mesangium, which consists of mesangial cells and densely, stained extracellular substance called mesangial substance.

These cells provide support for the capillary loops particularly at their branching points. In the same fig the podocytes was easily recognized, which have an extensive, branching pale-stained nuclei.

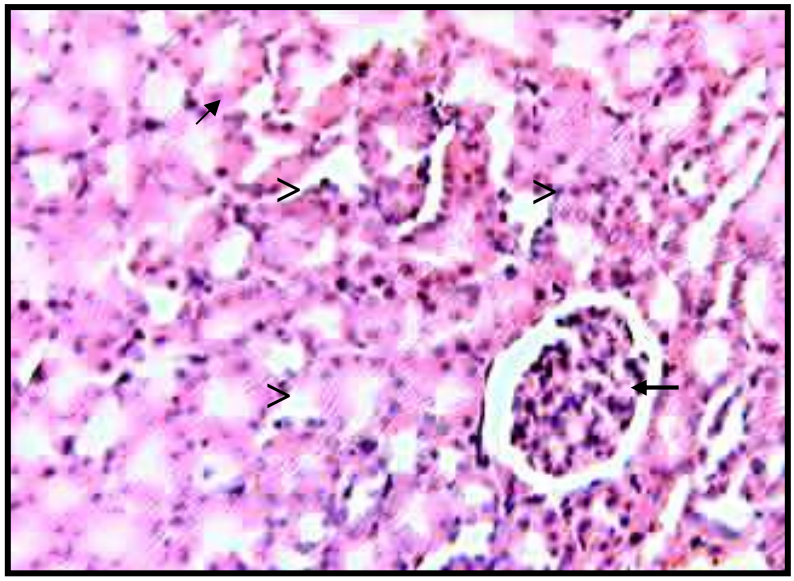


Fig (4.1): Section of kidney in control animal group. H&E (400X)

Renal Corpuscles (→) Kidney tubules (>)

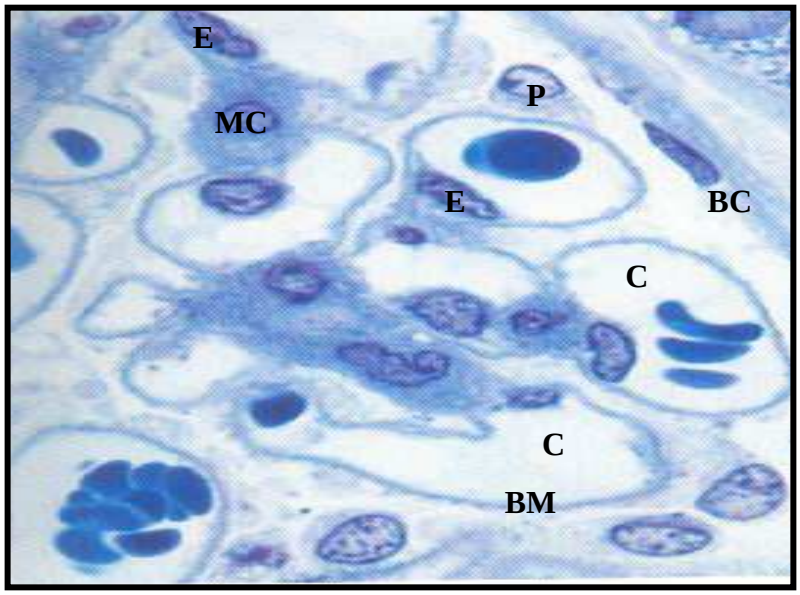


Fig (4.2): Semi-thick section of kidney in control animal group. Capillaries (C), Podocyte (P), Endothelial cell (E), Mesangial cell (MC), Mesangial substance (MS), Basement membeane (BM). Methylene blue (1000X).

4.2.2 ANIMALS TREATED WITH DICLOFENAC:

Light microscopic examination has shown many alterations in both renal corpuscles and kidney tubules.

These changes include the followings:

1- Several of the renal tubules showed necrosis of the epithelium. In these tubules the normal cuboidal epithelium has been replaced by eosinophilic, structureless debris in which cellular outlines as well as the nucleus are obscured. As well as hydrophobic degeneration was observed in some renal tubules **(Fig.4.3).**

2- A large spaces in interstitium component of some region of kidney structure were observed **(Fig.4.4)** which showed loss and sloughing of the tubular epithelial lining with granular casts within the extracellular blood capillaries (edema) **(Fig.4.5).**

Diclofenac sodium is a non-steroidal anti-inflammatory drug, which has been shown to have analgesic effect in various conditions, especially where tissue inflammation contributes to pain. It acts by inhibiting the prostaglandin (PG) synthesis **(Ejnell *et al*., 1992).**

Traditional diclofenac are thought to cause nephrotoxicity via the following mechanisms:

1) Dependant impairment of renal blood flow that can decrease glomerular filtration rate (GER) and increase creatinine levels in susceptible individuals.

2) Sodium and potassium retention leading to edema and hypertension **(Komhoff *et al*., 1997).**
3) Inhibition of cyclooxygenase (COX), the enzyme involved in PG synthesis **(Galli and Panzetta, 2002).**

All changes observed in. **fig (4.3,4.4,and 4.5)** are a typical example of acute tubular necrosis (ATN), which shows loss, and sloughing of the tubular epithelial lining as a result of PG inhibition via diclofenac **(Galli and Panzetta, 2002).**

The PGI2 is the major metabolite of cyclooxygenase pathway and with PGE2 (which are more widely distributed), it causes vasodilatation and potentiates edema formation **(Kumar *et al*., 2003).**

3- Several glomeruli demonstrate increased cellularity of entire glomerular tuft with occlusion of glomerular capillary lamina **(Fig.4.6 a & b).**

These changes reflects a proliferation of mesangial cells which are phagocyte **(Kumer *et al*., 2003)** and many workers have alluded to the importance of phagocytic capacity of the mesangial cell in various glomerulopathies **(Davison, 1973; Portch and Williams, 1973; Couser and Stilment, 1975)**.

4- **Fig.(4.7)** show focal segmental glomerulosclerosis with collapse of capillary loops in a portion of a single glomerulus (segmental capillary collapse) accompanied by epithelial cell proliferation.

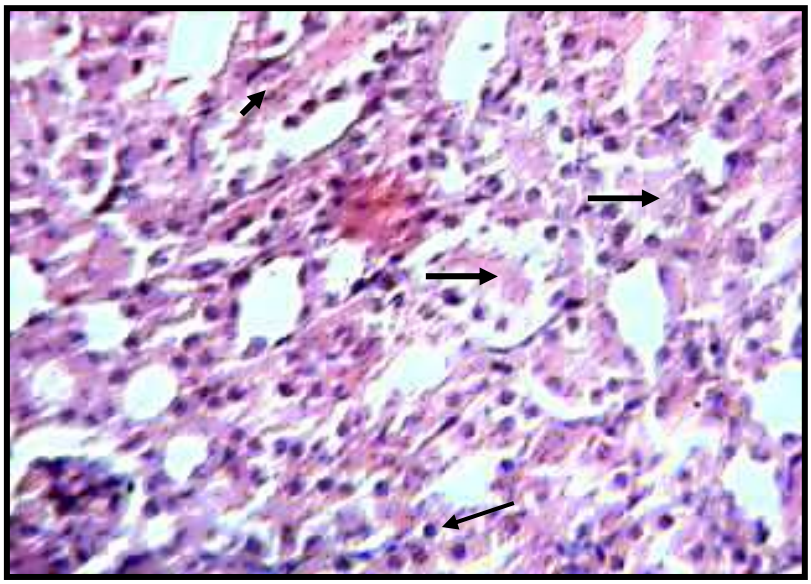


Fig (4.3): Section of kidney in animal treated with diclofenac. H&E (400X). Degeneration of kidney tubule (⟶) Hydrophobic degeneration (➝)

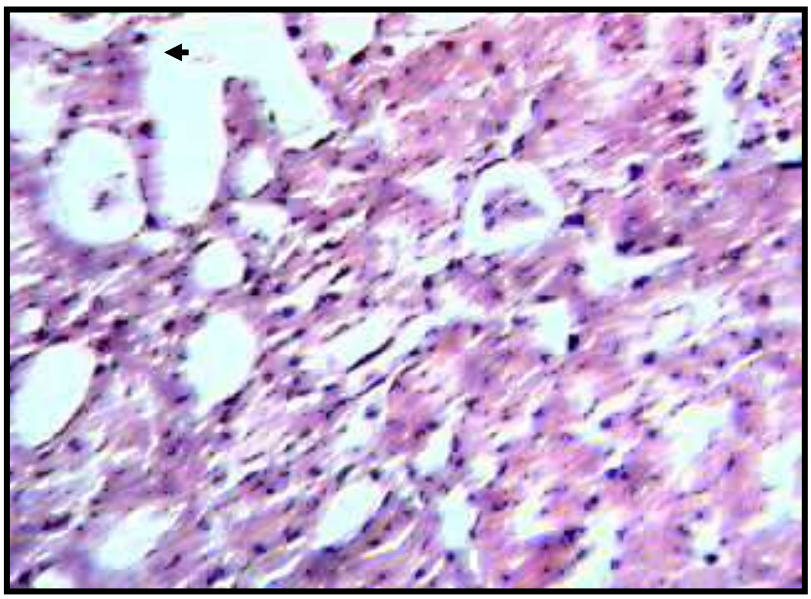


Fig (4.4): Section of kidney in animal treated with diclofenac. H&E (400X) Note large spaces in the interstitium (➝)

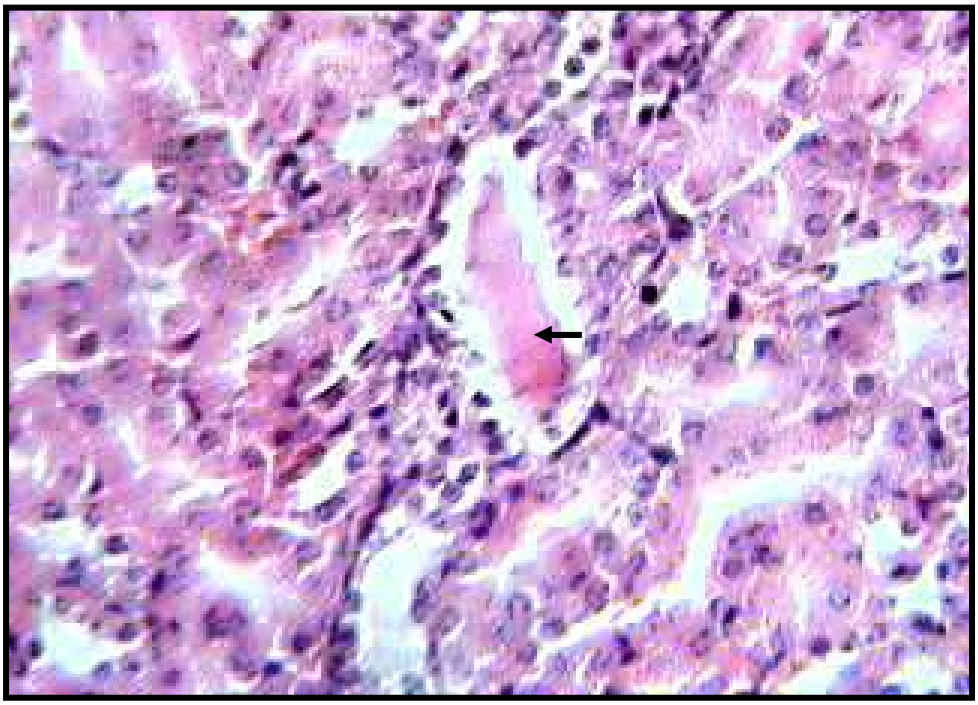


Fig (4.5): Section of kidney in animal treated with diclofenac. Note the edema (⟶) H&E (400X)

