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EFFECTS OF CIPROFLOXACIN ON RENAL CORTEX OF RABBITS AND THE ROLE OF VITAMIN C AS A PROTECTIVE AGENT: ANATOMICAL, HISTOLOGICAL, HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY

H. WHAB* ; A. KASSAB** and H. BAHGAT**

* Dept .of Histology, Faculty of Med., Minoufiya Univ., Egypt; ** Dept. of Anatomy and Embryology, Faculty of Vet. Med., Benha Univ., Moshtohor, Toukh, 13736, Egypt;

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SUMMARY

Ciprofloxacin is a fluroquinolone antibiotic commonly used to treat respiratory, urinary tract, skin and soft-tissue infections. The aim of the present work was to study the effects of ciprofloxacin on renal cortex of rabbits and the possible protective role of vitamin C. Twenty four adult male rabbits were used in this study and randomly divided into three groups: control, ciprofloxacin treated and ciprofloxacin plus vitamin C-treated group. This regimen of treatment was given orally for one month. At sacrifice, blood samples were drawn for biochemical study. The kidneys were observed macroscopically and sections were prepared for histological, histochemical and immunohistochemical studies. Ciprofloxacin treated rabbits showed a highly significant increase in Blood urea nitrogen and creatinine. The kidneys of ciprofloxacin treated rabbits appeared small in size with macroscopic evidence of fibrotic capsular thickening. Renal cortex of ciprofloxacin treated rabbits showed moderate tubular dilatation in some proximal and distal convoluted tubules with marked degeneration and vacuolization of their lining cells. Some glomeruli were hypertrophied while others were atrophied.

In summary, Ciprofloxacin can induce subtle renal damage which could be ameliorated by simultaneous use of vitamin C which is easily available and safe antioxidant. On the basis of our results, we advice Clinicians to use vitamin C in common with ciprofloxacin to avoid its potential complication.

INTRODUCTION

Ciprofloxacin (CFX) is a fluroquinolone of second generation used for the treatment of community-acquired infections. It displays broad-spectrum antibacterial activity against Gram-positive and Gram-negative pathogens (Suh and Lorber, 1995; Hardy et al., 1999; Boswell et al., 1999). Levofloxacin and ciprofloxacin are extensively used as empirical or directed therapy for a variety of infections in critically ill patients due to their excellent activity against common gram-negative pathogens and moderate activity against many gram-positive organisms as well (Rebecca et al., 2001).

Although the few side effects of CFX are well known, there is very few data on its histopathological findings on the kidney. However, kidney is a site for potential CFX toxicity, since it can be exposed to relatively high concentrations of CFX, whose elimination depends mainly on kidney function. Ciprofloxacin is commonly associated with acute renal insufficiency (ARI) and acute renal failure, but the majority of cases reported are in older adult patients (Lomaestro, 2000). The long term use of ciprofloxacin, which is an important fact considering that ciprofloxacin is a widely used antibiotic agent in treatment of urinary tract infection, may cause acute renal failure (Allon et al., 1990). Short-course antimicrobial therapy offers several theoretical advantages, including patient convenience and enhanced compliance, fewer potential adverse reactions, less potential for emergence of resistant bacteria in the gastrointestinal tract and lower costs (Hooton and Stam, 1991).

The majority of clinical references concentrate on the functional evaluation of kidneys; morphological aspects are studied as a matter of peripheral importance. The histopathological changes of the adverse drug reactions by CFX are not well known (Guzman et al., 2003). Only few reports reported that ciprofloxacin can induce pyelonephritis (Talan et al., 2000).

Bilimoria and Ecobichon (1992) found that cellular damage is prevented by the presence of adequate levels of antioxidant chemicals in the tissues. Vitamin C (ascorbic acid) is one of several compounds that form the first line of bodyís antioxidant defensive system (Guthrie and Picciano, 1995).Vitamin C is an effective chemoprotective agent against nephrotoxicity induced by cisplatin in Wistar adult rats (Greggi et al., 2000).

Thus, the aim of this work was to study the ef-

fects of long term treatment of ciprofloxacin on renal cortex of rabbits and the possible protective role of vitamin C.

