Original Article

Effect of doxorubicin on the histological structure of the kidneys in male albino rats

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Summary:

Background: doxorubicin is one of the most active cytotoxic agents in current use, it has a very potent effect in the treatment of various malignancy either used alone or combined with other cytocidal agents, the antitumor activity of this drug associated with cytotoxic effect such as cardiotoxicity, hepatotoxicity, nephrotoxicity, myelosuppression.

Objectives: - this study was designed to investigate the effect of the doxorubicin on the histological structure of the rats' kidney.

Patients and Methods:- one of the most important cytotoxic effect of doxorubicin is nephrotoxicity. Therefor sixteen adult male Wister albino rats have been used in this study, Eight of them were given 1 ml of intraperitoneal injection of normal saline alone daily for six consecutive days and served as a control group (A), The other Eight rats were given intraperitoneal dose of (1mg/kg body weight) doxorubicin, daily for six consecutive days and served as treated group (B), tow animals from group B kept alive without treatment for another week (observation period) as follow up animals to see the reversibility of the renal injury that are caused by doxorubicin. the animals were sacrificed at the end of the experiment, The body weight of the animals were recorded before starting the injection and recorded again just before killing the animals to see the changes in the body weight and the kidney sections were prepared and stained with hematoxylin and eosin stain, Periodic acid Schiff □s reagent (PAS), Orcin VanGieson stain, and examined by light microscope.

Results: Doxorubicin cause reduction in the body weight and changes in the histological structure of the kidney which are atrophy of some glomeruli with widening of the urinary space between the glomerular tuft and the bowman's capsule , other glomeruli show expansion of glomerular tuft with obliteration of the urinary space, degenerative changes in the tubular epithelium, increase rate of apoptosis, necrosis, cystic changes of the tubules and vacuolation of the tubular epithelial cells cytoplasm, protein cast formation in the tubular lumen, infiltration of inflammatory cells in the interstitium, proliferation of collagen fibers(fibrosis) in the interstitium and glomerulosclerosis, the follow up animals show persistence and more extensive lesion than group B.

Conclusion: doxorubicin causes marked changes in the normal histological architecture of the rats' kidney, and these changes are irreversible after cessation of doxorubicin injection (observation period).

Key words: doxorubicin, rat kidney, histological changes.

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Introduction:

Doxorubicin (the trade name is Adriamycin) is anthracycline antibiotic , It is used as an anticancer agent in the treatment of many solid malignancies and lymphomas [1]. while they generate acceptable outcome in chemotherapy of some cancers, they also exhibit severe toxicity and undesirable side effects [2]. as cardiac, renal and hematological toxicities [3], therefore its use has been limited Since it cause disturbance in oxidant-antioxidant systems which has been demonstrated with lipidperoxidation (LPO) and protein oxidation results with tissue injury [4]. Although the exact mechanism of DXR-induced nephrotoxicity remains unknown, it is believed that the toxicity may be mediated through free radical formation, iron-dependent oxidative damage of biological macromolecules, membrane LPO, and protein oxidation [5].Intercalation into DNA leads to disturbances of DNA synthesis, DNA- dependent RNA synthesis and protein synthesis Doxorubicin interference with topoisomerase II enzyme and induction of DNA double strand breaks and DNA fragmentation [6]. This followed by growth arrest in G1 and G2 lead to programmed cell death and apoptosis [7-8]. Adriamycin cause direct toxic damage to the glomeruli followed by tubulointerstitial injury because it cause damage to the glomerular filtration barrier which are composed of glomerular endothelial cells which contain glycocalyx, glomerular basement membrane and the podocytes, doxorubicin lead to decrease in the thickness of glycocalyx, increase in the size of the holes in the glomerular endothelial cells, glomerular charge selectivity is reduced and the foot process of the podocytes fused with each other's all these changes end with passage of macromolecules as protein lead

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to proteinuria and decrease the GFR [9].

