TOXICO-PATHOLOGICAL STUDY OF GENTAMICIN BY INTRAMUSCULAR INJECTION IN EXPERIMENTAL RABBITS

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ABSTRACT

In the current study, 16 rabbit was divided into two groups. Animals of Group I (untreated control) were maintained without any treatment. Group 2 animals (treated group), the gentamicin were injected in thigh muscle /I.M. at 25 mg/kg body weight twice a day (morning and evening) for two months. During experimental period anorexia, emaciation, and death some animals were seen. Histopathological examination showed that there was proliferated of cortical renal tubules cells and degeneration with necrosis of some them as well as congestion of sinusoid in liver and proliferation of bile duct. There was also vacuolated and congestion of myocardial tissue. In conclusion, the administration of gentamycin for long time could serve as a source of harm to animal organs such as kidney, liver, bile duct, and heart

INTRODUCTION

Gentamicin, an aminoglycoside antibiotic, is a mixture of basic, water soluble compounds containing the aminocyclitol 2-deoxystreptamine and additional amino sugars. The three major active components are designated Clf, Cj, and Cla, but other minor active components which may be presented include gentamicins A, B, and X. The gentamicin C complex is normally formulated as the sulfate salt, a white-to-buff colored, odourless, and water-soluble powder. Ion exchange procedures are

frequently used for the separation and purification of gentamicin on a commercial scale, after pH adjustment of the fermentation broth and filtration [1].

Gentamicin is widely used for the treatment of various infectious diseases in veterinary and human medicine. It is used for the treatment of a variety of susceptible bacterial infections in swine, poultry, bovines, and equines (e.g., colibacillosis and peritonitis in swine; for mastitis, urinary tract disease, respiratory disease and septicemia in bovines)[2]. Various formulations of gentamicin have been developed for the treatment of food producing animals. Some of these in combined with other antibiotics such as penicillin G, ampicillin, and cloxacillin. Formulated products typically contain 4-80 mg/ml of gentamicin and are available for administration by injection, mammary infusion, oral treatment or as an additive to drinking water [3]. It's injected or oral administration leads to high concentrations in renal cortex and internal ear. The frequent clinical findings of gentamicin toxicity included nephrotoxicity and ototoxicity [4]. Most of the aminoglycoside injected into the body is excreted into the urine without being metabolized. However, the remainder of the injected dose accumulates selectively and abundantly in the renal cortex. Aminoglycoside taken up by renal proximal tubular cells remains there for an extended period, leading to renal damage, such as structural changes and functional impairment of the plasma membrane, mitochondria and lysosome [5].

Aminoglycosides (Gentamicin) is absorbed into the renal proximal tubular cells across the brush border membrane. So far, results have indicated that aminoglycosides are taken up by receptor-mediated endocytosis following the binding of aminoglycosides to the brush-border membrane. There is also evidence that aminoglycosides directly disturb glomerular physiology, reducing SNGFR by lowering both the Kf and the afferent glomerular arteriolar output [6]. It has been found that vascular, glomerular, and tubular targets are involved in drug-induced nephrotoxicity [7], as a result of mechanisms that disrupt normal cellular structures and functions (mitochondria, membrane integrity, etc.), induce renal injury through intratubular obstruction (crystal deposition), promote cellular swelling, and tubular luminal occlusion (through osmotic effects).

Very few studies of histopathology were reported in literature regarding histopathology of gentamicin in rabbits. This work aimed to study the toxicological pathology of gentamicin by using rabbits as experimental model to help in avoiding any side effects in human and to open the way for further research in toxicological pathology of gentamicin and any related aminoglycoside antibiotics for the benefits of human and other veterinary uses.