MATERIALS AND METHODS

Animals

Twenty four adult male rabbits weighing 700-1300 grams were used in this study. They were randomly divided into three groups; the first group served as normal control. The second group (ciprofloxacin treated) was given ciprofloxacin in a dose of: 200 mg/kg body weight (Wise et al., 1986). CFX used was in the form of tablets (Cipro 500 mg). The third group was given simultaneous doses of CFX and vitamin C. Vitamin C in a dose of 100 mg/kg body weight (Levine et al., 1999 and Greggi et al., 2000). Vitamin C was in the form of tablets (Cevarol 500 mg, produced by Memphis Company). Both CFX and vitamin C were dissolved in distilled water and given orally by a modified plastic syringe daily for one month. All animals were housed under the same conditions and allowed food and water ad-libitum.

Sampling, sectioning and staining

Twenty-four hours after the last dose, blood samples were taken from the ear vein to determine blood urea nitrogen (BUN) and creatinine using standard laboratory techniques. Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function.

The rabbits were anaesthetized with sodium pentobarbital (30-40 mg/kg) and subsequently killed by exsanguinations from the abdominal aorta without regaining consciousness following the opening of the abdominal cavity with a median incision and exposing kidneys.

Both kidneys of each animal were taken and observed grossly. Small pieces from the outer cortex were taken, fixed in 10% neutral buffered formol, dehydrated and embedded in paraffin. Sections of 5-6 µm in thickness were obtained and stained with Haematoxylen and eosin (H&E) for routine histological examination and periodic acid Schiff reaction (PAS) for mucopolysaccharides (Drury and Wallington, 1980).

For immunohistochemistry, obviously, the ABC method with HRP/DAB detection system was applied. 5µm paraffin sections were deparaffinized, hydrated, washed, heated in buffered citrate and incubated for 30 minutes with rabbit serum. Then they were incubated with anticaspase-3 mouse monoclonal antibodies (MAbs) (Lab vision, USA) for one hour in humid chamber at room temperature (Makio and Togari, 2003). Then slides were washed in phosphate buffered saline (PBS), incubated with biotinylated rabbit anti-mouse Ig antibodies for 30 minutes and washed in buffered

phosphate solution. DAB chromagen was applied for few minutes, and then sections were counter-stained with Harris Haematoxylen, dehydrated, cleared and mounted.

All statistical analyses were accomplished with the statistical package for the social sciences (SPPS 9.0, SPSS Inc. Corp., Chicago, IL, USA) computer package.

RESULTS

Using of ciprofloxacin for long-term treatment resulted in typical macroscopic and histological changes in the rabbit kidney.

General appearance

Ciprofloxacin-treated rabbits showed anorexia, easily removal of fur from different parts of their body, mild diarrhea, and general emaciation with a progressive and highly significant decrease in body weight (table. 1). The severity of these symptoms reduced in the CFX+vitamin C-treated group.

Biochemical changes

Blood urea nitrogen and serum creatinine showed a highly significant increase in ciprofloxacin-treated rabbits (table. 2).

Gross findings of the kidneys

On macroscopic examination, the kidneys of ciprofloxacin-treated rabbits appeared small in size and little friable (as compared to those of the controls) and typically described as bilaterally symmetrical decreased size. The kidneys were also congested and showed macroscopic evidence of fibrotic capsular thickening.

Histological findings

All individuals of ciprofloxacin-treated rabbits revealed moderate tubular dilatation in some proximal and distal convoluted tubules with marked degeneration and vacuolization of their lining cells (Figs. 1, 2, 4 and 6). Some cells were exfoliated in the tubular lumen (Fig. 2). Nuclei were shrunken or dissoluted (Figs. 2 and 4). Hyaline cast and necrosis of some tubules were observed (Fig. 4). Some glomeruli were hypertrophied and markedly lobulated with extravasations of their blood while others were atrophied and shrunken (Fig. 5). The glomerular basement membrane was slightly thickened with narrowing of Bowman's capsule (Fig. 5). The proportion of the tubular interstitial changes was more than that of the glomerular changes. Renal blood vessels showed marked dilatation and congestion and appeared as longitudinal streaks between the tubules (Figs. 3 and 6). There was marked mononuclear cell infiltrations and hemorrhage at some areas of the interstitium of the cortex (Figs. 3 and 10). The amount of collagen fibers around Bowman's capsule and in the interstitial tissues increased in the group of rabbits that treated with ciprofloxacin in

comparison with the control group (Figs. 8, 9, 10 and 11). These changes were consistently found in all individuals of this group.