Patients and Methods:

In this study, sixteen adult male Wister albino rats of (200-220 g) body weight, aged three months were taken from the animal house of Experimental Research Unit, Collage of Medicine, University of Mosul, Mosul, Iraq. The animals were allowed to acclimatize to laboratory conditions one week before the experiments begin and caged in a quite temperature controlled room; they were allowed to drink tap water and fed daily by pellet. The animals were divided into two experimental groups eight rats for each group.Group A (n=8) Was given 1 ml of
intraperitoneal injection of normal saline alone daily for six consecutive days and served as a control group.Group B (n=8) Was given intraperitoneal dose of (1mg/kg body weight) doxorubicin, daily for six consecutive days. The animals were sacrificed at the end of the experiment under light ether anesthesia. The body weight of the animals were recorded before starting the injection and recorded again just before killing the animals to see the changes in the body weight. two rats from group B were kept alive without treatment for one week (observation period) to see if the changes caused by doxorubicin reversible or not, the animals were very tiered, cannot withstand to keep them more time so sacrificed at the end of the observation period and consider these animals as follow up animals.

The dissection of the rat starts by making incision from the neck and continues downward toward the tail, After opening the abdominal cavity. The kidneys were seen to be large bean shaped organs located at the posterior abdominal wall on each sides of the vertebral column. And pieces of 4-5 mm were taken from each kidney and kept for 24 hrs. In 10% Neutral Buffered Formalin ,then Dehydration of the slices was made in ascending ethanol series (70%, 80%, 90% and 100%) one hour for each. After dehydration the slices cleared with two changes of Xylene for 1 hr. each, and then embedded in three changes of paraffin with a melting point of 60 °C 1hr each. Finally the pieces were embedded in paraffin blocks. After that from each block, a serial sections of about 5 microns in thickness were obtained by microtome, then mounted on glass slides and then deparaffinaized in tow changes of Xylene for 5 minutes each, followed by immersion in descending grades of ethanol (100%,90%,80%and 70%) two minutes for each. Finally some sections were stained by hematoxylin and eosin stain, others by PAS stain and Orcin-vangieson stain, then examined by light microscope and micrographs were taken at (X 400 and X600) magnification.The statistical analysis were performed by paired t-test to see the changes in the body weight before and after the injection (table 1) and by using post Hoc test for comparing the changes in the body weight between group A and B (table 2).

Results:

The histological features of kidney sections of group A (control group) showed to be absolutely normal without any change (figure1), there were no significant change in body weight of group A before and after the injection (p=0.197) Table (1).

In group B there were very highly significant reduction in body weight before and after the injection (p=0.000) table (1). And very highly significant reduction in the body weight when compared with the control group A P=0.000 (table 2). The light microscopic examination showed most of the glomeruli were atrophied with widening of the urinary space The proximal and distal convoluted tubules undergo degenerative changes, necrosis and increase rate of apoptosis, infiltration of inflammatory cells (figure 2), glomerulosclerosis and interstitial fibrosis (figure 3), cytoplasmic vacuolation of epithelial cell of the tubules and marked thickening in the basement membrane of the glomeruli and tubules (figure 4),other glomeruli showed mesangial matrix expansion and adherence of the glomerular tuft to the Bowman's capsule with obliteration of the urinary space (figure 5), protein cast formation and cystic changes in the tubules (figure 6). The follow up animals show persistence and more extensive lesion than group B The Histological examination revealed more number of affected glomeruli (figure 5), more extensive Degenerative changes, apoptosis and necrosis of the tubules, protein cast and cystic changes(figure 6) , infiltration of mononuclear cells more sclerotic changes in the glomeruli, fibrosis in the interstitium.

Discussion:

The kidney sections of the control group (group A) show normal histological feature of the kidney similar to those observed by other researchers on rats [1-10].The reduction in the animals body weight in group B may be attributed to the cytotoxic effect of doxorubicin since it cause myelosuppression, anemia, loss of appetite and poor feeding, or it may inhibits protein synthesis [10].The results in the present study were seen at 1-2 weeks after doxorubicin injection and become sever with the time in agreement with [9-11], but in contrast to [12] who mentioned that mesangial expansion, infiltration of inflammatory cells and glomerulosclerosis seen at the 4-6 weeks after doxorubicin injection, these difference in the results probably due to difference in the dose, in the duration of treatment and in the type of animals.