MATERIALS AND METHODS

Experimental design:

The study was carried out on both sexes of rabbits (1000-1250 g body weight and 6-12 month old). They were collected from local markets and kept for an adaptation period (2 weeks). Rabbits were housed in wire mesh cages under ambient light conditions in the animal house of the Veterinary Medicine College\ Basrah University. During the experimental period, rabbits were provided with water and green fodder. They were divided into 2 groups each of 8 rabbits (4 male and 4 female). Animals of Group I (untreated control) were maintained without any treatment. Group 2 animals (treated group), the gentamicin (Gentafar®, 10% Farvet, Holland) was injected in thigh muscle /I.M. at 25 mg/kg body weight twice a day (morning and evening) for two months. During experimental period, clinical signs were monitored. At the end of the experiment, animals were sacrificed. Immediately after sacrificed, the kidneys, liver, bile duct, and heart were isolated and fixed in 10% formalin at room temperature for 24 h. The specimens were then removed from the buffered-formalin and dehydrated through a graded series of ethanol and xylene prior to paraffin embedding. After that, the specimens were embedded in paraffin, sectioned at the thickness of 5 microns using rotary microtome, mounted on slide, and stained with haematoxyline-eosin as described by [8]. The slides were then examined under light microscope (Olympus) to detect and describe any histopathologic changes induced by the treatment with the gentamicin.

RESULTS

Clinical examination:

Anorexia, subsequent emaciation, and death in some experimental animals were noticed during experimental period.

Histological and Histopathological examination:

Histological and Histopathological examination revealed that there was lesions in kidney and other organs like liver, bile duct, and heart .

Kidney sections of control rabbits showed normal histological structures of the glomeruli and renal tubules in the cortical and medullary portions (Figure1) compare with treated group. Animals treated with gentamicin at 25 mg/kg body weight for 60 days showed vacuolation of renal cells and dilatation cortical tubules (Figure 2,3,4,and 5). There was dramatic renal injury with necrosis of proximal convoluted tubules (Figure 6, 7, and 8). Moreover, there was degeneration and necrosis in the epithelial cells of renal tubules at the sub capsular zone of kidney (Figure 9). The current study also revealed vacuolation and necrosis of proximal convoluted tubules (Figure 10, 11, and 12). Dilatation medullary tubules with vascular lumen impacted by haemolysed blood was also seen (Figure 13, 14).

Liver sections of control group showed normal histological structures of hepatocytes, bile duct, and central vein (Figure 15) compare with treated group. Animals treated with gentamicin at (25) mg/kg body weight for 60 days showed congestion sinusoids with central vein and minimal vacuolation of hepatocytes (Figure 16) as well as proliferation of bile duct (Figure 17,18).

Heart sections of control group showed normal histological structures of myocardial muscle cells (Figure 19) compare with treated group. Animals treated with gentamicin at (25) mg/kg body weight for 60 days showed vacuolation and congestion of myocardial muscle cells (Figure 20).

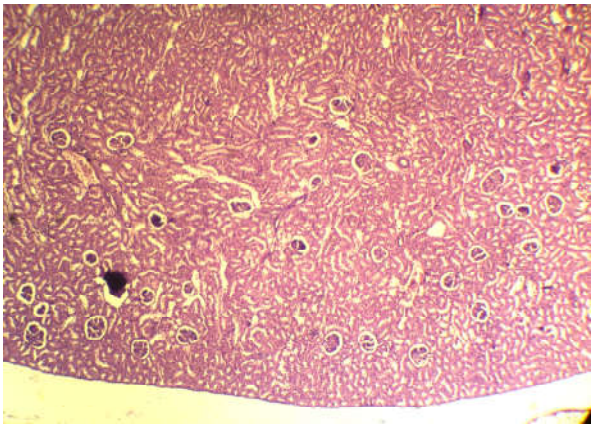

Fig 1: Section of Rabbit Kidney. Control group, with normal structure (H & E, 4X).

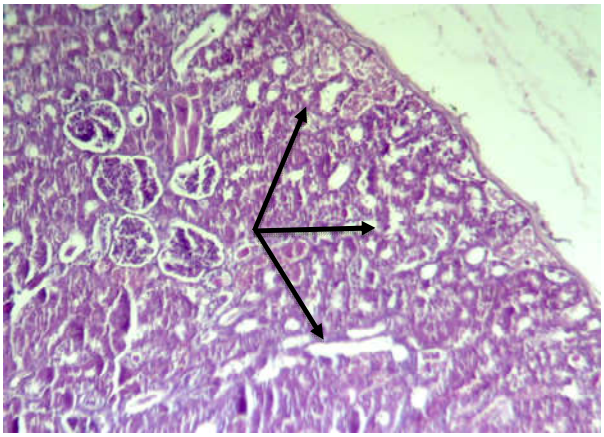

Fig 2: Section of Rabbit Kidney. Dilatation and vaculation of cortical tubules (H & E, 10X)