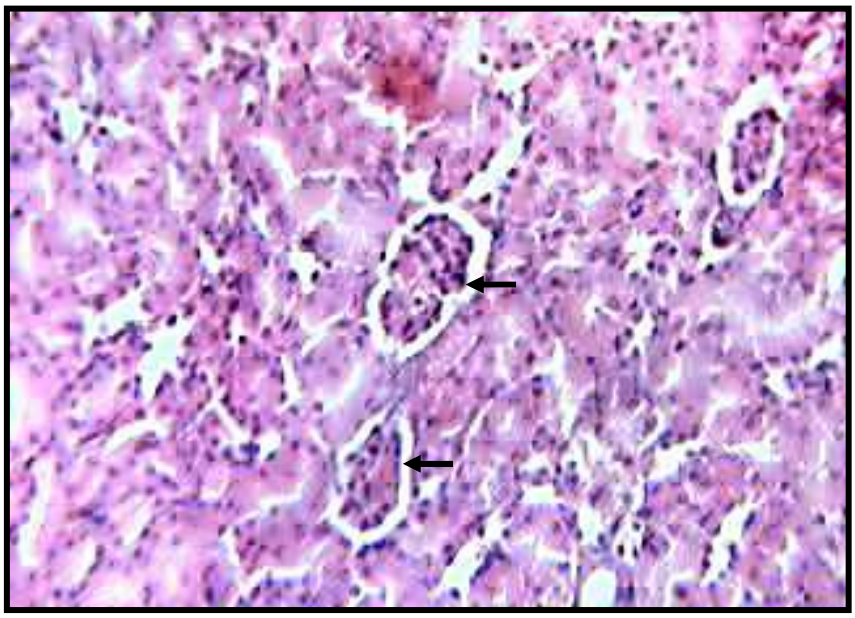


Fig (4.6a): Section of kidney in animal treated with diclofenac. Note the increasment of cellularity of glomerular tuft (►) H&E (250X)

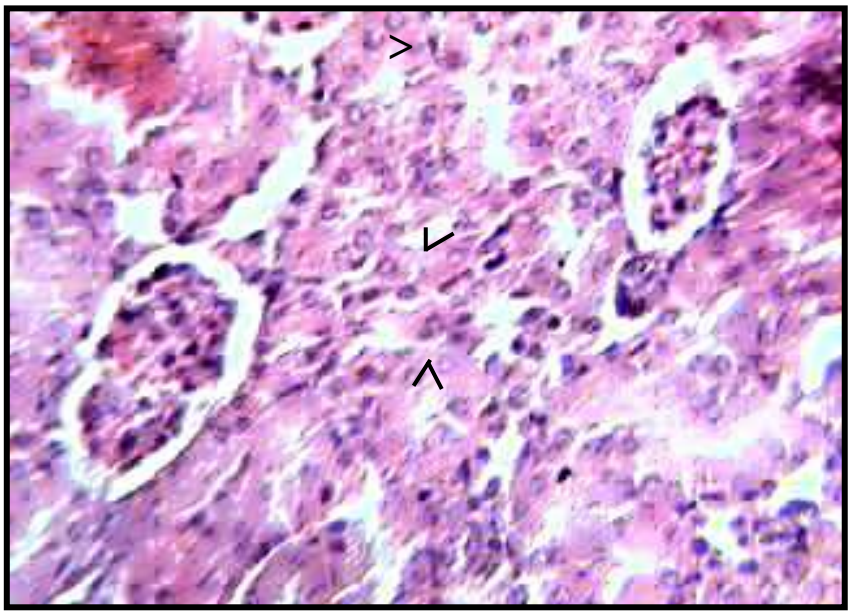


Fig (4.6b): Higher magnification of above. Note lysis of tubule nuclei (>) H&E (400X)

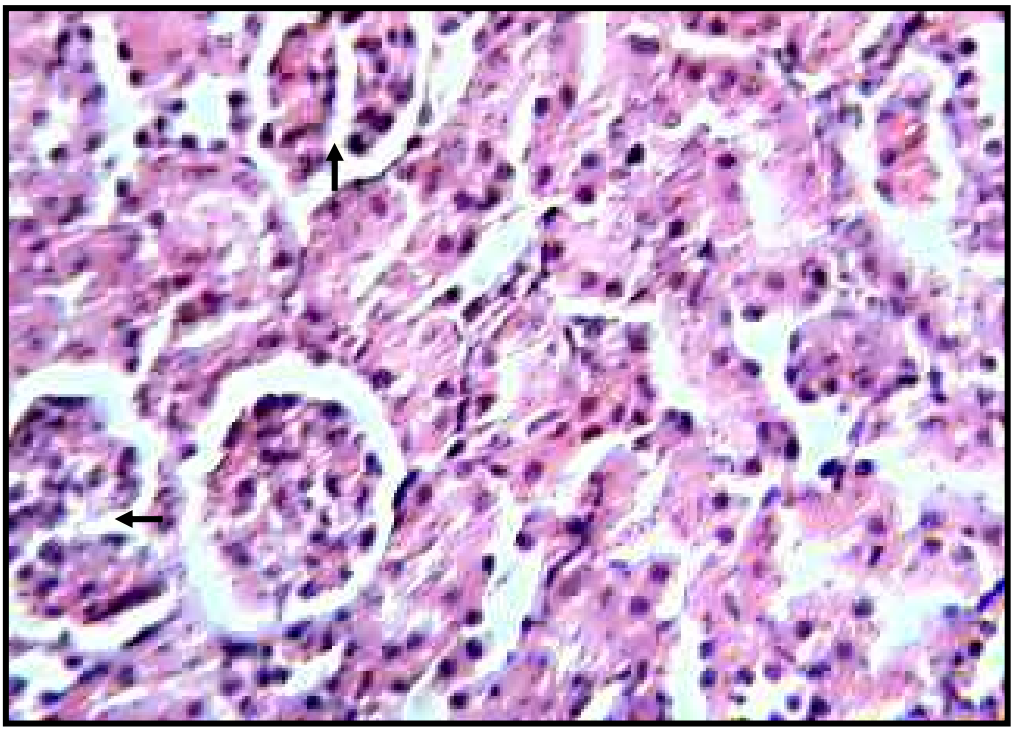


Fig (4.7): Section of kidney in animal treated with diclofenac. Note the segmental glomerulosclerosis (⟶) H&E (400X)

These changes reflects the side effects of diclo., since **Fong and Cohen (1982)** demonstrated that renal insufficiently due to enhanced vasoconstriction is the main consequence of diclofenac use.

5- Besides the above changes **Fig. (4.8)** show congestion in blood capillaries of renal corpuscles compained by neutrophils infilteration within glomeruli. This finding probably due to the increased intrarenal pressure and hydronephrosis of affected kidneys **(Claesson *et al*., 1989; Gonnermann *et al*., 1990; Peters *et al*., 1993)**. Diclofenac may also cause more substrate to be metabolized through the lipoxygenase pathway, leading to an increased formation of inflammatory leukotriens **(Hecker *et al*., 1989)**.

6- **Fig.(4.9)** shows some atrophied glomeruli accompanied by enlargement of renal spaces. These data for the first time suggest that diclofenac induced nephrotoxicity may involve production of reactive oxygen species leading to oxidative stress and massive genomic DNA condensation, and these two free radical mediated events may ultimately translate into cell death of renal corpuscles **(Hickey *et al*., 2001)**.

7- On the other hand the histological examination have shown that some of renal corpuscles and kidney tubules have normal appearance similar to the histological examination of control groups. This finding indicates that diclofenac effects only the active regions, since not all the renal corpuscles share in the functional activity simultaneously.

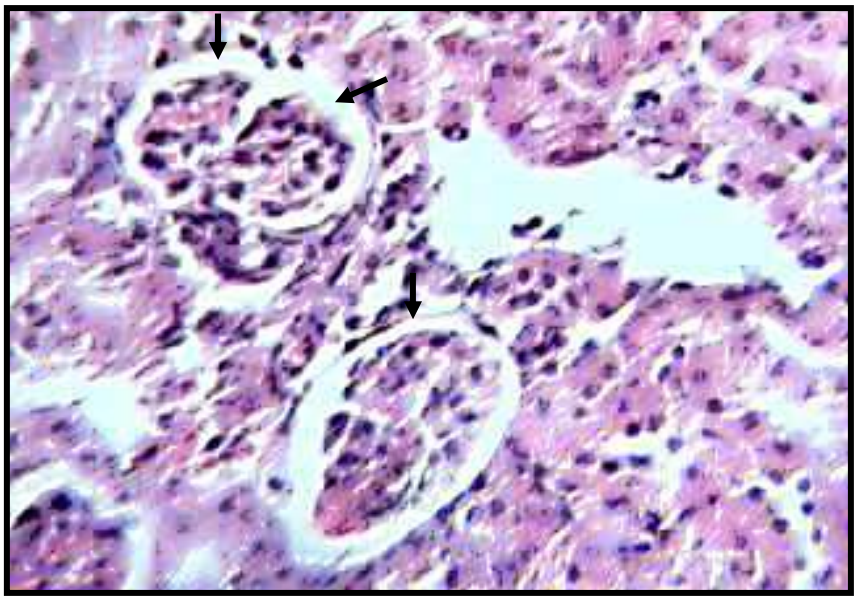


Fig (4.8): Section of kidney in animal treated with diclofenac. Note congestion in blood capillaries (⟶) H&E (400X)

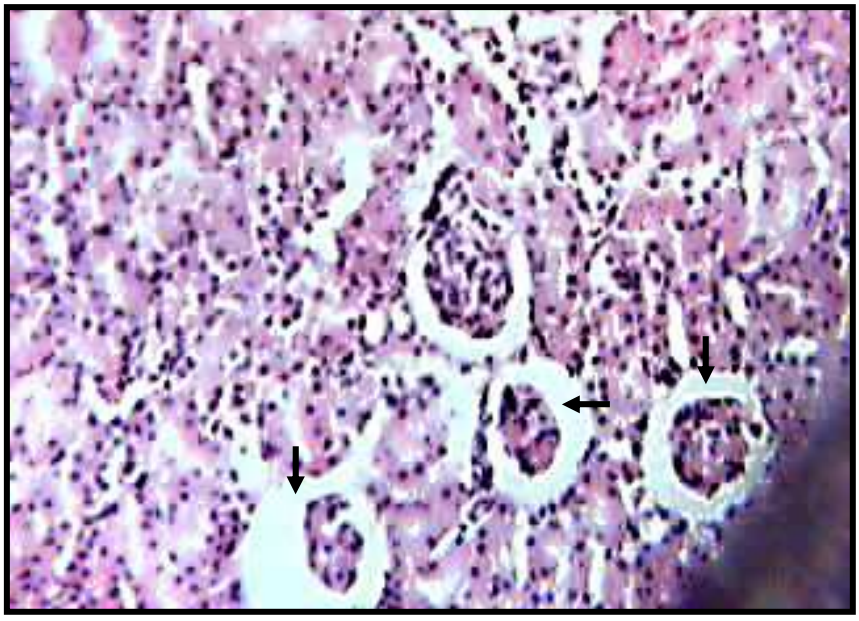


Fig (4.9): Section of kidney in animal treated with diclofenac Note the atrophied glomeruli (⟶) H&E (250X)

4.3 ULTRASTRUCTURE EXAMINATION:

4.3.1 Control group:

Fig.(4.10) reflect the ultrastructure components of kidney in control animal group. This figure shows the structure of capillary loops, that recognized by their content of erythrocytes. The capillary is lined by thin layer of fenestrated endothelial membrane. Their nuclei can be seen bulging into the capillary lumina. Mesangial cells and dense mesangial substance provides support for the capillary loops. The nuclei of several podocytes was observed, their primary processes giving rise to numerous secondary foot processes, which rest on the glomerular basement membrane. **Fig (4.11)** shows the Bowman's space with the parietal cell.

Examination of proximal convoluted tubules in **fig.(4.12)** reveals profuse, tall microvilli constituting the brush border. The cytoplasm immediately beneath the brush border contains many pinocytic vesicles, crowded elongated mitochondria and lysosomes. The nucleus lie near the basement membrane.