Doxorubicin administration cause vasoconstriction in the blood vessels in order to control and maintained the concentration of the drug in the blood and as protective mechanism to prevent the tubular cell damage this will lead to atrophy of the glomerular tuft and widening of the urinary space [13].Adriamycin (ADR) cause damage to the glomerular filtration barrier therefor it allow the passage

of macromolecules as protein which continue for several months [14-15], the presence of this protein in the tubular lumen stimulate the proximal tubular cells to reabsorbed more protein until it reach a state of saturation this lead to accumulation of protein material, protein cast formation, obstruction of the tubule with increase the intratubular pressure cause dilatation of the tubule and sometime rupture of the tubular basement membrane and leaking of this protein to the interstitium [16-17], which trigger the inflammation[18], in addition to that the proteinuria cause injury to the mesangial cells, which in turn cause mesangial proliferation and more production of mesangial matrix causing glomerular expansion and [19-20].

Inflammatory cells infiltration consists mainly of macrophages and T lymphocytes [12]. The macrophage stimulate the release of transforming growth factor B (TGF-B), interleukin 1 (IL-l) [18], and thromboxane A2 (TXA2) and platelet derived growth factor (PDGF), release from platelets This in turn may contribute to the process of mesangial proliferation and glomerular sclerosis[21-22],The transforming growth factor – B produced by macrophages causes up regulates ECM production and down regulates degradation of the ECM, so lead to overproduction of ECM, mesangial expansion, fibrosis and focal segmental glomerulosclerosis, in addition to that tubulointerstitial cells were induced to differentiate into fibroblast – like cells by activated TGF-B in ADR- treated rats which in turn release more ECM into the intercellular space between renal tubules and lead to interstitial fibrosis [23-11]. The vacuoles in the tubular cytoplasm are formed probably due to the disturbance in the fat metabolism which are caused by doxorubicin and accumulates inside the cell and appear under the light microscope as vacuoles in the cytoplasm [24] .

Apoptosis is a cell death or suicide in a controlled fashion, is a normal physiological event, it was an important pathological change seen in this study in group (B) and follow up animals. Apoptosis, is evidenced by diminished cell size, reduced cytoplasmic volume with condensed eosinophilic cytoplasm and condensed nuclear chromatin, apoptosis end with tubular a trophy and renal failure [25]. Necrosis was another obvious change noticed in group (B) and follow up animals in the current study and the most common reaction leading to the cell necrosis is the formation of covalent bonds between a reactive metabolite of the parent compound and cell protein or DNA [26].furthermore, as a result of doxorubicin toxic damage to the glomerular filtration barrier and losing the selectivity of filtration, the accumulation of amorphous material between the tuft and the capsular basement membrane causing obliteration of the urinary space, this material may reach around the tubule neck and this material may reach the interstitium along the outer aspect of the tubule basement membrane and Bowman's capsule, so the underlying basement membrane appeared to be thickened [27].

The follow up animals that given doxorubicin then kept for observation without treatment showed persistence and more extensive lesion in the renal tissue this indicate that the doxorubicin cause irreversible renal damage even in the absence of doxorubicin exposure these in agreement with [28-29] who found that the Rats that are given doxorubicin develop heart failure as well as a self-perpetuating glomerular nephropathy, which has a relatively early onset even in the absence of continued doxorubicin exposure, the glomerular damage progresses and late-onset tubular lesions are observed. The explanation of these renal damage even in the absence of continuous doxorubicin exposure is when doxorubicin enters the mitochondria it generates reactive oxygen species (ROS) that cause damage to mitochondrial DNA (mt DNA) which have been associated with oxidative stress, which in turn may generate additional ROS, these ROS may attack the respiratory chain itself or again cause damage to mtDNA through continuous cycle even in the absence of doxorubicin exposure [1]. In conclusion doxorubicin cause damage to the renal tissue which developed with the time even after discontinuation of doxorubicin injection.

Table (1):- statistical analysis the changes in the animal's body weight before and after injection in each group.

Groups	N	Before injection mean ± SD	After injection mean ± SD	*P- values
Control	8	204.88 ± 7.00	205.25 ± 6.94	0.197
Doxorubicin	8	205.00 ± 5.98	188.00 ± 6.94	0.000

*Paired t-test; P > 0.05 =NS (not significant); P ≤ 0.05 = S (significant); P = 0.000 VHS (very high significant).

Table (2):- comparison between the animal's body weight of two groups after the injection.

Groups	After injection Mean ± SD	*Significancy
Control (A)	206.13 ± 6.402	A with B ; *P=0.000 (VHS)
Doxorubicin (B)	188.75± 6.944	

* Post Hoc tests; P > 0.05 =NS (not significant); P ≤ 0.05 = S (significant); P = 0.000 VHS (very high significant).