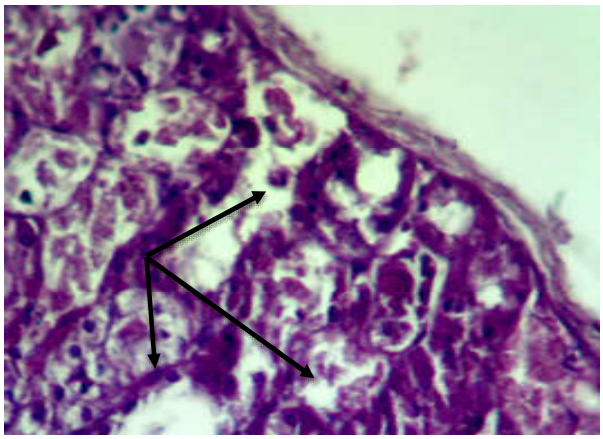

Fig 3: Section of Rabbit Kidney. Dilatation and vaculation of cortical tubules (H & E, 40X)

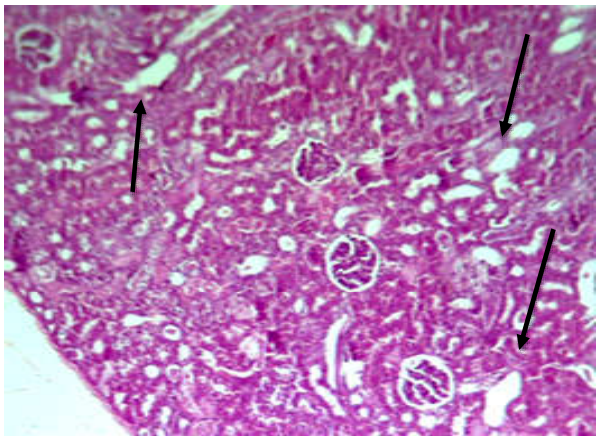

Fig 4: Section of Rabbit Kidney. Dilatation and vaculation of cortical tubules (black arrow)(H & E, 10X)

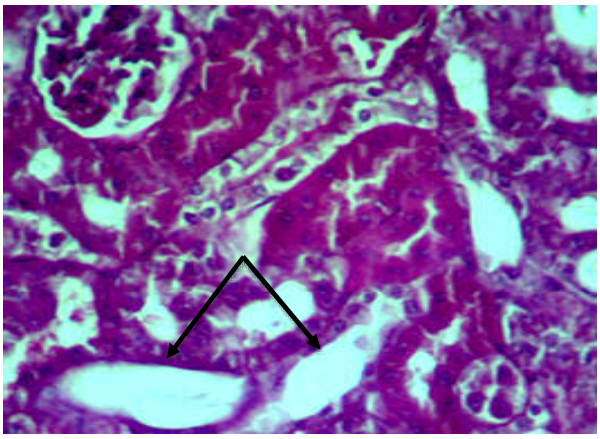

Fig 5: Section of Rabbit Kidney. Dilatation and vaculation of cortical tubules (black arrow)(H & E, 40X)

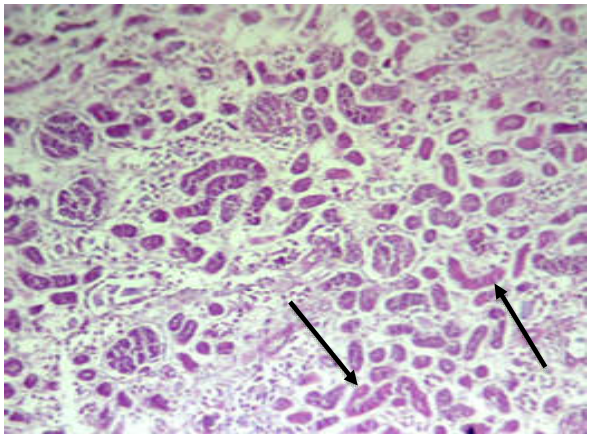

Fig 6: Section of Rabbit Kidney. Dilatation and vaculation of cortical tubules (black arrow)(H & E, 4X)