Fig.(4.13) reveals the structure of distal convoluted tubule (DCT) the most striking feature of DCT that the DCT lacks a brush border, having only a few irregular microvilli at the luminal surface. The nucleus lies closer to the luminal surface and consequently tend to bulge into the lumen.

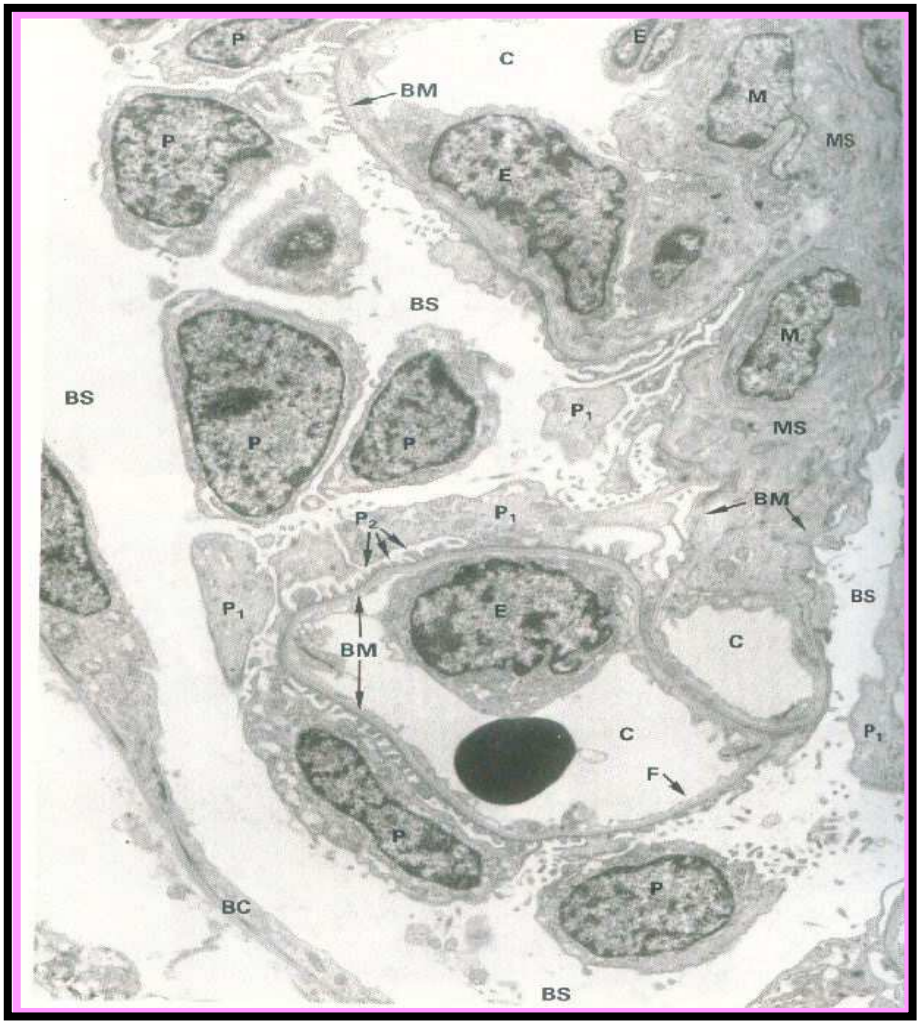


Fig (4.10): Ultrastructure of glomerulus in control animal group. Uranyl acetate and lead citrate (4800X). Copillary loops (CL), Mesangial Substance (MS), Mesangial cell (Mc); Endothelial cell (E), Podocyte (P); P_1 its primary and secondary P_2 foot process; Basement membrane (BM), Bowman's Capsule (BC); Bowman's space (BS) Wheater *et al*., 1987.

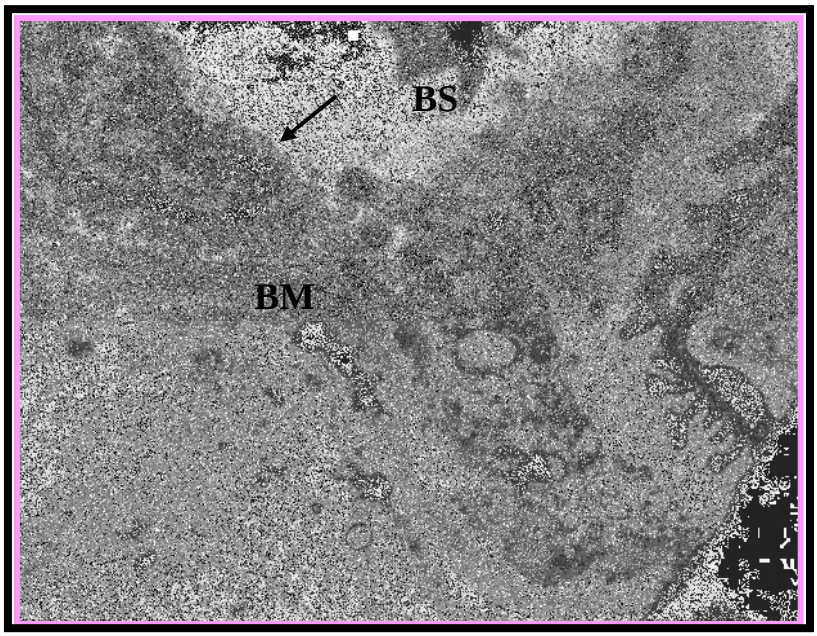


Fig (4.11): Parietal cell (↗) of glomerulus control animal group. Uranyl Acetate lead citrate (6200X). Bowman's space (BS), Basement membrane (BM).

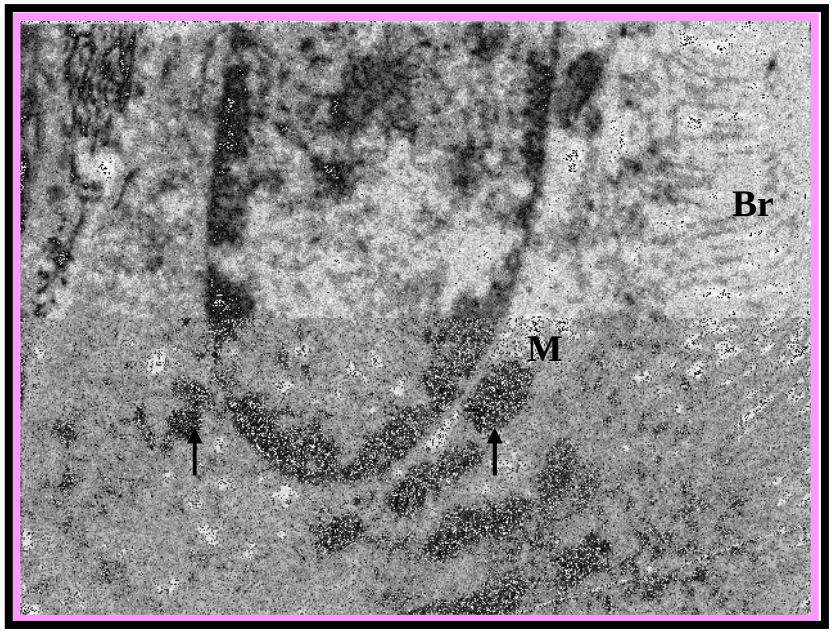


Fig (4.12): Proximal convoluted tubules in control animal group. Uranyl acetate and lead citrate (8700X). Brushed border (Br), Mitochondria(M), Succinic dehydrogenase activit y (↗).

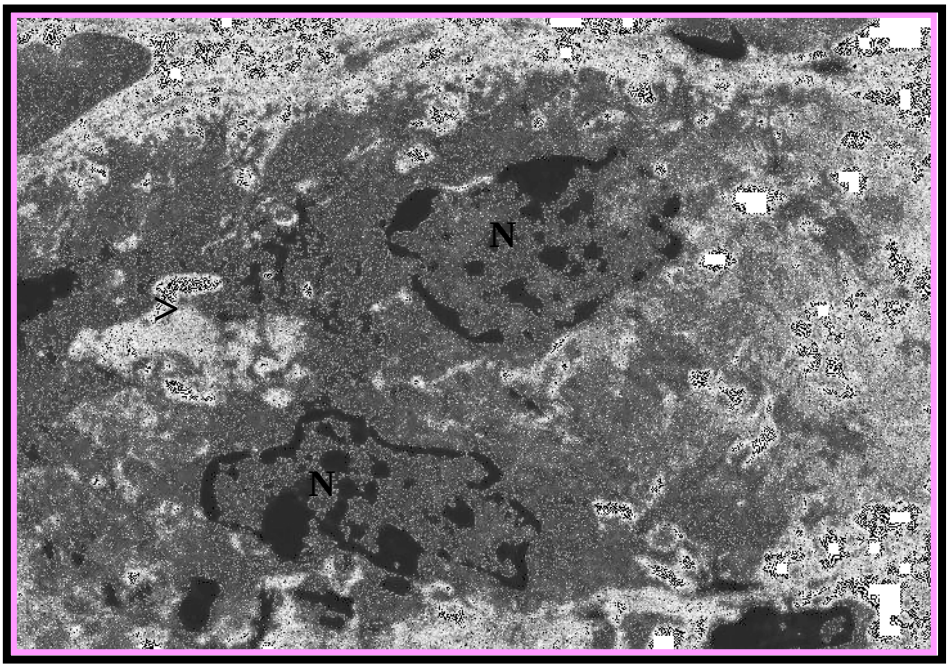


Fig (4.13): Distal convoluted tubule in control animal group. Uranyl acetate and lead citrate (4400X). Nucleus (N), Microvilli (>)

4.3.2 Animals group treated with Diclo.:

4.3.2.1 Kidney tubules:

The tubular cells showed a spectrum of changes from apparently normal to franz-necrosis. In some region, the tubular cells appeared well preserved with well defined mitochondria and presence of brush border in the proximal tubule **(Fig.4.14 and 4.15).**

On the other hand, the epithelial cells of another proximal tubule showed well swollen with irregular vacuolation of cytoplasm, tubular lumens appeared dilated and sieve-like, some of inflammatory cells were observed in the interstitium **(fig.4.16).**

While in another, tubules atrophy of tubular cells was more conspicuous. It was characterized by disintegration or disappearance of nuclei and fuzzy appearance, with undistinct outlines and often in association with casts deposition in the lumen, increment in the mitochondrial number with the vacuolation of the cytoplasm was markdly observed. The epithelial cells resting on intact or disrupted basement membrane with uneven thickness and with high electron density **(Fig. 4.17).**

The term acute tubular necrosis (ATN) was introduced by **Bhuyan, 1980** and associates to signify acute tubular disfunction In acute renal failure (ARF). In our study gross anatomic alteration and impaired tubular function abnormalities was found following diclo. treatment. These changes reflect ARF presences.

Brush border pathologic changes are prominent when renal failure is established. Since numerous transport processes involve

brush border membranes, it is tempting to attribute at least part of the transport difficulty to lesions at this site. Furthermore, disturbed proximal tubule transport many curtail filtration by increasing solute delivery to the distal tubule, thereby stimulating renin release by the macula densa **(Thurau and Boylan, 1976; Wright and Briggs, 1979)**.

The presence of cast's deposition in the tubule lumen, might cause partial obstruction of tubular fluid flow. The obstruction has been postulated to lead to an increased resistance to outflow, increased intraluminal pressure, and ballooning nephrons to form cysts **(Gardner, 1988).**

Tubular necrosis appears important at the post glomerular level. It is related to tubular blockage by cast formation and rupture of tubular basement membrane resulting in fluid leakage with interstitial edema, which was observed in light microscopic examination.

