Pathological study of kidney in male rats treated with doxorubicin in Diyala province

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Abstract. Doxorubicin is the anti-neoplastic drug, used in treatment of many type of tumor like breast and ovary carcinoma. In this study, the goal was to identify the histopathological changes of doxorubicin on kidney of male rats weighing 200-225g, and the doxorubicin injected intraperitoneally. The rats classified into two main group, The first primary group (n=30) subdivided into sex groups (each group= 5 rats). The first subgroup giving 0.3 ml of physiological normal saline every 84 hours for three weeks. Other subgroups were injected in peritoneum at a dose (1,2,3,4,5) mg / kg of doxorubicin twice weekly (each dose = 5 rats), every 84 hours for three weeks. The second primary group (n=20) also subdivided into four group (each group= 5rats), The first subgroup giving 0.3 ml of physiological normal saline every 84 hours for six weeks. Other subgroups injected intraperitoneally in a dose (1,2,3) mg/kg of doxorubicin twice weekly for six weeks. Anatomy was performed on male rats after 48 hours of the last injection. The histopathological lesions were degeneration, thrombus, tubular casts, congestion, cells vacuole of glomerular tuft and hemorrhage of blood vessels. The score of injury showed significantly increased of the glomeruli injury degree of the male rats, which injected with doxorubicin in peritoneum at a dose of 5 mg/kg of body weight for three weeks and there is a significant increase in the degree of glomerulus injury at a dose 3 mg/kg of body weight when injected of doxorubicin for six weeks. In this study, we mentioned the histopathological effect of doxorubicin on the male rat kidney.

Keywords: Doxorubicin, Histopathology, male rats, dose, kidney, vacuole.

1. Introduction

The injury of the renal system particularly kidney can lead to high morbidity and mortality rate in the animals because of the kidney consider important organ due to their functions include detoxification and excretion of drug and toxic material, regulation function, elimination of urine and creatinine (1). Kidney was exposed to the toxic effect of drug lead to renal damage and other lesion as nephrosis of nephron, interstitial nephriris and glomerular nephriris (2). Doxorubicin (also called Adriamycin) anticancer therapy used for treatment of tumor in different organs and tissue including, osteosarcoma, Hodgkins lymphoma, breast cancer, thyroid tumor and neoroblastoma (3). This drug influenced on some biochemical activities of the kidney, in the previous studies showed a decrease of sodium and increase of potassium, urea in serum when injected of rats with different doses of doxorubicin.
Also, increase in inorganic phosphors (5) and represented of protein urea, hypoalbumiaemia and hyperlipidaemia (6,7). Another study in which injected of rats with doxorubicin in single dose 5mg/kg of body weight that showed increase secretion of lysosomal enzyme which consider signs of renal tubule injury (7,8). An increase of fibronectin, glucosminoglycan and cholesterol in plasma was observed (6).

Another study conducted on Atlantic hagfish maxima glutinosa for clarification of initial pathomechanisms which responsible of alteration in metabolism of protein in glomeruli injected with Adiamycin (trade name of doxorubicin) showed increased protein build-up in glomerular cells and decreased of proteolytic enzyme. There was also a lack of RNA building due to the lack of protein degradation, which leads to the increase of the total protein of the glomerulus, which explains the pathological mechanism responsible for the increase protein glomerular and the occurrence of glomerular hardening of injected animals with ADR (9).

A number of researchers pointed to the role of the complement in the renal degeneration caused by the drug, as the activation of the complement plays a role in reducing the damage of the glomerular glomerulosclerosis in the body and the lack of CD59, which regulates the attack complex in the membrane leads to tubulointerstitial injury (10). The reasons for the hardness of glomeruli was the increase of gene expression of advanced glycation end product in the product of glomeruli, RAGE can contribute to product injury when glomerular stiffness, such as partial glomerular sclerosis at the ADR injection (9). Because of the biochemical and genetic effects of doxorubicin on the kidneys we therefore decided to study the histopathological lesions of doxorubicin on kidneys in male rats.