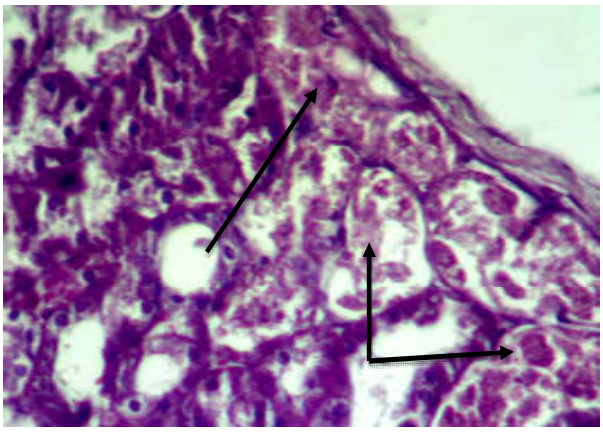

Fig 7: Section of Rabbit Kidney. Dilatation and vaculation of cortical tubules (red arrow)(H & E, 10X)

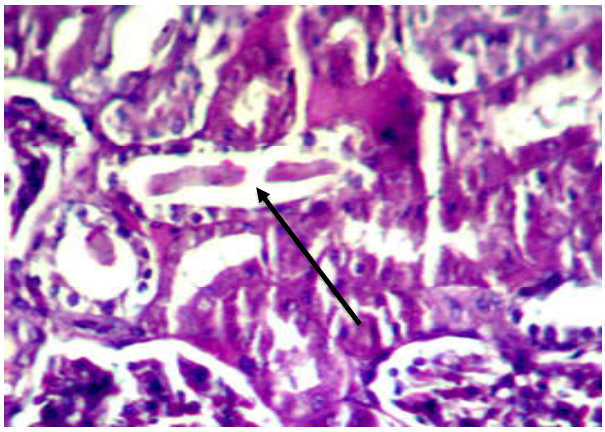

Fig 8: Section of Rabbit Kidney; necrosis of proximal convoluted tubules (red arrow)(H & E, 40X)

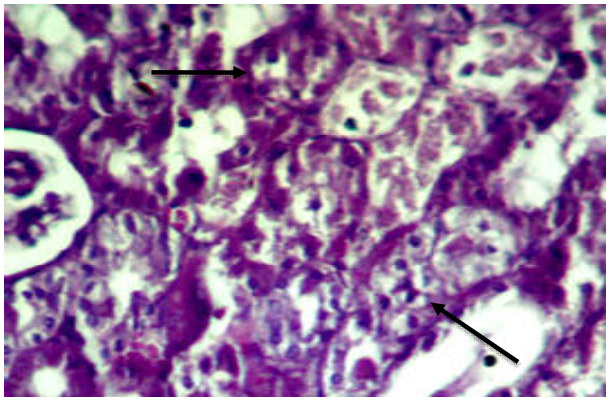

Fig 9: Section of Rabbit Kidney. Degeneration and necrosis in the epithelial cells of renal tubules at the sub capsular zone(red arrow) (H & E, 40X)

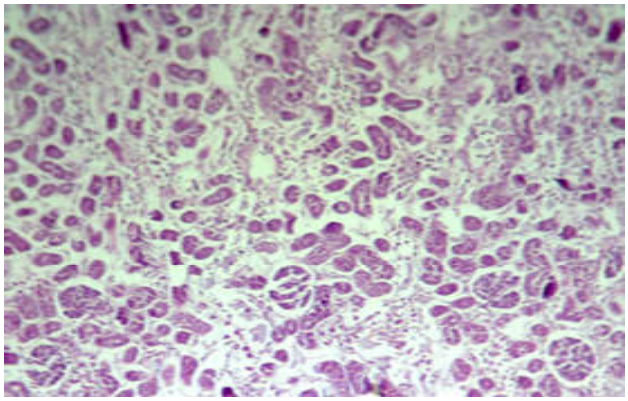

Fig 10: Section of Rabbit Kidney. Vacuolation and necrosis of proximal convoluted tubules(H & E, 4X)