Fig.(4.18) showed epithelial cells of distal tubule, it was characterized by cuboidal cells resting on intact basement membrane, with well preserved nucleus. The cytoplasm was fill with high electron density mitochondria, with some apical pinocytotic vessicals, and often associated with hyaline casts in the lumen. While **fig. (4.19)** showed another distal tubules cells with uniformaly flat epithelial cells but with necrotic nuclei, frayed cytoplasm with undistinct outlines. The tubular lumen appeared dilated and swollen with pigmented granular casts. In **fig. (4.20 and 4.21)** the presence of red blood corpuscles (RBC), with infiltration of inflammatory

cells were more conspicuous in the interstitium. The presence of pigmented and hyaline casts in the distal tubule lumen reflects the obstruction of renal tubule which produces greater decrements in glomerular filteration rate **(Wilson, 1980).**

Treatment with diclo. causes injuries in different renal tubules type (proximal and distal) which is possibly related to the alterations in the metabolic state of the epithelial cells, these changes considered as a stimulus causing accumulation of inflammatory cells. The presence of inflammatory cells in the kidney after tubular obstruction is characteristic of this disorder **(Nagle *et al*., 1976; Jonas *et al*., 1983).**

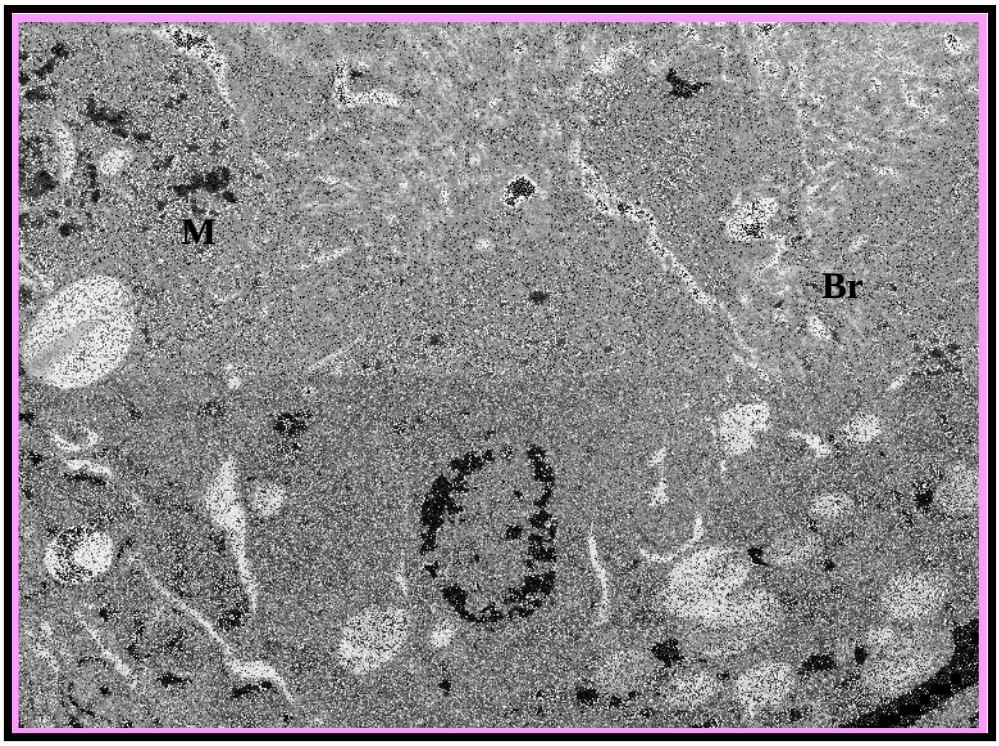


Fig (4.14): Proximal convoluted tubule of animal treated with Diclo. Uranyl acetate and lead citrate (3400X) Brush border (Br), Nucleus (N)

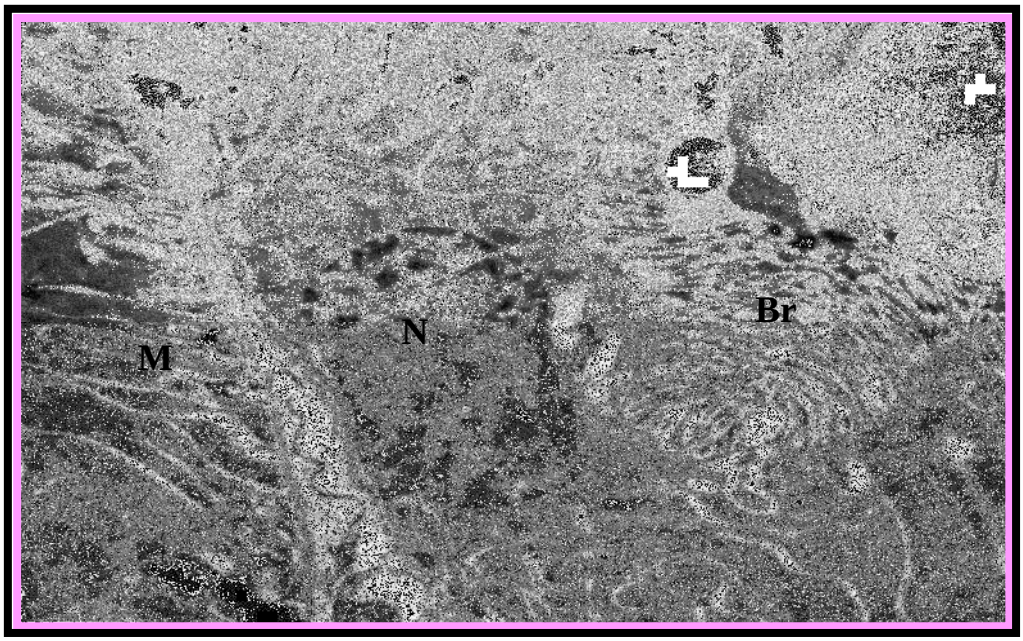


Fig (4.15): Proximal convoluted tubule of animal treated with Diclo. Uranyl acetate and lead citrate (4400X).Brush border (Br), Nucleus (N), Mitochondria (M)

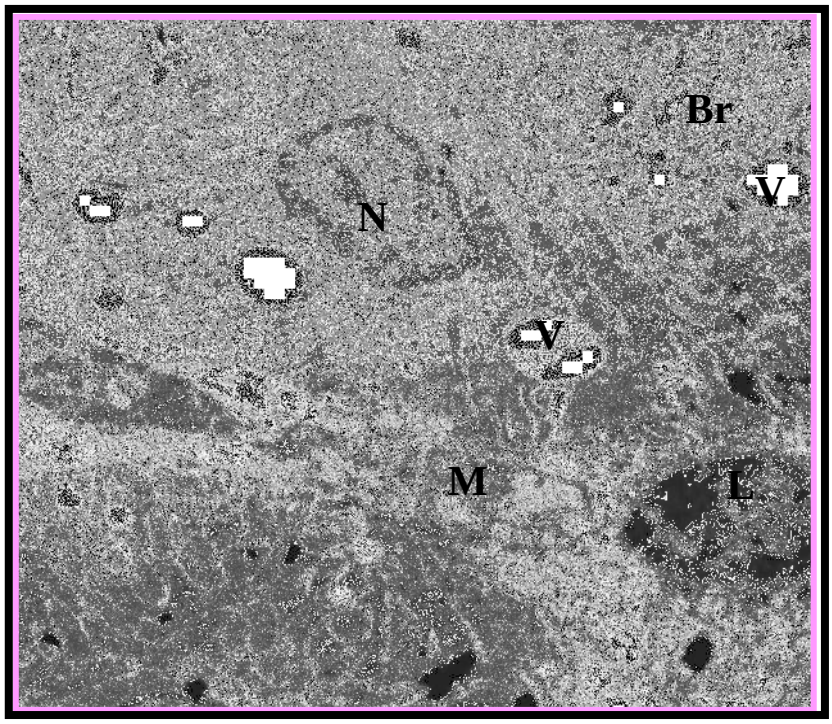


Fig (4.16): Proximal convoluted tubule of animal treated with Diclo. Uranyl acetate and lead citrate (3400X). Brush borde (Br), Nucleus (N), Mitochondria(M),Vacuole(V), Inflammatory cell(I).

Fig (4.17): Tubules of animal treated with Diclo. Uranyl acetate and lead (3400X). Basement membrane (Bs), Nucleus (N), Vacuole (V), Luminal Cast (LC).

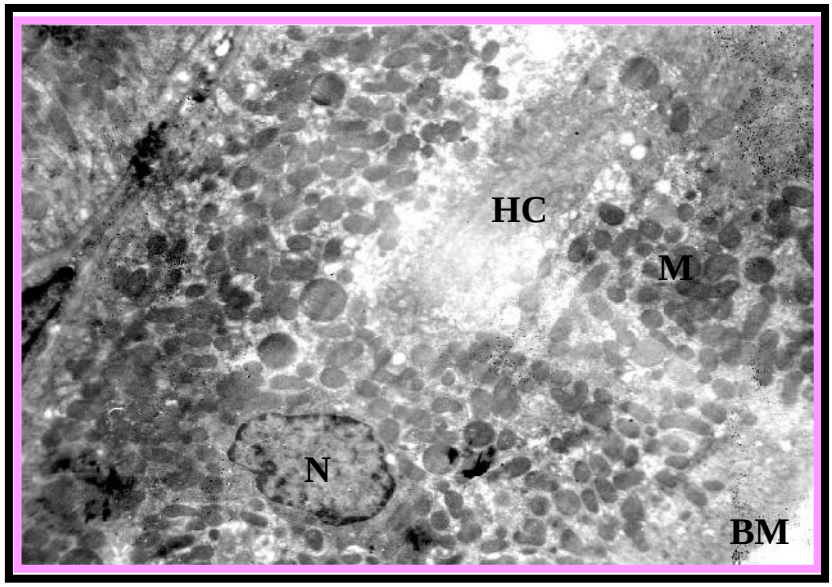


Fig (4.18): Distal tubule of animal treated with Diclo. Uranyl acetate and Lead citrate (3400X). Basement membrane (BM), Nucleus (N), Mitochondria (M), Hayline Cast (HC).

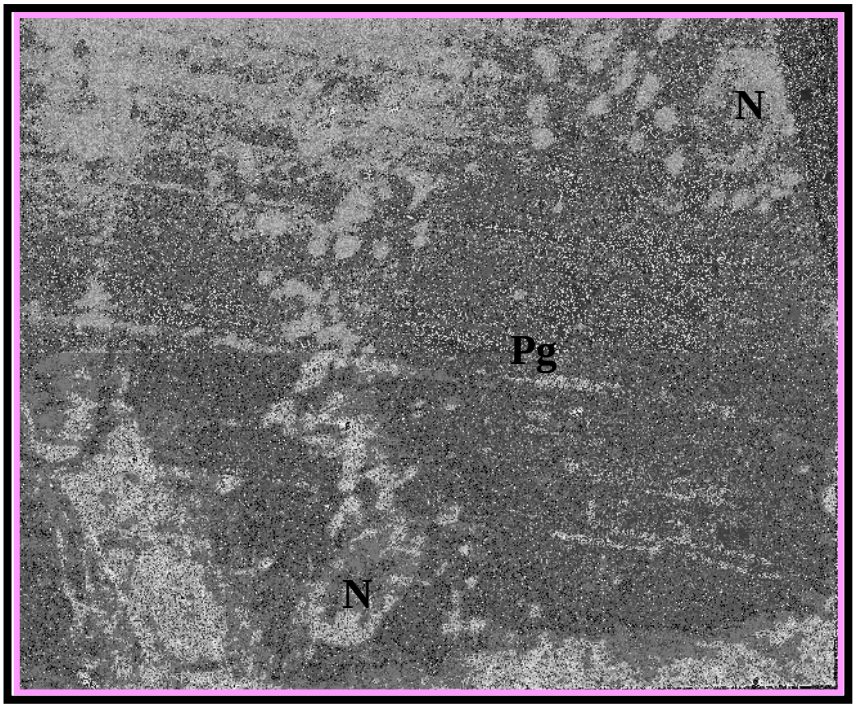


Fig (4.19): Distal tubule of animal treated with Diclo. Uranyl acetate and lead citrate (3400X). Nucleus (N), Pigmented Casts (Pg).