2. **Material and methods**

2.1. **Chemical drug.**

In the current study, doxorubicin hydroxy daunorubicin hydrochloride used to treat certain cancers. The drug used was in the form of injections containing 50 mg in 25 ml of 0.9% hydrochloric acid produced by Ebewe pharmacy. b. h. Unterach Austria The property was given twice weekly (10). The doses were used in different concentrations, with doses of 1 mg / kg (11), 2 mg / kg (12) and the dose was also used 3 mg / kg (15).

2.2. **Experimental animals**

This study performed on Iraq republic, Diyala university, college of veterinary medicine. All experimental, surgical and pathological procedures performed in diyala university, college of veterinary medicine. All male rats in a temperature 20-22 C°, fed standard and water and cycle of light (12 hours light and dark). fifty male rats, weighing 200-225g and aged 2.5-3.5 months, were divided into 2 main groups. The first main group used 30 male rats and was injected with 6 doses twice weekly for three weeks and was divided into six subgroups of each group containing 5 rats; The first main group was injected with 0.3 ml of physiological saline solution, the second group was injected with doxorubicin at 1 mg / kg concentration, the third group was injected with doxorubicin with a concentration of 2 mg / kg, the fourth group injected the same drug with a concentration of 3 mg / kg, The drug was concentrated at 4 mg / kg and injected with 5 mg / kg of doxorubicin The second main group: 20 male rats were injected with 12 doses within 6 weeks at a rate of twice a week. They were divided into four subgroups of each group containing 5 rats; The first group was injected with 0.3ml of physiological saline solution, the second group was injected with doxorubicin at 1 mg / kg twice weekly for 6 weeks, the third group was injected with doxorubicin at 2 mg / kg twice weekly for 6 weeks, Doxorubicin was injected with 3 mg / kg twice weekly for 6 weeks. After 48 hours of the last injection (15), the animal was anesthetized with diethyl ether and then the thoracic cavity was opened. The kidney was extracted and washed with a normal physiological saline to remove the blood. The samples were dried with the filter paper and then placed in the neutral formal solution 10%.

2.3. **Histopathological study**

The kidneys were preserved natural formalin solution (10%) for 24 h., washed with tap water
for 15 minutes and then passed by a series of 70%, 95% and 100% ethyl alcohols, embedded in paraffin waxed at 59 °C for 3 hours and in two phases. The microtome (Leica RM2235) was sectioned the sample with a thickness of 4-5 micrometers, and then the slides were stain with haematoxylin and eosin stain. Novel microscope was used to examination of slides (17).

2.4. Injury score

The number of glomerular damage was calculated on the basis of a 20-60 glomerular examination. In each cross-section, 40 glomeruli were taken from the beginning of the cortex to the depth. Twenty samples were taken in injected animals with 5 mg / kg for 3 weeks In the contrast with control group and animals injected with 3 mg / kg for 6 weeks. The severity of the lesion was calculated from 0 to 4 + depending on the percentage of glomerular damage, the 1+ (lesion) includes 25% damage of the glomeruli, while 4+ indicates 100% glomerular damage. Injury score is obtained by multiplying the severity of damage (0 to 4+) by the percentage of glomeruli affected by the same degree of harm. Therefore, the extension of the damage to each tissue sample is obtained by adding this number.

For example, if 5 out of 40 glomeruli have 1 + lesion and 10 from 40 have 3 + lesion, The injury score will be (1*5/40) + (3*10/40) * 100 = 87.5% Is the percentage of glomerular damage for each sample according to the method (19).