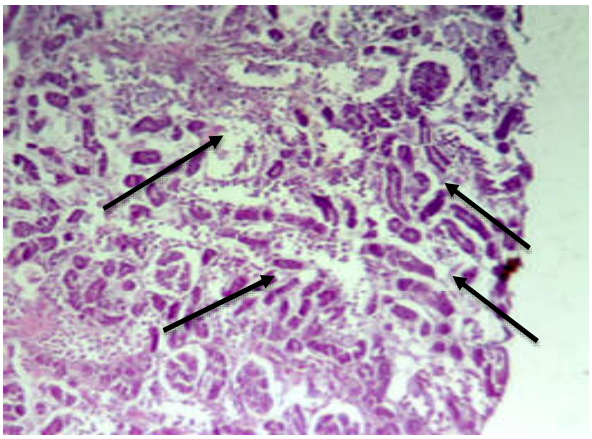

Fig 11: Section of Rabbit Kidney. Vaculation and necrosis of proximal convoluted tubules (H & E, 4X)

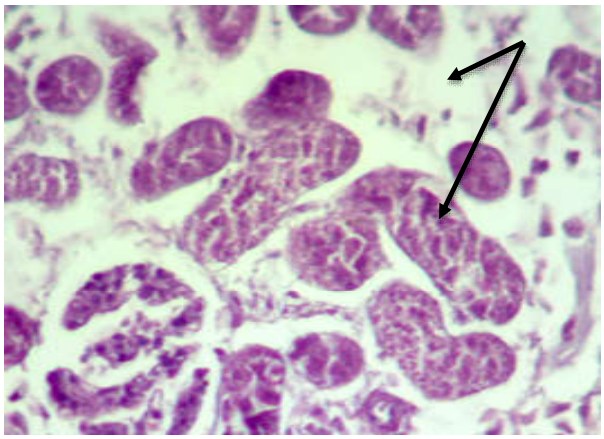

Fig 12: Section of Rabbit Kidney. Vaculation and necrosis of proximal convoluted tubules(H & E, 40X)

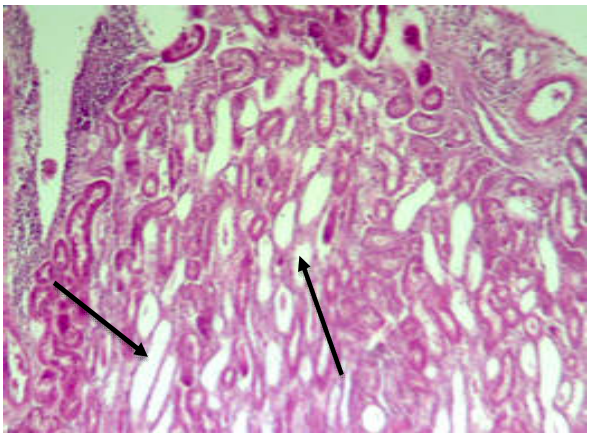

Fig 13: Section of Rabbit Kidney. Dilatation medullary tubules with vascular lumen impacted by haemolysed blood (red arrow) (H & E, 10X)

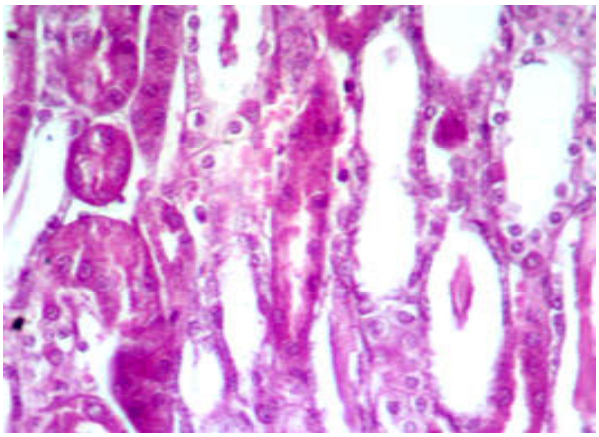

Fig 14: Section of Rabbit Kidney. Dilatation medullary tubules (H & E, 40X)