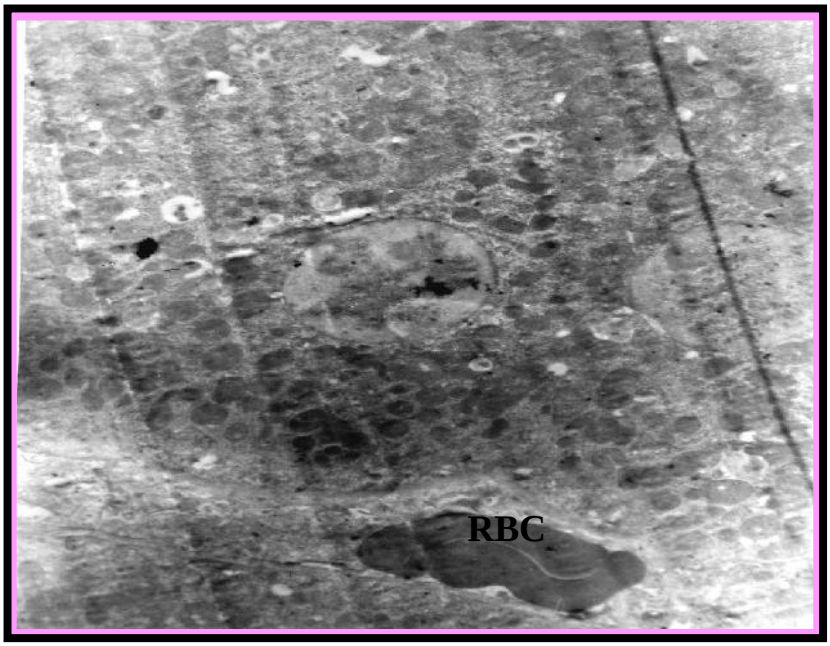


Fig (4.20): Distal tubule cells of animal treated with Diclo. showing the presence of RBC in the interstitium. Uranyl acetate and lead citrate (3400X). Red Blood Corpuscles (RBC).

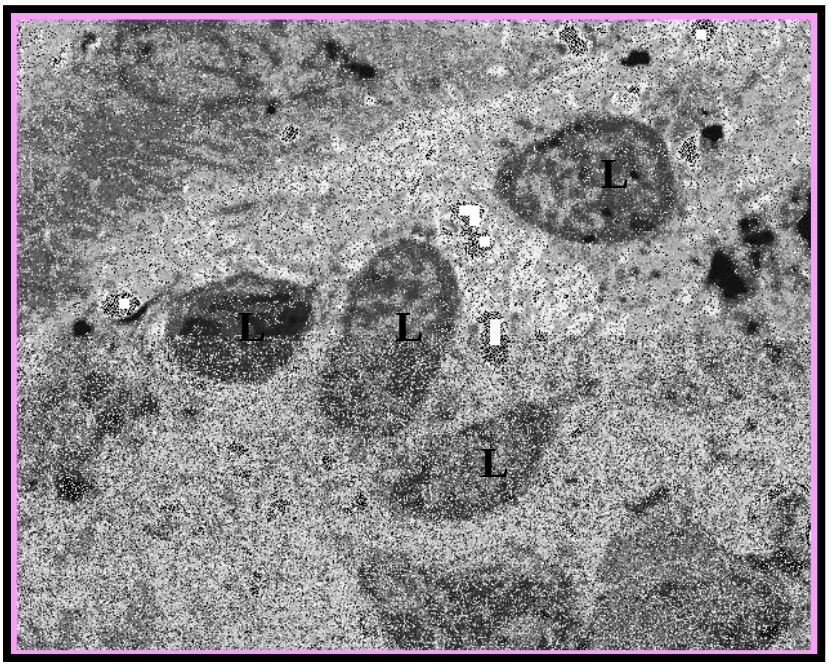


Fig (4.21): Infilteration of the interstitium with inflammatory cells of animal treated with Diclo. Uranyl acetate and lead citrate (3400X).. Lymphocyte (L).

4.3.2.2 Renal Corpuscles:

The renal corpuscles showed a variable changes in renal corpuscle cells, these changes include the followings:

1- Small circumscribed irregularities are present in the basement membrane of blood capillary. The basement membrane was irregularly thickened and cell processes, presumably of mesangial cells, project into the thickened basement membrane producing a segmental double contour appearance. Small spherical particles of varying size are also enclosed in the basement membrane **(Fig. 4.22 and 4.23).** These particles may represent organelles from degenerate cell processes in the basement membrane **(Smith, 1975**).

Changes in the thickness of basement membrane may cause alteration in the glomerular filtration rate, and these are a characteristic of focal and segmental hyalinosis **(Smith, 1975).**

2- Degeneration of podocyte cells were markedly observed, in which cytoarchitacher of podocyte was lost, with the elongation, widening, and fusion of the major foot processes **(fig. 4.24).**

It is well known fact that the podocyte (visceral glomerular epithelial cell) is one of the major cell types responsible for maintenance of the structure and function of the glomerular filter, these podocyte processes serve to counter balance the pulsatile hydrostatic force of blood pressure during the filteration process and tending to expand the glomerular capillary lumen **(Kriz *et al*., 1994).**

Normal function of the filter requires the maintenance of foot-process structure. **Olsen, 1992** suggest that injury to the glomerulus is usually associated with leakage of protein across the filter into the urine and with disappearance (effacement) of podocyte foot processes either locally or generally, thus the anatomic position of the podocyte at the gateway of the glomerular filter is well situated to control the glomerular pressure/ filteration rate relationship.

If this were to be the case, then defects of podocyte function may span a broader set of clinical condition, later we can said that podocyte dysfunction causes increased leakiness of the glomerular filter, which might infests clinically by proteinuria and nephritic syndrome, and this need further study.

3- A considerable increase in the mesangial cells were observed, and the normal distinct between the mesangial area and glomerular capillaries was lost. The glomerulus was bloodless and capillaries were almost entirely occluded by the swollen mesangial cells. An increase in the mesangial matrix was found with a small spherical structures which vary in size and electron density **(Fig. 4.25).**

The phagocytic capacity of the mesangial cell has been demonstrated by several investigaters **(Mellors and Brzosko, 1962; Latta and Maunbach, 1962; Michael *et al.*, 1967)**. It has been suggested that an important function of the mesangial cell is to remove and catabolize material which has become deposited within the glomerular capillary wall, thus restoring the integrity of this

structure **(Farquhar and Palade, 1962).** In this study it is possible to speculate that the mesangial cells were unable to cope with the volume of material being deposite within the wall, thereby enlarging and obstructing capillaries.

The small spherical structures, which vary in size and electron density, might represent deposition of immunological material and this need further study.

4- Polymorphonuclear cells (Neutrophil) were easily recognized by their multilobed irregular nuclei, as well as a monocyte cell were well preserved within the renal corpuscles as show in the electron micrograph **(Fig. 4.26 and 4.27).**

In **fig.(4.27)** the glomerular mesangial regions were seen to contain very few cells, many were represented by cytoplasm containing varying numbers of organelles, with no visible nuclei.

The presence of inflammatory cells (neutrophil, monocyte, lymphocyte) was related simply to the presence of a foreign substance, possibly it is related to alterations in the metabolic state of epithelial and endothelial cells as a result of the fall in glomerular blood flow. The presence of inflammatory cells in the kidney after treatment with diclo. is characteristic of this disorder **(Jonas *et al*., 1983).**

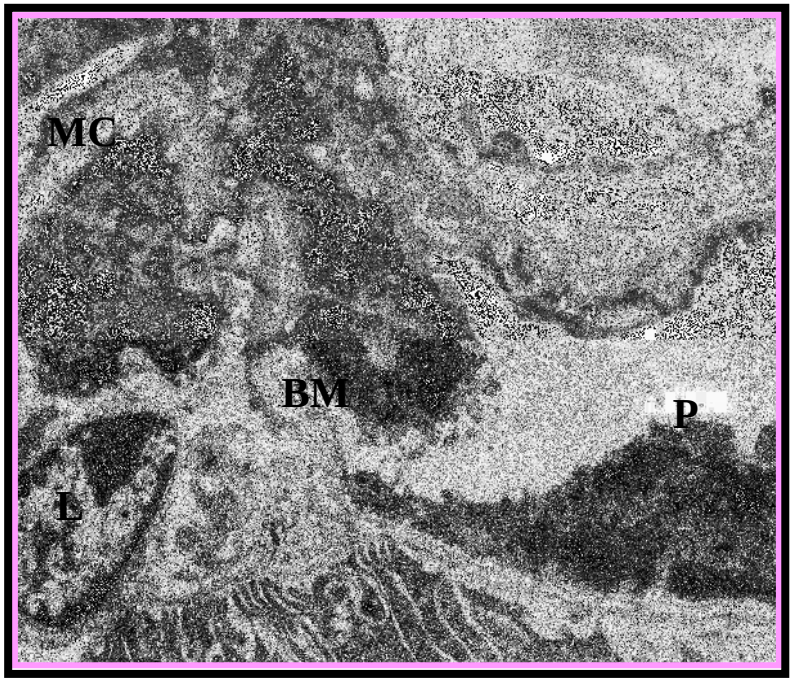


Fig (4.22): Irregular thickening of basement membrane of animal treated with Diclo. Uranyl acetate and lead citrate (5400X). Mesangial cell (MC), Basement mrmbrane (BM) ,Parietal cell (P), Lymphocyte(L).

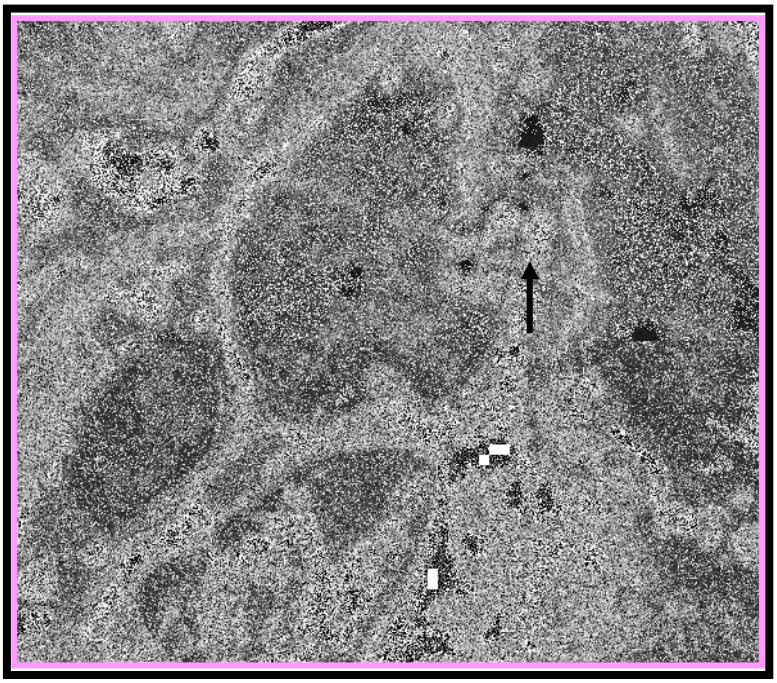


Fig (4.23): Higher magnification of above. Uranyl acetate and lead citrate (6200X). Cell Processes (→)

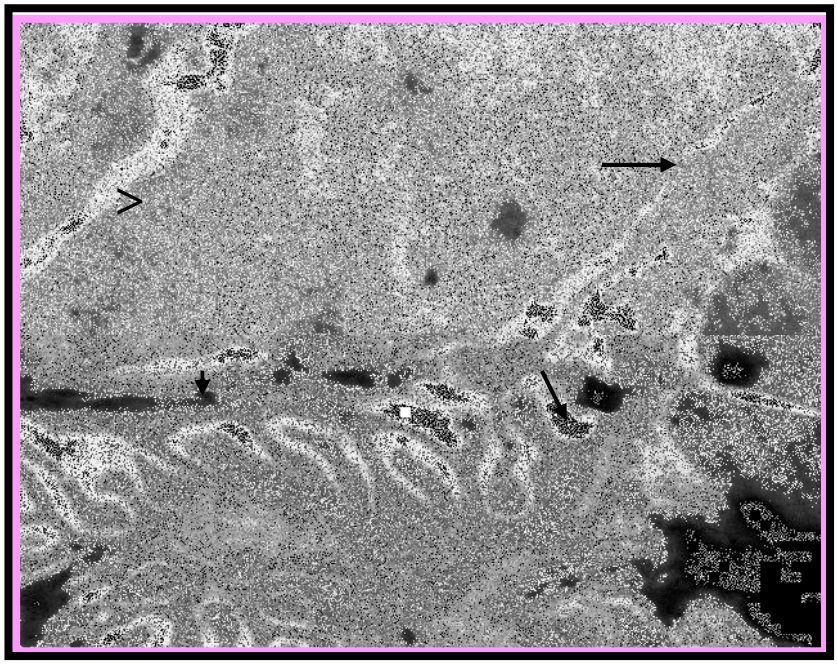


Fig (4.24): Degenerated Podocyte (>) of animal treated with Diclo. Uranyl acetate and lead citrate (8700X). Elongated foot processes (➔), Widening foot processes (➚).