3. Results

The results of the histopathological examination of the kidney from control group rats showed the appearance of the normal structure of the kidney cortex (Figure 1). The tissue sections of the kidneys treated with the drug concentration of 1 mg / kg, was observed the emergence of congestion in the blood vessels, contraction of the glomerular tuft, distention of Buman capsule, mesangial cells necrosis, picknosis of glomerular tuft, degeneration, necrosis of renal tubular epithelia, infiltration of interstitial inflammatory cells, swelling of tubular epithelia and cyst (figure 2). At 2 mg / kg concentration for 3 weeks, an expansion was observed in Bowman's capsule and renal tubules, congestion, hemorrhage, mesangial cell necrosis, lobulated of glomeruli tuft, fibrosis with eosinophils and focal infiltration of inflammatory cells (figure 3). When treated with 3 mg / kg dose for 3 weeks, congestion was observed with thickening of the vessel wall, necrosis of the glomerulus, degeneration of epithelial cells of the renal tubules, lobulated and cyst (figure 4). Infiltration of inflammatory cells around the renal glomeruli, hemorrhage, basement membrane breakdown of renal glomeruli, narrowing of the renal tubules and expansion of others, presence of vacuole and hemorrhage (figure 5). When treated with a dose of 4 mg / kg for 3 weeks, congestion was observed in the blood vessels, interstitial tissue inflammation, epithelial cell swelling of the renal tubules and expansion of other tubules, hemorrhage and presence of tubular hyaline casts (figure 6). Cysts, vacuole of glomeruli and distention of Buman capsule (figure 7). While injected of the rats with 5 mg/kg of doxorubicin for three weeks, showed fatty changes in the epithelia of renal tubules (figure 8). Congestion, infiltration of inflammatory cells, lobulated (figure 9). The injection of male rats with 1mg/kg for 6 weeks, the effects were less severe compared to the previous concentration, was observed congestion of blood vessels and narrowing some of the renal tubules (figure 10), and treated of animal with a dose 2 mg/kg of the same drug for 6 weeks, noted severe necrosis and injected with a dose 3mg/kg for 6 weeks showed segmental glomerulosclerosis (figure 11). Hemorrhage between renal tubules and (figure 12), glomerular hyaline casts (figure 13) and vacuole of the glomerular tuft (figure 14).

The injury score:

The results of the present study showed a significant difference in the probability of p <0.05 in the percentage of glomerular lesions in animals treated with doxorubicin at a concentration of 5 mg / kg for three weeks compared to control group (168.12 ± 5.735) while in the control group was (168.12 ± 11.076)%. There was also a significant difference in the percentage of glomerular lesion in treating
animals at a concentration of 3 mg / kg for six weeks, reaching (133 ± 8.623) compared to the control group which is (93 ± 14.75)% (Table 1).

Table 1: represent the percentage of injury score ± of renal glomeruli in a dose of 5mg/kg of doxorubicin for 3 weeks and 3mg/kg for six weeks compared with the control group.

| Drug concentration  | Dose of 5 mg/kg       | Dose of 3 mg/kg       |
|---------------------|-----------------------|-----------------------|
| Control group       | 77.5% ±11.076 (A)     | 93% ± 14.75 (A)       |
| Experimental group  | 168.12% ± 5.735 (B)   | 133% ± 8.623 (B)      |

The numbers followed by different letters showed a significant difference at the probability level (p <0.05), according to Duncan test.

Figure 1: Histopathological section of the kidney showed normal structure of epithelial cells lining renal tubule with normal structure of glomeruli (H&E stain. 100X).

Figure 2: Histopathological section showed the infiltration of inflammatory cells particularly phagocytes and lymphocytes in the interstitial tissue (INF), cystic (cyst), degeneration (DE), necrosis (N) and renal tubule hemorrhage (H), (H&E stain. 400X).
Figure 3: Histopathological section of the kidney showed sever necrosis of renal tubule epithelia (N), mesangial cells necrosis and atrophy of Boman capsule (AT), lobulation (LOB) and inflammatory cells infiltration (INF), (H&E stain. 400X).

Figure 4: Histopathological section of the kidney showed thrombus in blood vessel (THR), congestion (CON), inflammatory cells infiltration (INF) and deletion of blood vessel (DEL), (H&E stain. 400X).