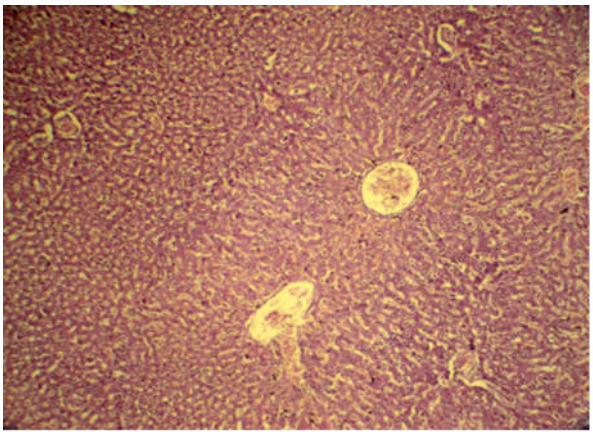

Fig 15: Section of Rabbit Liver . Control with normal structure. (H & E, 4X)

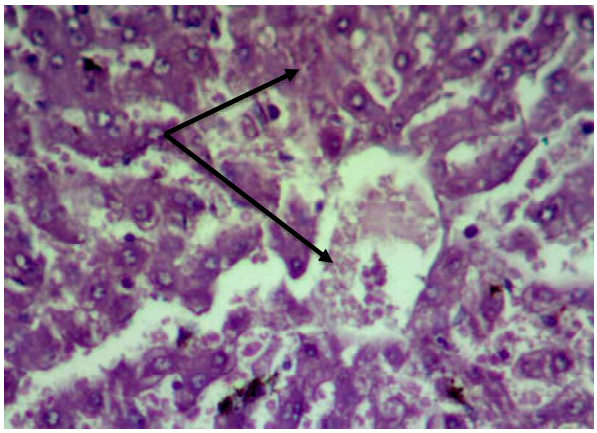

Fig 16: Section of Rabbit liver. Congested sinusoids with central vein and minimal vaculation of hepatocytes (H & E, 40X)

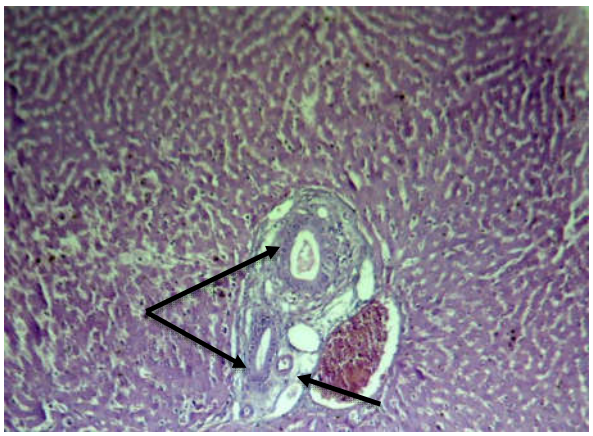

Fig 17: Section of Rabbit liver. Proliferation of bile duct (red arrow(H & E, 10X)

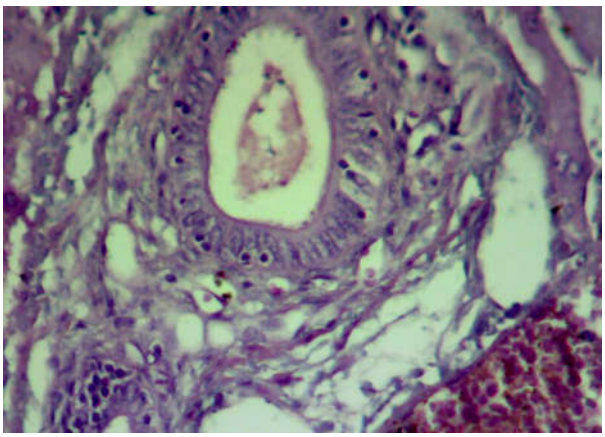

Fig 18: Section of Rabbit liver. Proliferation of bile duct (red arrow((H & E, 40X)

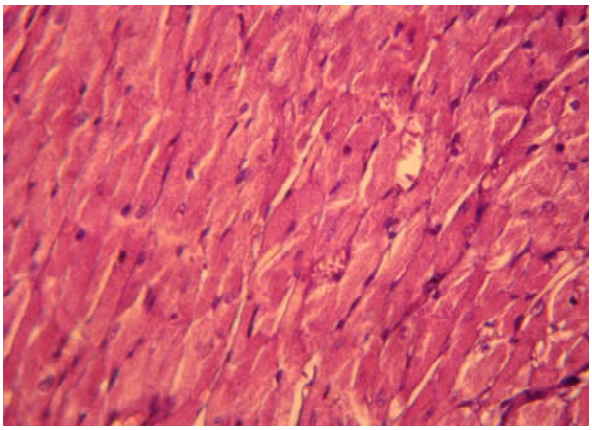

Fig 19: Section of Rabbit heart. control group, with normal structure (H & E, 20X)