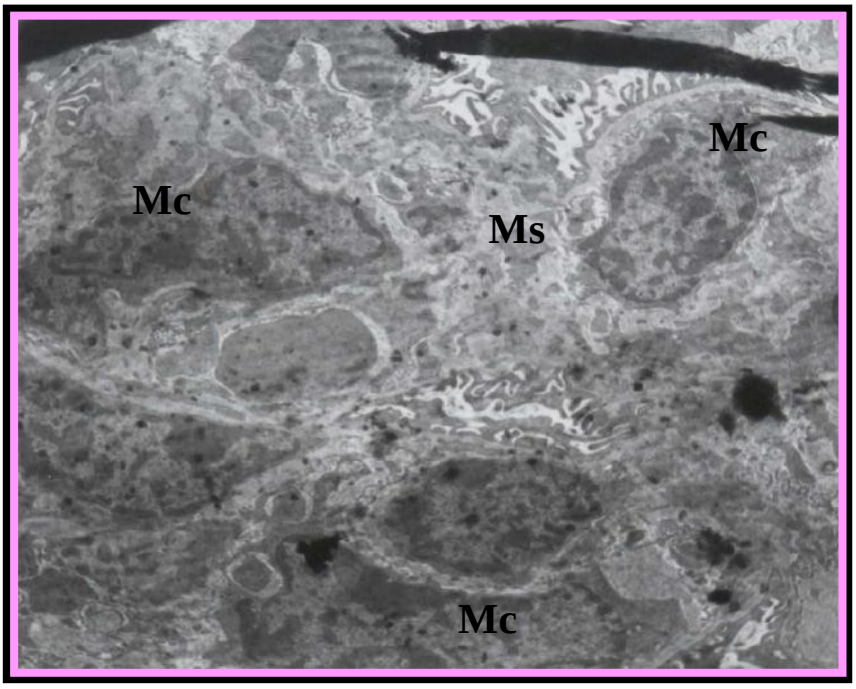


Fig (4.25): Mesangial cell proliferation (Mc) of animal treated with Diclo. Uranyl acetate and lead citrate (4400X). Mesangial substance (Ms).

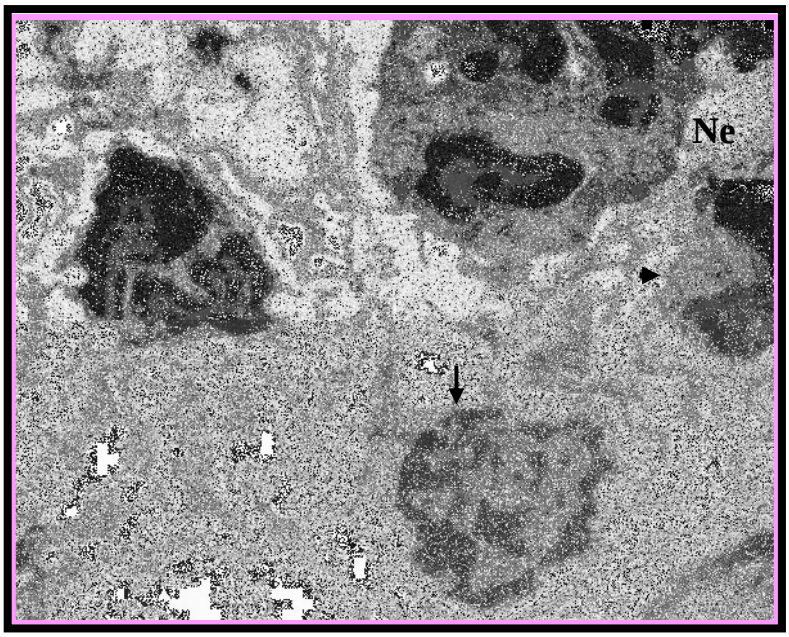


Fig (4.26): Inflammatory cells within renal corpsules of animal treated with Diclo. Uranyl acetate and lead citrate (6200X). Neutrophil (Ne), Monocyte (➝), Drgenerated podocyte (↗).

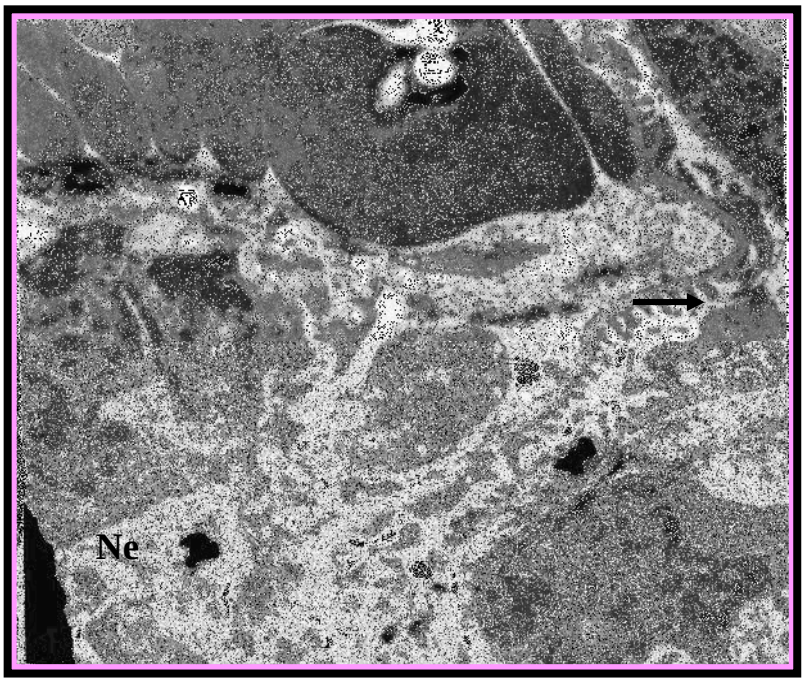


Fig (4.27): Inflammatory cells within renal corpsules of animal treated with Diclo. Uranyl acetate and lead citrate (6200X). Neutrophil (Ne), Degenerated podocyte (↗)

4.3.3 Succinate Dehydrogenase (SDH) activity:

To confirm the identity of succinate dehydrogenase , a negative control and a positive control, were established. In negative control, absence of black particles formazan was notice on the inner membrane of mitochondria, while in positive control extensive black particles were observed which were attaching the inner membrane of mitochondria.

4.3.3.1 Control animals group

Electron microscope examination of SDH activity, in proximal and distal convoluted tubule of this animal group, showed the mitochondria in different size and shape (small and large size, rounded and elongated shape) with moderately dense matrix.

The black particles formazan slightly presented on the inner membrane of the mitochondria as black dotes **(Fig. 4.12, 4.28a and b).**

4.3.3.2 Animals treated with diclo.

In this animal group, the electron microscope examination showed an increase in the number of mitochondria in both proximal and distal convoluted tubule. **Fig.(4.29)** showed normal epithelial cell of proximal tubule, the mitochondria was enclosed and occupying intense black particles of formazan. While **fig.(4.30)** showed epithelial cell of proximal tubule with normal brush-border,

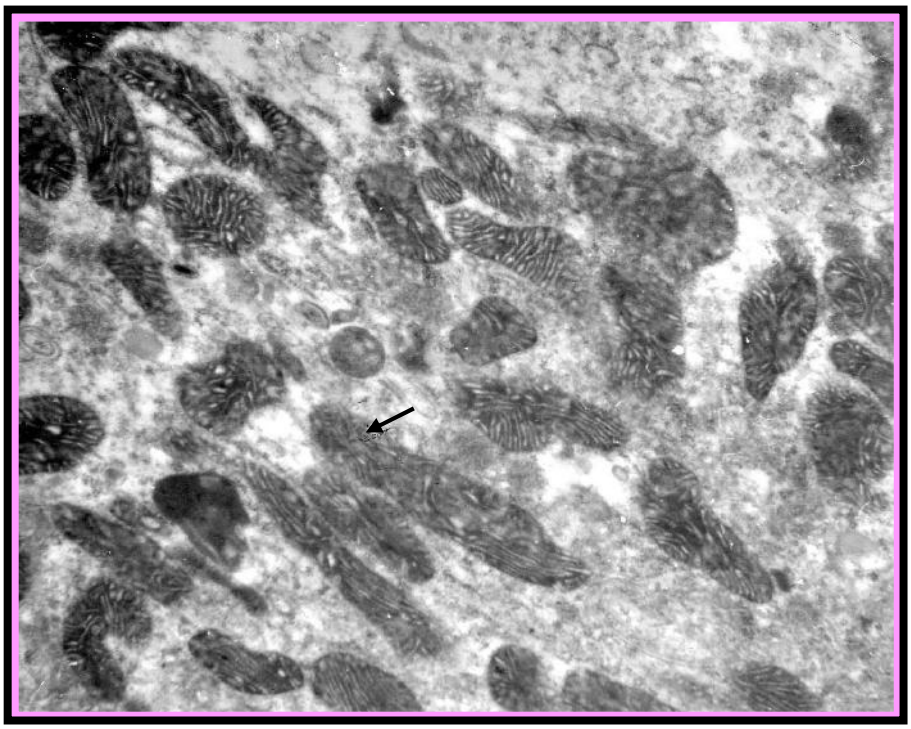
but the cytoplasm contain fuzzy nucleus with huge number of inclusion bodies, the mitochondria contain moderate precitpitate of black particals formazan.

Fig.(4.31) showed epithelial cells of distal convoluted tubule. In these cells a huge number of mitochondria with high electron density were observed , the high electron density of the mitochondria represent the intense activity of SDH. As well as **fig. (32 a & b)** showed an epithelial cell from distal convoluted tubule which elucidate the presence of black particles formazan that bordered the mitochondria and within the inner –membrane on the cristae.