Renal cortex of the CFX+vitamin C-treated group revealed slight tubular dilatation. There was slight thickening of glomerular membrane but with slight increase or normal sized glomeruli. There was minimal amount of collagen fibers (Fig. 12). So, the pathological changes of the various renal structures (glomeruli, Bowman's capsule, tubuli, interstitium and blood vessels) reduced in the CFX+vitamin C-treated individuals.

Microscopic examination of the medullary portion of the kidney was relatively unremarkable with no abnormalities and only isolated foci of interstitial mononuclear inflammatory infiltrates.

Histochemical findings

Periodic acid Schiff's reaction: sections of ciprofloxacin treated group showed very strong PAS reaction in the basement membranes and brush borders of both proximal and distal convoluted tubules and in the parietal epithelium of the glomeruli (Fig. 7).

CFX+vitamin C-treated group exhibited strong PAS reaction like that of the control.

Immunohistochemical findings

The control group of rabbits showed no immunoreactivity for caspase-3 in the tubules and glomeruli (Fig. 13).

In ciprofloxacin treated group, there was a strong positive immuno-reaction for caspase-3 in both proximal and distal convoluted tubules (Fig. 14).

In CFX+vitamin C-treated group, there was a moderate positive reaction in some tubules and negative reaction in the others (Fig. 15).

Table 1. Body weight (in gram) in all groups

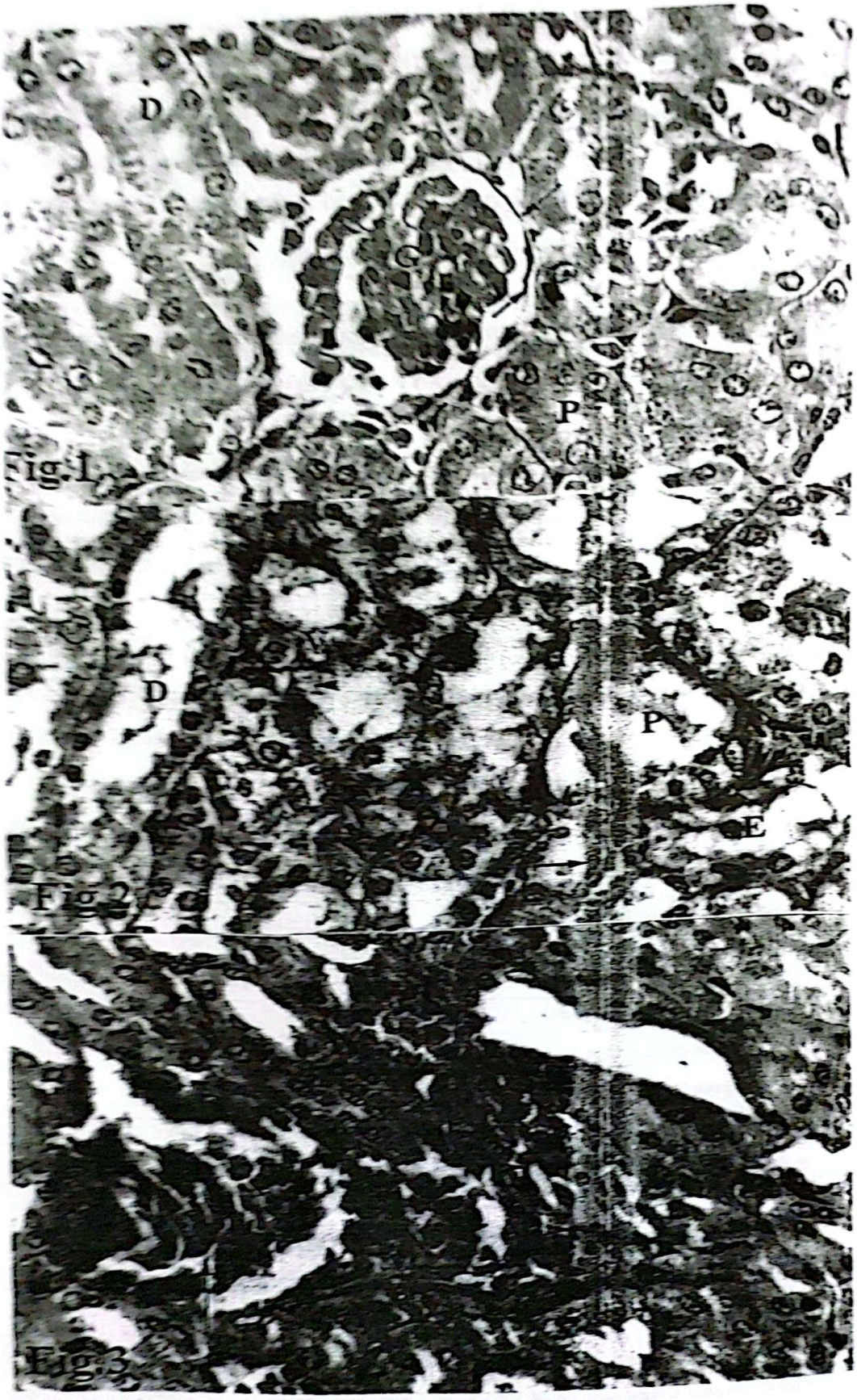
Group	Mean ± SD	P-value
Control	2005 ±3.68	
Ciprofloxacin-treated	1650 ± 2.5	< 0.001
CFX+vitamin C-treated	1850 ± 4.5	< 0.05