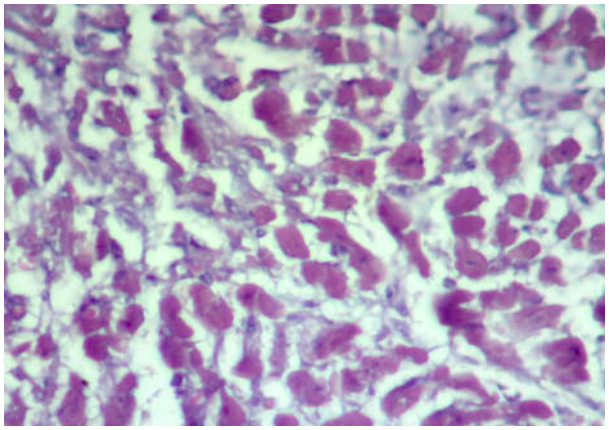

Fig 20: Section of Rabbit Heart. Clearly vaculation and congestion of myocardial muscle cells (H & E, 40X)

DISCUSSION

The present study revealed that animals treated with gentamicin at (25) mg/kg body weight for 60 days induced histopathological changes in various organs involved prominent clinical signs.

In the current study, microscopic examination revealed prominent alterations in the kidney such as dilatation, degeneration and necrosis in renal tubules (cortical tubules, medullary tubules and proximal convoluted tubules). These alterations could be due to the cytotoxic effects of gentamicin. Tubular cells necrosis was the prominent features in rabbits given gentamicin. This finding is in agreement with previous studies, in which there was tubular necrosis as a result of gentamicin toxicity [9,10,11,12]. It has been found that gentamicin treated rats, monkeys, and avian revealed tubular epithelial damage with intense granular degeneration involving > 50% of renal cortex. Animals given gentamicin showed tubular necrosis, degeneration, dilation together with regeneration of proximal tubular cells, cell infiltration in interstitium, and hyaline cast formation in the tubular lumen [9,10, 11,12]. More than half of proximal tubules showed desquamation of necrosis, but involved tubules easily found, complete or almost complete tubular necrosis. Tubular necrosis, basal membrane disruption, mesangial cell contraction, proliferation and apoptosis indicated by a decrease in glomerular filtration and alteration in intraglomerular dynamics.

The current study also revealed that gentamicin produced severe nephrotoxicity. This finding is in agreement with previous studies [2,4,10], in which gentamicin produced nephrotoxicity. Previous studies showed that aminoglycoside antibiotics are nephrotoxic and the underlying mechanism to explain the pathogenesis is increased oxidative stress [2,4,10]. Some free radical scavengers provide marked functional and histopathological protection against toxicity. This suggests the possibility that free radical metabolism in the renal tissue is greatly affected by gentamicin. It has been found that the enzymatic free radical defense mechanism has been greatly disturbed in renal tissue exposed to gentamicin. Antioxidant protection from nephrotoxicity possibly is scavenging toxic free radicals. It has been found that once-daily administration of gentamicin or tobramycin was significantly less toxic than more frequent (i.e., twice or three times daily) dosages or continuous infusion [17, 18].

Drug-induced nephrotoxicity is an important cause of renal failure. Hydroxyl radicals play a role in the pathogenesis of gentamicin nephrotoxicity. Gentamicin can induce suppression of Na(+)-K(+)-ATPase activity and DNA synthesis in rats proximal tubules leading to renal injury. This injury may be relevant to reactive oxygen metabolites generated by gentamicin. Renal cortical mitochondria are the source of reactive oxygen metabolites, which induces renal injury [10].

Gentamicin is aminoglycoside broad spectrum antibiotic used against pathogenic gram negative and positive bacteria [19]. The aminoglycoside when improperly used, could serve as a source of harm to animal organs such as kidney, liver, bile duct, and heart [20]. This finding may be attributed to the fact that all the amino-glycoside used especially gentamicin produced significant increase level of nitrogenous wastes, (urea, uric acid and Creatinine) in the blood [21]. It has been found that administration gentamicin into rats induced impairment of renal function through liberation of oxygen free radical. Rats treated with gentamicin at 60mg/kg body weight and 80mg/kg body weight revealed disorders in some biochemical parameters (serum urea, creatinine, and uric acid) which lead to harmful animal organs.