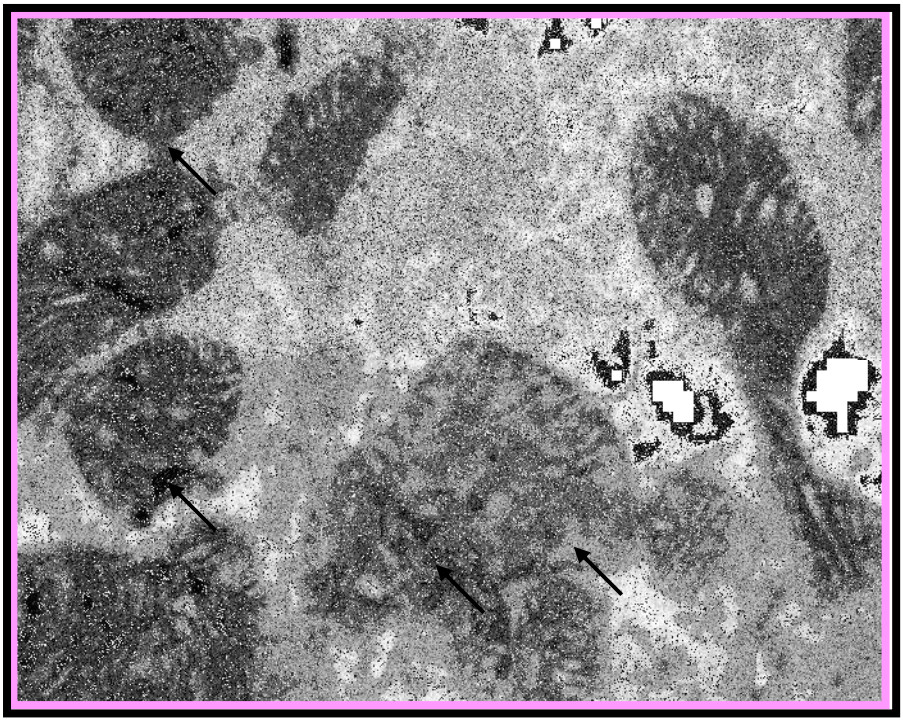
Electron microscopic examination revealed that the mitochondria is a structurally complex organelle, in which certain oxidative enzyme systems are localized. The SDH is particular has been shown to be situated almost in the inner mitochondria membrane **(Davidson and Sittman, 1999).**

The presence of black particles formazan which was noticed in the current study, in both control and animal treated with diclo., indicated that these cells were presented in metabolic active state in both groups. While the packing of theses particles which was noticed in the mitochondria of the experimental cells, give an opinion that the diclo. may cause:

1- Enhancement in the enzyme activity leading to more metabolically active cells.
2- Increament of mitochondrial biogenesis which increased SDH activity.
3- Diclo. effects on the protein synthesis, since accumulation of SDH was conspicuously observed in animal treated with Diclo. This result put a light on further studies concerning Diclo. effects on other mitochondrial enzymes such as lactate dehydrogenase (LDH), ATPase…., which either diminish or increase.



a
12500X



b
30000X

Fig (4.28 a & b): Mitochondria in renal tubule of animal control group. Uranyl acetate and lead citrate. Succinic dehydrogenase activity (⟶).

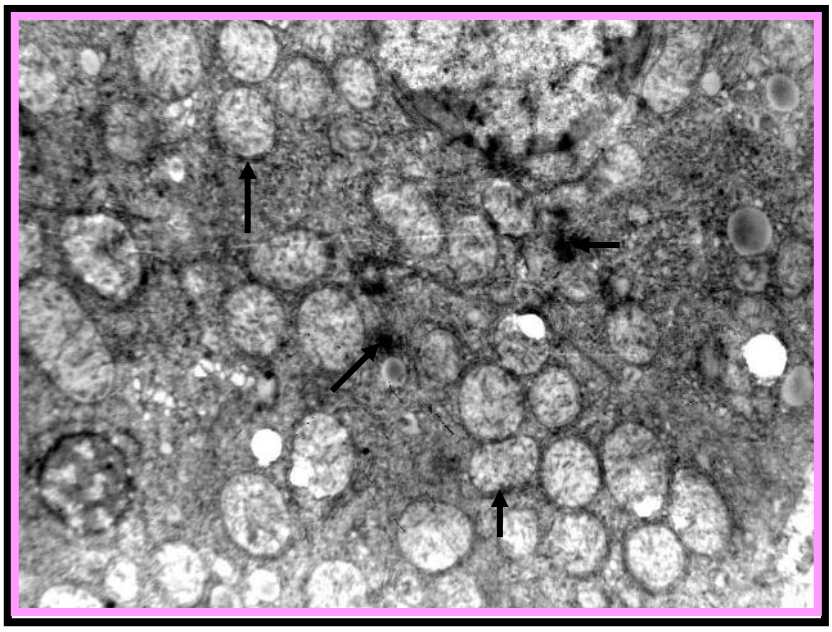


Fig (4.29): Epithelial cell from proximal tubule of animal treated with Diclo. Uranyl acetate and lead citrate (8700X). SD (↗).

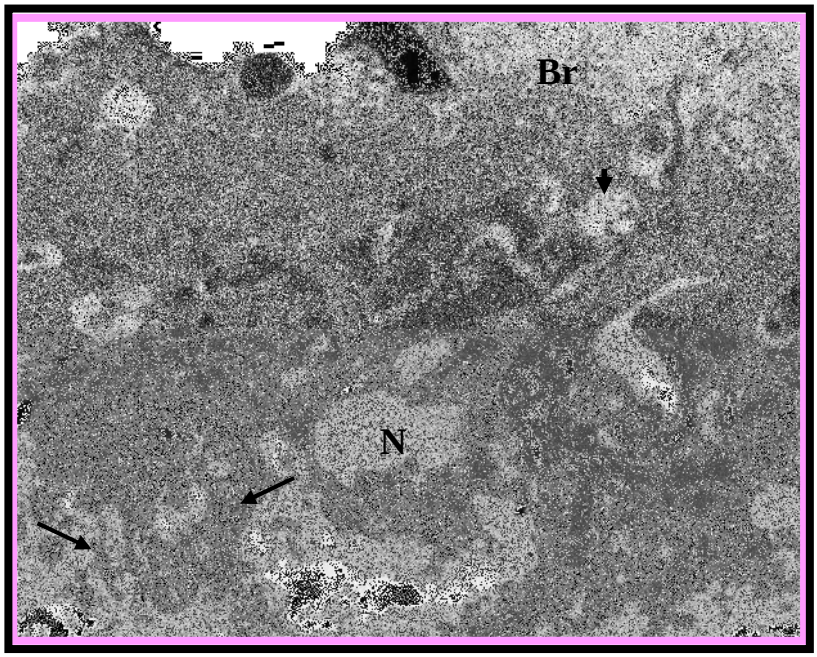


Fig (4.30): Epithelial cell from proximal tubule of animal treated with Diclo. Uranyl acetate and lead citrate (8700X). Note fuzzy nucleus (N), inclusion body (➤), SD (↗), Brush border (Br).

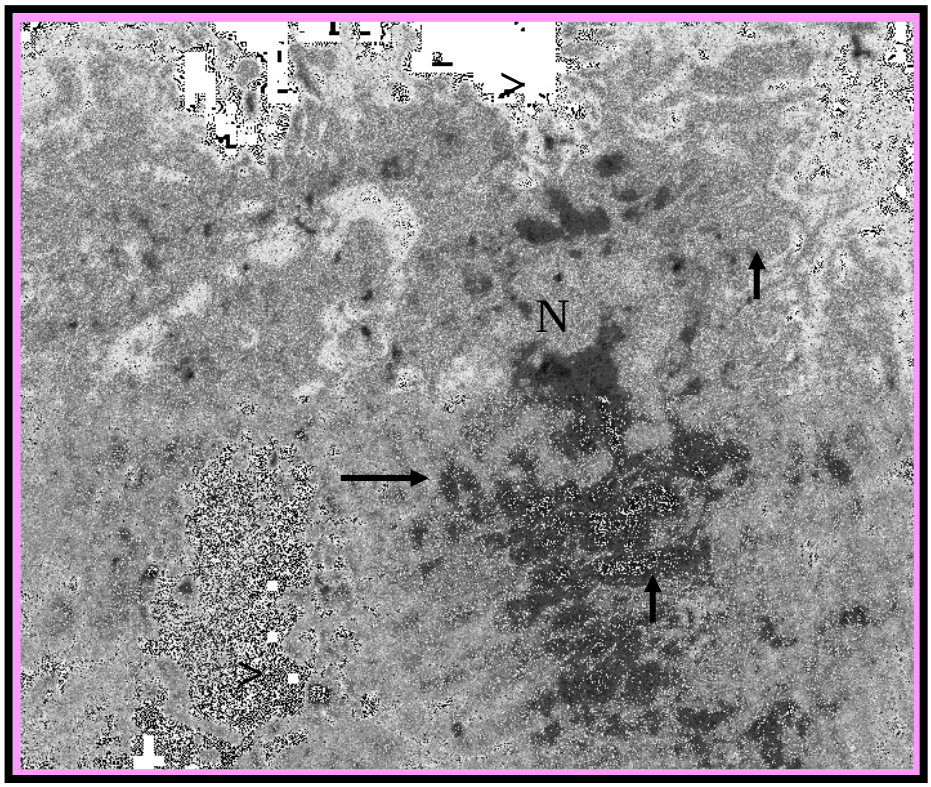
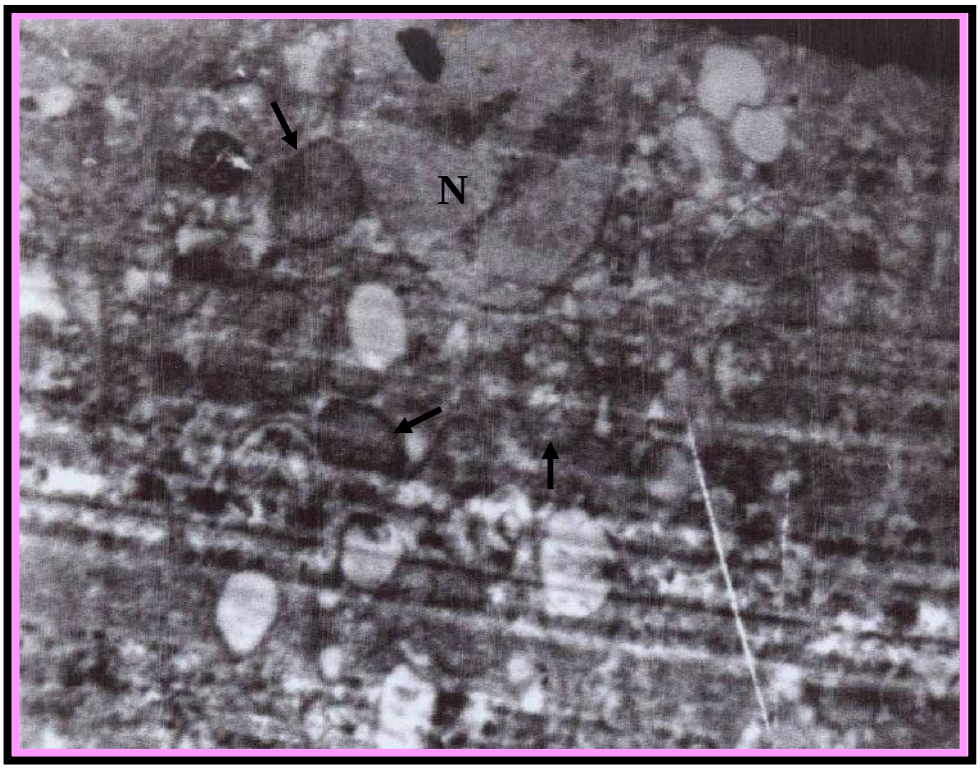
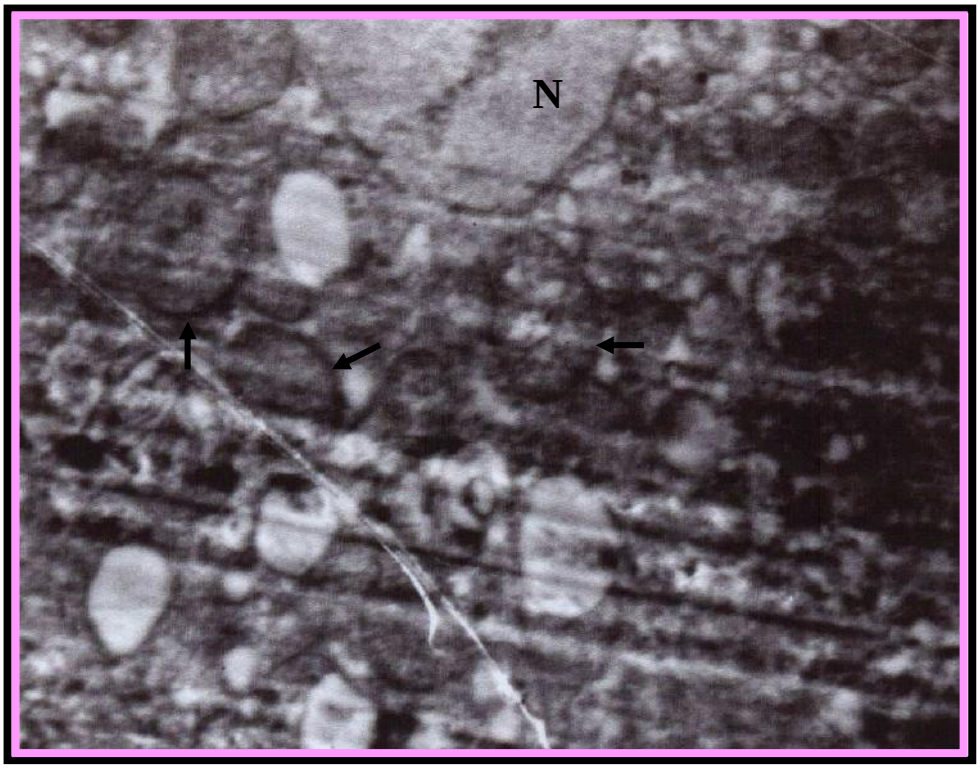