P value > 0.05 = Non-significant
P value < 0.05 = Significant
P value < 0.01 = highly significant

Table 2. Blood urea nitrogen (BUN) and creatinine in all groups

Group	BUN (mg/dl)		Creatinine (mg/dl)	
	Mean ± SD	P-value	Mean ± SD	P-value
Control	17.8 ±3.2		0.67 ± 0.29	
Ciprofloxacin-treated	32.8 ± 5.9	< 0.001	2.07 ± 0.30	< 0.001
CFX+vitamin C-treated	24.3 ± 3.5	< 0.05	0.79 ± 0.33	< 0.05

P value > 0.05 = Non-significant
P value < 0.05 = Significant
P value < 0.01 = highly significant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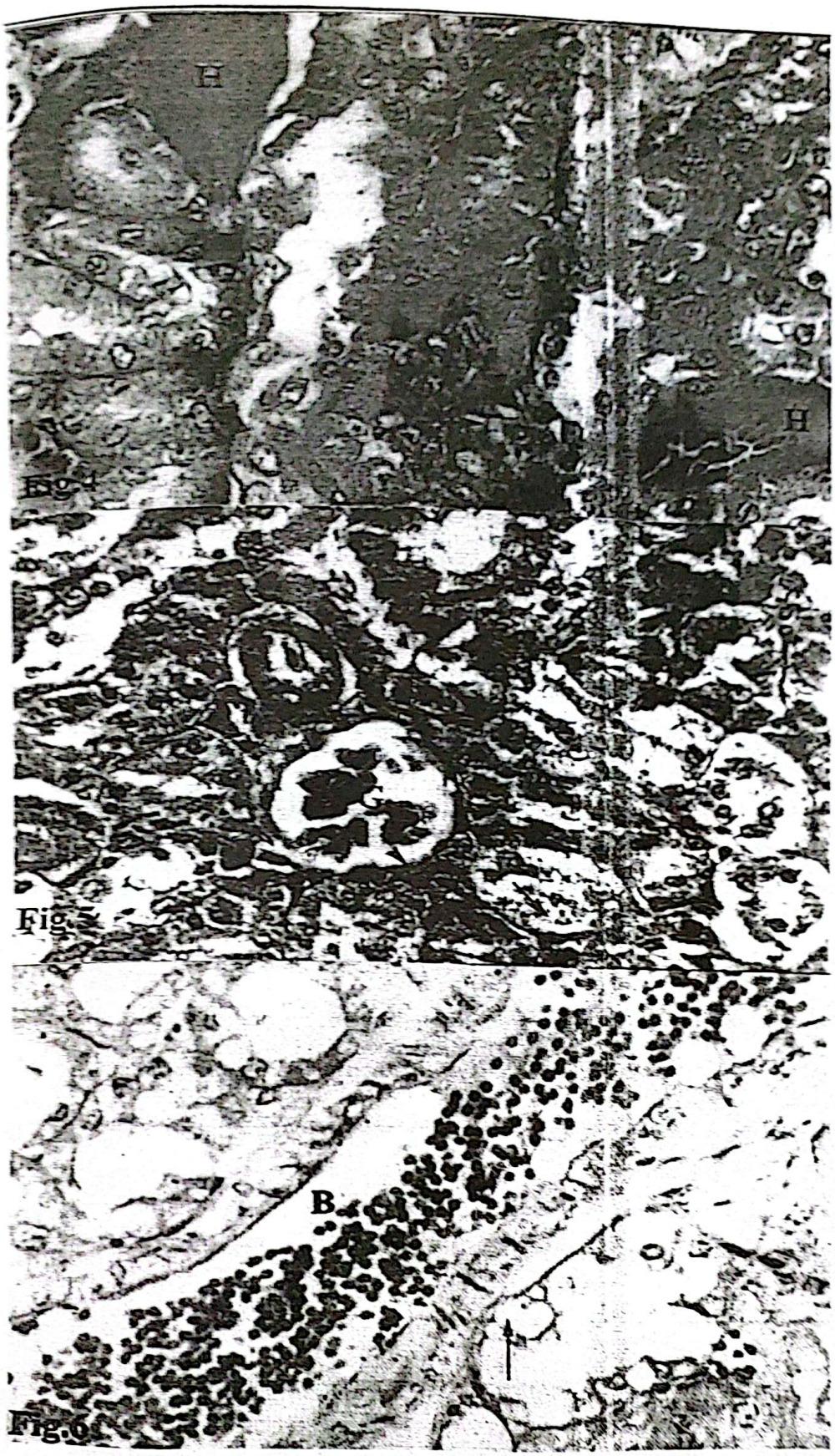