In the current study, many pathological alterations of the rabbit's liver showed vacuolation, minimal degeneration of hepatocytes ,and congestion sinusoids with

central vein under the effect of gentamicin. This is justifiable since the hepatocytes are particularly sensitive to toxic influences, for this drug. The hepatocytes also come in contact with toxic chemicals during their metabolism by the liver. This finding is in agreement with previous studies, in which experimental and clinical gentamicin toxicity in commercial White Leghorn birds has been reported to produce high mortality and toxico-pathological changes in kidneys and liver such as vacuolar degeneration of hepatocytes [22]. It has been found that the patho-morphological changes in liver at 30 mg /kg gentamicin comprised of fatty change which increased in severity and frequency with increase in dose levels, and severe congestion and fatty change of hepatocytes after administration of gentamicin in pectoral muscles of broiler birds at 40, 50 and 70 mg/kg body weight, respectively [1].

As serum proteins synthesis occurs in liver, a severely damaged liver may also lead to decrease protien synthesis. Liver of the birds administered 30 mg and above levels of gentamicin showed an injurious effect and accompanied with anorexia in the birds [23]. This association of the lesions suggested that kidney and liver injuries followed by gentamicin administration could be the major reasons for anorexia and subsequent emaciation of the rabbits.

The present study also revealed that treatment rabbits with gentamicin induced vacuolation and congestion of myocardial muscle cells. This finding is in agreement with previous study, in which emaciation observed in different groups which treated with gentamicin was accompanied by swollen kidney, liver and hemorrhages on the different organs including heart [1]. Atrophy and edema fluid is present in interstitial tissue during metabolic reaction or cellular growth disturbances, degeneration with or without myocytolysis lead to atrophy, myocardial necrosis associated with sever degeneration of myocardial cells and with systemic circulation disturbances of all body due to adminstration of this drug[24].

Taken together, the present study revealed toxic effects of gentamicin on the kidney, liver, bile duct, and heart during the use of this drug. Therefore, more researches must be done on other organs of the body to highlight its effects on these organs.

دِرَاسَة سُمُية ـ إمرَاضِيه الحَقنِ العَضَلي للجِنتَاميسين سَلفات في الأرانبِ المُختَبَرية.

صالح كاظم مجيد ، زينب وحيد خضير ، محمد عبد العباس حسن ، .ألاء طارق عبد

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فرع الصحه العامه البيطريه ، كلية الطب البيطري ، جامعة البصرة ، البصره ، العراق

الخلاصه

اظهر هذا البَحْث تأثير حقن تركيز (٢٥ مجم/كجم/وزن الجسم) من الجنتاميسين سلفات في عضلة الفخذ لمُدةِ شهرينِ وبواقع مرتينِ يومياً (صباحاً ومساءاً) على التركيب النسيجي للكلى ،الكبد والقلب في ذكور وإناث الأرانب البالغ عددها ١٦ بواقع مجموعتين. المجموعه الاولى مثلت عينه السيطره والمجوعه الثانيه المجوعه المعامله بعقار الجنتاميسين. تم مشاهدة علامات سريريه مثل فقدان الشهية والهُزال مع نفوق بعض الحيوانات من المجموعة المعاملة بالمادة العلاجية والذي ربما يعود سببه إلى الفشل الكلوي الناتج عن الجنتاميسين. وقدْ أظهَرَتْ الفحوصات النسيجية لمجموعة الأرانب التي عُومِلَتْ بهذا التركيز من المادة توسع وتجوف الأنيبيبات اللّحَائية الكُلوية وتَنَكُسْ وتَنَخُرْ الأنيبيبات المُلْتَفَة الدانية. كما شُهِدَتْ الجيوب والخلآيا الكبدية محتقنة ومتجوفة والآقنية الصفراء منتشرة بشكل واضح خلال الدراسة. كذلك تَمَ ملأحظَة تجوف مع احتقان الخلآيا المكونة لعضلة القَلْب.

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