Figure 5: Histopathological section of the kidney showed vacuole (V), Hemorrhage (H), lumen narrowing of renal tubule (L) and dilation (DIL), (H&E stain. 400X).
Figure 6: Histopathological section of kidney showed inflammatory cells infiltration (INF), casts of hyaline (CAST), hemorrhage (H) and vacuole (V), (H&E stain. 400X).

Figure 7: Histopathological section of the kidney showed seclerosis (SCL), cysts between lobules (cys), infiltration between tubules (INF) and vacuole (V), (H&E stain. 100X).

Figure 8: Histopathological section of kidney showed fatty changes of endothelium renal tubule, (H&E stain. 400X).
Figure 9: Histopathological section of kidney showed hemorrhage (H), hypertrophy of endothelium (HYP), lobulated of glomeruli (LOB), increase space of human capsule (INC) and infiltration of inflammatory cells, (H&E stain. 400X).

Figure 10: Histopathological section of kidney showed infiltration of inflammatory cells (INF) and hemorrhage (H), (H&E stain. 400X).

Figure 11: Histopathological section of the kidney showed sclerosis of glomeruli (SCL), (H&E stain. 100X).
Figure 12: Histopathological section of the Kidney showed expansion of renal tubules (EXP), vacuole forming (V), (H&E stain. 400X).

Figure 13: Histopathological section of the kidney showed cast of hyaline (CAST) and vacuole (V), (H&E stain. 400X).

Figure 14: Histopathological section of the kidney showed vacuole in the glomeruli, (H&E stain. 400X).
4. Discussion

The result showed occurred of vacuoles in glomerular tuft and renal tubules. The results were identical to the results of (20), which indicated the formation of vacuoles in renal tubules, as indicated by a study to be vacuole in the glomerular (18). Another study injected rats with a single dose showed forming small vacuole that increased in size in later stages (6). The results showed that there was congestion, hemorrhage, and thrombosis, and the results were identical to those of (21) indicating severe congestion and hemorrhage among renal tubules when injected with 1 mg / kg dose once a week for four weeks. Our results also showed the occurrence of fibrosis in the tubular interstitial tissue. The fibrin is fibrous protein present in deferent inflammation and take a eosin stain (22). Our results were identical to those of other studies, other study observed decrease of endothelial nitric oxide synthatase, which results in the appearance of proteinuria, glomerular sclerosis and interstitial tissue fibrosis when injected of rats with doxorubicin (23), and renal fibrosis (24). Our results showed degenerative changes in the glomerulus, glomerular tuft shrinkage, distention of Buman capsule, narrowing of renal tubules and It is identical to what he found by (18). While another study reported that when injected into the rats with doxorubicin in a dose of 3 mg / kg, glomerular tuft is present in a small site of Buman capsule, which gives the glomerulus the appearance of the sac containing the tuft residue. It is also noted that the proximal renal tube is suffering from atrophy which lead to expansion of its lumen (28). A previous study indicated a slight glomerular sclerosis and hyaline casts and infiltration of inflammatory cells in interstitial tissue, (24) mentioned that glomerular sclerosis was rare, Others reported that glomerulosclerosis was observed nine months after injection with a single dose and also indicated severe changes in the tubular interstitial tissue (20), Others have shown that the epithelial cell damage to the renal tubules and proteinuria, which initially consists of tubular casts, leads to damage to the interstitial tissue, where the tubule will obstruct and break down the basal membrane, which leads to tubular inflammatory reaction (26). The use of single dose 10.5 mg / kg of the drug led to changes in tissue, including interstitial damage and stiffness of the glomerulus and formed of hyaline casts (25). The tubular cast block the cavity of collecting tubules and these casts causes atrophy and desquamation of epithelial cells and surrounded by phagocytes and multinucleate giant cells (27). Our results have shown that the collection of lipid in tubular epithelial cells of the renal tubules, that the appearance of natural fat and lipid in epithelial cells that can result from the absorption by tubular cells can lead to lipid nephrosis associated with diabetes or hypoxia due to toxic injury.

5. References

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