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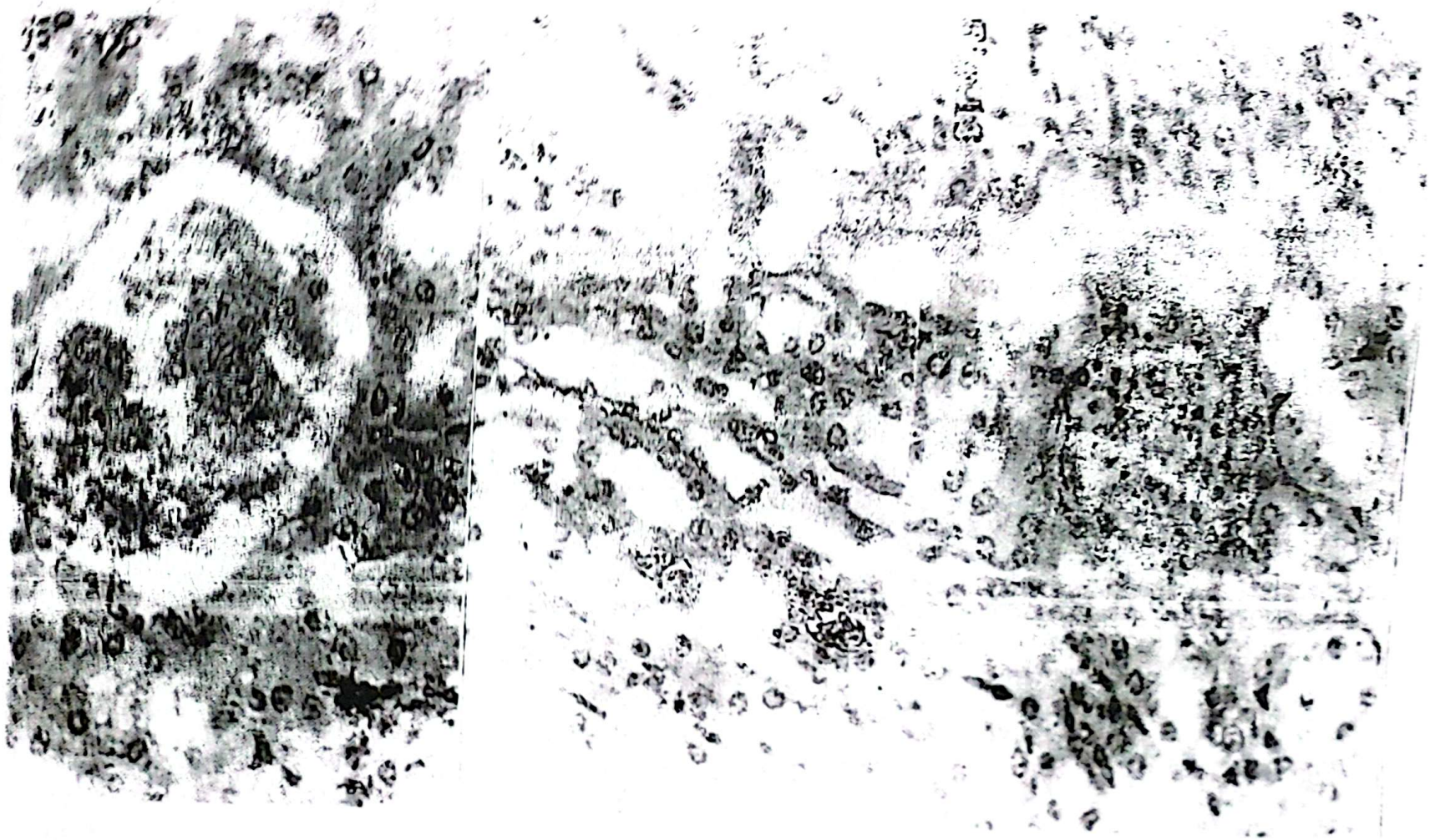