Fig (4.31): Distal convoluted tubule of animal treated with Diclo. Note the huge number of mitochondria with high electron density . SD (↗), Microvilli (>), Nucleus (N). Uranyl acetate and lead citrate (6200X).



a
12500X



b
30000X

Fig (4.32 a & b): Epithelial cell from distal convoluted tubule of animal treated with Diclo. Uranyl acetate and lead citrate. Nucleus (N), SD (↗).

CONCLUSIONS

From this study we conclude the followings:

1. The ultrahistochemical study of SDH in control animal group appears as a black granular particles attaching to the inner membrane of the mitochondria.
2. The ultrastructural investigation of SDH activity reflects an increment of the enzyme activity in the proximal and distal convoluted tubule.
3. From the light microscopic examination, we conclude that diclo. have been not affected all of nephrons of the right kidney.
4. Treatment with diclo. caused a focal segmental glomerulosclerosis with collapse of capillary loops, accompanied by epithelial cell proliferation.
5. Animal treated with diclo. showed obvious changes in cytoarchitecture of renal corpuscles.
6. Diclo. have a marked effects on the renal function, since it cause an increase in the thickness of basement membrane of kidney tubule and on the basement membrane of renal corpuscles.

7. Diclo. have a marked effects on the proximal tubule, since they cause degeneration of brush border which play an important role in the transport activity.
8. Treatment with diclo. caused a casts deposition in the lumen of proximal and distal convoluted tubule, which might cause obstruction of tubular fluid flow.
9. Treatment with diclo. cause an enhancement of renal corpsules cellularity.
10. Treatment with diclo. cause an infiltration of renal corpuscles with inflammatory cells, which reflects an alteration in the metabolic state of epithelial and endothelial cells as a result of the fall in glomerular blood flow.

ألأسم :ريم أثير طه القيسي
الجامعة : النهرين
الكلية :العلوم
القسم :التقانة الأحيائية
العنوان : حي الزهراء محله ٣٢٧ زقاق ٥٩ دار ٢٩
الهاتف:الموبايل ٠٧٩٠١٩٨٧٦٨٨
المشرفة: دكتورة كوكب سليم القيسي
عنوان الاطروحه: دراسة تأثير العقار غير ستيرويدي (دايكلوفيناك) في فعالية أنزيم السكسنيت دي هايدرو جينيز في كلية الفئران البيض
(دراسة بالمجهر الألكتروني)
تاريخ المناقشه:٢٥-٦-٢٠٠٦

Declaration

This is to that the organization and preparation of this thesis have been made by the graduated student **Reem Atheer Al-Kaisy** under my supervision at the College of Science, University of Al-Nahrain, Biotechnology Department in partial fulfillment of the requirement for the degree of Master of science in Biotechnology.

Signature

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature

We, the examining committee certify that we have read this thesis entitled **“ A study on the Effect of Voltaren Drug (Diclofenac) on the Succinic Dehydrogenase Activity in the Kidney of Albino Mice (An Electron Microscopic Study)”,** and we have examined the graduate student **“Reem Atheer Al-Kaisy”** in its contents and that in our opinion, it meets the standard of a thesis for the degree of

Signature

Signature **Signature**

I hereby certify upon the decision of the examining committee

Dr. Laith Al-Anni

Dedication

To All Iraqi People who suffered and are

Still suffering…

To my family… especially my father and

my mother who gives meaning to my life…

and all whom I love … I present this modest

research – work with love and gratitude.

Reem

List of abbreviation

BC	Bowman`s Capsule
BM	Basement Membrane
Br	Brush border
Bs	Bowman`s space
C	Capillaries
C	Capillary loops
Diclo.	Diclofenac
E	Endothelial Cell
H and E	Harris hematoxyline and Eosin
HC	Hayline Cast
I	Inflammatory Cell
L	Lymphocyte
LC	Luminal Cast
M	Mitochondria
MC	Mesangial Cell
MS	Mesangial Substance
N	Nucleus
Ne	Neutrophil
P	Podocyte
P1	Primary Foot Processes
P2	Secondary Foot Processes
PC	Parietal Cell
Pg	Pigmental Casts
RBC	Red Blood Corpuscles
SDH	Succinic Dehydrogenase
V	Vacuole

List of abbreviation

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RECOMMENDATIONS FOR FURTHER STUDY

First of all, using swiss rats as a model to design a new experiment in which the following data could be estimated:

1- Diclo.effects on the heamatological parameters.

2- Diclo.effects on the biochemical parameters, such as urea, creatinin, sodium, potassium and calcium ion concentration in the blood sera.

3- Diclo. effects on the glomerular-filteration rate.

4- Examination of immuno-complex precipitate in the glomerular mesangial cells by using immuno-flourecent technique.

5- Cytogenesis study to investigate the effects of Diclo. on specific cogmponent of the kidney.

6- Enzymehistochemical study of many other enzyme such as LDH, ATPase, also its important to estimate the brush-border enzyme (Alkaline Phosphatase).

7- A morph metric study of the renal space corpuscles ratio to the glomerular tuft.

8- A Chromatography technique is useful for carrying out the qualitative and quantitative estimation for enzyme and other metabolites.

8- Similar study could be carried out on the left kidney.

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SUMMARY

Studies concerning the effects of diclofenac on the renal function were published. Since no information on its effect on the succinate dehydrogenase enzyme (which play an important role in the renal transport activity) have taken by consideration, which was the goal of this study.

In this study (12) healthy adult male mice were used, (6) mice were injected intramuscularly with 0.5 ml diclofenac sodium (0.05 mg/kg) one time in the day for 7 days, and the remained considered as control group.

After one week all animal groups were sacrificed by spinal dislocation. Sample from right kidney were obtained and preceded for light microscopic and electron microscopic examination.

The results have shown the following:

1. A significant decrease ($p > 0.05$) in the body weight as well as in organ/body weight ratio was observed.
2. Light microscopic examinations have shown many alterations in both renal corpuscles and kidney tubule, which include: necrosis of several renal tubules with the formation of large spaces in the interstitium component of some region of kidney structure.

As to the glomeruli, a focal segmental glomerulosclerosis with collapse of capillary loops was observed.

3. Electron microscopic examination have shown a spectrum of changes in kidney tubule from apparently normal to Franz-necrosis, which reflect a state of acute renal failure.

On the other hand renal corpuscles have shown a variable changes in renal corpuscle cells, represented with irregularities and increment of basement membrane thickness, proliferation of mesangial cells, and degeneration of podocytes.

4. The ultrahistochemical study of succinic dehydrogenase enzyme (SDH) reflect an increament of the enzyme activity in the proximal and distal convoluted tubule of animal treated with diclofenac.

From this study it has been concluded that:

1. Diclofenac treatment caused acute renal failure.
2. Treatment with diclofenac caused a focal segmental glomerulosclorosis with collapse of capillary loops.
3. Treatment with diclofenac has a marked effect on (SDH) activity, which plays an important role in transport activity of renal tubule.

بِسْمِ اللَّهِ الرَّحْمَنِ

عَلَّمَ

الأِنْسِنَ

مالَمْ يَعلَمْ

الخلاصة

بينت الدراسات السابقة تأثيرات عقار الدايكلوفيناك في وظيفة الكلية، ونظراً لعدم وجود دراسات عن تأثيره في انزيم السكسنك دي هايدروجينيز (الذي يلعب دوراً مهماً في فعالية النقل الخلوي للكلية) والتي استهدفته هذه الدراسة.

وفي هذه الدراسة تم استعمال (١٢) فأراً ذكراً صحياً ثم حقن ٦ فئران في العضلة من دايكلوفيناك صوديوم بجرعة مقدارها 0.5 ML (0.5 Mg/Kg) مرة واحدة لمدة ٧ أيام واستخدمت باقي الحيوانات سيطرة، وبعد اسبوع شرحت الحيوانات بطريقة خلع النخاع، واخذت نماذج الكلية اليمنى وحظرت للفحص بالمجهر الضوئي والالكتروني واظهرت النتائج ما يلي:

١- وجود انخفاض معنوي ($P< 0.05$) في وزن الجسم وفي نسبة وزن العضو/ وزن الجسم.

٢- اظهرت نتائج الفحص بالمجهر الضوئي حدوث تغيرات في الكبيبة الكلوية وفي نبيبات الكلية والتي تتضمن: تنخر العديد من نبيبات الكلية مما أدى إلى تكون فراغات في النسيج البيني للكلية لبعض المناطق وبالنسبة للكبيبة لوحظ وجود التهاب كبيبي تصلبي مع انخماص في الاوعية الدموية الشعرية.

٣- اظهرت نتائج الفحص بالمجهر الالكتروني تغير واضح في النبيب الكلوي من الوضع الطبيعي إلى تنخر واضح والذي يعكس حالة من الفشل الكلوي الحاد. من ناحية اخرى اظهرت الكبيبة الكلوية تغيرات ملحوظة في خلايا الكبيبية

الخلاصة

الكلوية المتمثلة بعدم انتظامها مع زيادة في سمك الغشاء القاعدي، مع تكاثر في الخلايا الوسطية وتنكس الخلايا القدمية.

٤- ان الدراسات المستدقة الكيميائية النسيجية لأنزيم السكسنك دي هايدروجينيز يعكس زيادة فعالية هذا الانزيم في كل من النبيب الملتوي البعيد والقريب في الحيوانات المعاملة بالدايكلوفيناك.

من هذه الدراسة تم التوصل إلى النتائج التالية:

١- المعالجة بالدايكلوفيناك صوديوم يسبب فشل كلوي حاد.

٢- المعالجة بالدايكلوفيناك صوديوم يسبب التهاب كبيبي مع انخماص بالاوعية الدموية الشعرية.

٣- المعاملة بالدايكلوفيناك صوديوم له تأثيرات واضحة في فعالية انزيم السكسنك دي هايدروجينيز والذي يلعب دور مهم في عملية النقل الخلوي في النبيب الكلوي.

دراسة تأثير العقار غير ستيرويدي (دايكلوفيناك) في فعالية انزيم السكسنيت دي هايدروجينيز في كلية الفئران البيض (دراسة بالمجهر الالكتروني)

رسالة مقدمة إلى

كلية العلوم جامعة النهرين وهي جزء من متطلبات نيل

درجة الماجستير في علم التقانة الاحيائية

تقدمت بها

ريم اثير القيسي

٢٠٠٦ م ١٤٢٧ هـ