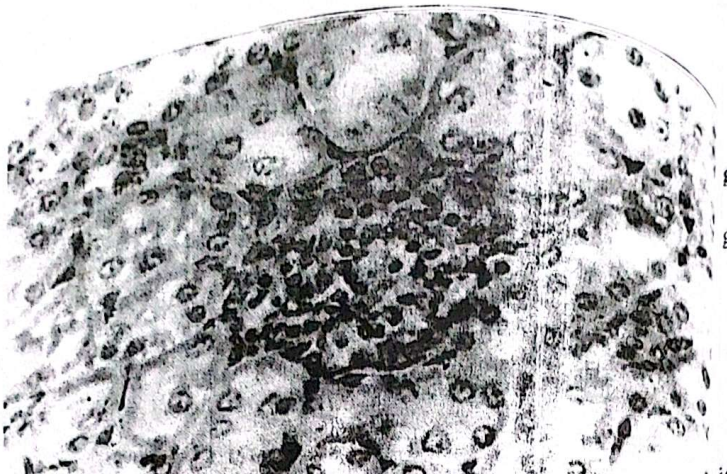
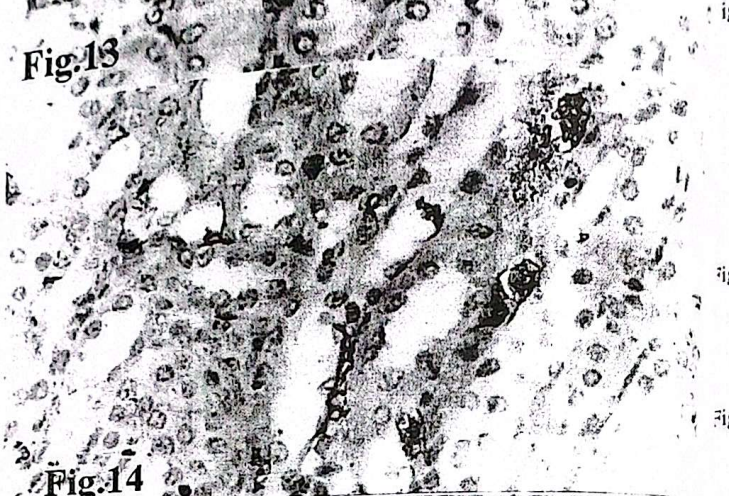


Fig.13



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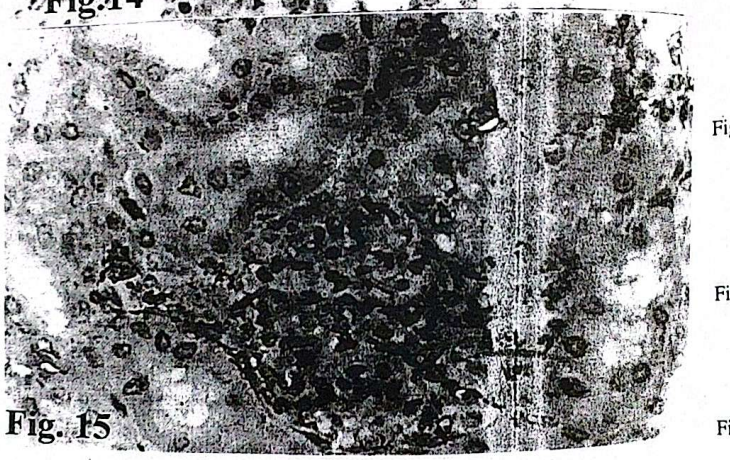

Fig. 15

Figure legends

Fig. 1. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing a glomerulus (G) surrounded by Bowman's membrane (arrow) and group of dilated proximal and distal convoluted tubules. PCTs are lined by pyramidal cells with narrow lumen (P). DCTs are lined by cuboidal cells with wide lumen (D). (H & E X400)

Fig. 2. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing dilatation in proximal (P) and distal (D) convoluted tubules with degeneration and vacuolation of their lining cells (V). Some nuclei are shrunken (arrows) while others are dissoluted leaving ghosts (arrow heads). Notice exfoliation of some cells in the tubular lumen (E). (H & E X400)

Fig. 3. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing dilatation and congestion of a blood vessel (arrow). Notice: mononuclear cell infiltration (arrow heads) (H & E X400)

Fig. 4. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing areas of acidophilic hyaline cast (H). Degeneration and vacuolation of some lining cells of the convoluted tubules (arrows). Notice: some nuclei are dissoluted leaving ghosts (arrow heads). (H & E X400)

Fig. 5. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing shrunken glomerulus (G) with thickening of Bowman's membrane (arrow head). Notice: degeneration of lining cells of convoluted tubules (arrows). (H & E X400)

Fig. 6. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing congestion of a blood vessel (B). Notice: degeneration and vacuolation of lining cells of the convoluted tubules (arrows).(H & E X400)

Fig. 7. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing very strong PAS reaction in the basement membranes of the PCT and DCT (arrows) and glomerulus (G) but mild reaction and even negative reaction (arrow head) in their brush borders. (PAS X400)

Fig. 8. Photomicrograph of control rabbit renal cortex showing minimal amount of collagen fibers (blue color) around Bowman's capsule and in-between convoluted tubules. (M. T. X400)

Fig. 9. Photomicrograph of control rabbit renal cortex showing minimal amount of collagen fibers (blue color) in the adventitia of a blood vessel (arrow) and in-between convoluted tubules. (M. T. X400)

Fig. 10. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing large amount of collagen fibers in the interstitial tissues (arrows) accompanied with cellular infiltration (arrow heads). (M. T. X400)

Fig. 11. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing large amount of collagen fibers in the adventitia of a blood vessel (arrows). (M. T. X400)

Fig. 12. Photomicrograph of a CFX+vitamin C-treated rabbit renal cortex showing minimal amount of collagen fibers around Bowman's capsule and in-between convoluted tubules. (M. T. X400)

Fig. 13. Photomicrograph of a control rabbit renal cortex showing no immunoreactivity for caspase-3 in the tubules and glomeruli. (PAP X400)

Fig. 14. Photomicrograph of a ciprofloxacin-treated rabbit renal cortex showing strong positive immunoreactivity for caspase-3 in the convoluted tubules (brownish punctuate in the cytoplasm). (PAP X400).

Fig. 15. Photomicrograph of a CFX+vitamin C-treated rabbit renal cortex showing moderate positive immunoreactivity for caspase-3 in some tubules and negative reaction in others. (PAP X400).

DISCUSSION

The kidney is one of the most pharmacological targets for antibiotics, it is important to characterize the effect of ciprofloxacin on the kidney. In the present study, we have examined the effects of CFX on the kidneys of rabbits since CFX exhibits greater distribution to the kidney and other tissues compared with other quinolones.

The renal toxicities associated with ciprofloxacin had been well described and depending upon the insult can have multiple presentations including occult hematuria, decreased renal function, increased renal weight, crystalluria and acute interstitial nephritis leading to acute renal failure (Patterson, 1991).

The renal changes in the present study are mainly due to the effect of ciprofloxacin; On the contrary, in the past, the true incidence of ciprofloxacin-induced renal changes was difficult to determine since many patients are taking other medications that may affect renal function (Lomaestro, 2000).

In this study blood urea nitrogen (BUN) and creatinine showed a highly significant increase in CFX treated rabbits, which passed in line with the previous findings of Allon et al. (1990) and Basaran et al. (1993).

Renal cortex of ciprofloxacin treated rabbit showed moderate tubular dilatation in some proximal and distal convoluted tubules with marked degeneration and vacuolization of their lining cells and pyknosis or dissolution of their nuclei as had been previously reported by Dharnidharka et al. (1998). Cellular degeneration could be referred to renal ischemia due to ciprofloxacin-induced vascular injury as has been previously reported by Shih et al. (1995). It could be also explained by direct toxic effects of CFX on cell membranes leading to cell damage. Cytoplasmic vacuolization could be explained by cell membrane alternation secondary to lipid peroxidation induced by CFX leading to shift of extracellular fluid into the cells with intracellular fluid accumulation leading to hydropic degeneration of cells manifested by presence of Cytoplasmic vacuoles. This explanation coincided with Weyers et al. (2002) who found that lipid peroxidation might be one of the mechanisms of CFX toxicity on renal tubules. Dilatation and congestion of renal blood vessels and interstitial hemorrhage in CFX treated rabbits could be explained by direct toxic effect of CFX on the wall of blood vessels leading to their dilatation and leakage of blood from their necrotic wall to the interstitium. Marked mononuclear cell infiltration was noticed in interstitium of ciprofloxacin treated rabbits as a result of interstitial nephritis and this coincided with the previous results of Lien et al. (1993) who found granulomatous interstitial nephritis in renal biopsy from pa-

tient presented with acute renal failure and skin lesions following a 14-day course of CFX treatment.

Control and CFX+vitamin C-treated rabbits showed strong PAS reaction in basement membranes and brush borders of both proximal and distal convoluted tubules. While ciprofloxacin-treated rabbits showed very strong reaction in the basement membranes while mild and even negative reaction in the brush borders. Increased reaction in basement membrane could be attributed to increase its thickness while the weak reaction in brush borders was due to its destruction by ciprofloxacin.

Control rabbits showed no immunoreactivity for caspase-3 in most of the cells lining the tubules and glomeruli. Green (1998) reported a definitive role of caspases in apoptosis and inflammation. Nearly all physiological cell deaths in animals were preceded by the process of apoptosis (programmed cell death), during which the dying cells vanish without a trace, silently cleared without any accompanying inflammatory response. The final stage of apoptosis, called execution, occurs through the activation and function of caspases (Makio and Togari, 2003). Venardos et al. (2004) assessed the level of apoptosis by examining caspas-3 activity in tissue homogenates. In ciprofloxacin-treated rabbits, there was a strong positive immunoreaction in both proximal and distal convoluted tubules which could be attributed to renal apoptosis. This agreed with the previous findings of Dharnidharka et al. (1998) who reported prominent distal nephron apoptosis in renal biopsy obtained from a case of acute renal failure resulting from CFX overdose.

In view of this potential complication, renal function should be closely monitored in patients receiving ciprofloxacin therapy, especially if other potentially nephrotoxic drugs are prescribed concomitantly.

In the present study, ciprofloxacin can induce subtle renal damage which could be ameliorated by simultaneous use of vitamin C (which is easily available and safe antioxidant). However, recently, Goswami et al. (2006) have demonstrated that reactive oxygen species are involved in the antibacterial action of ciprofloxacin and that different antioxidant compounds including vitamin C provided substantial protection of E.coli against CFX. According to Goswami et al. (2006) dietary intake and cellular levels of antioxidants including ascorbic acid may affect the effectiveness of CFX-treatment.

Therefore, we advice Clinicians to use vitamin C in common with ciprofloxacin to avoid its potential complication. But according to Goswami et al. (2006), further investigations surrounding the intake of antioxidants on the effects of fluroquinolones for the treatment of infections caused by E. coli are necessary in the future.

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