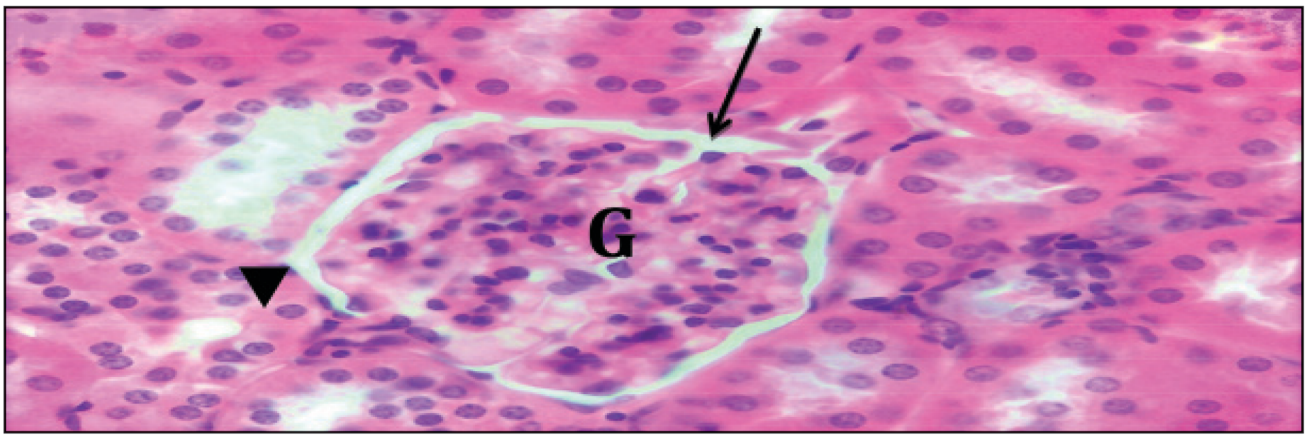

Figure 1: histological section of kidney rat tissue of group A (control group) showed the renal corpuscle composing of glomerulus (G) surrounded by urinary space (arrow) and basement membrane of glomerular (Bowman's) capsule (arrow head). (H&E X600).

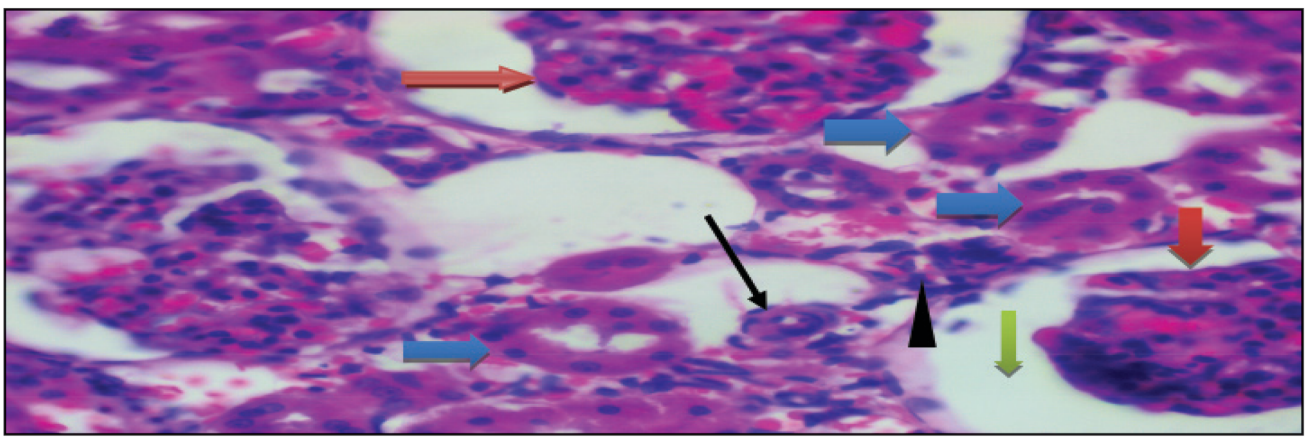

Figure 2: histological section of kidney rat tissue of group B showed atrophy of the glomerular tuft (red arrow), widening of the urinary space of the glomeruli (green arrow), apoptosis of the epithelial cells of the tubules (blue arrows), necrosis of the epithelial cells of the tubules (black arrow), infiltration of inflammatory cells (arrow head) (H&E X600).

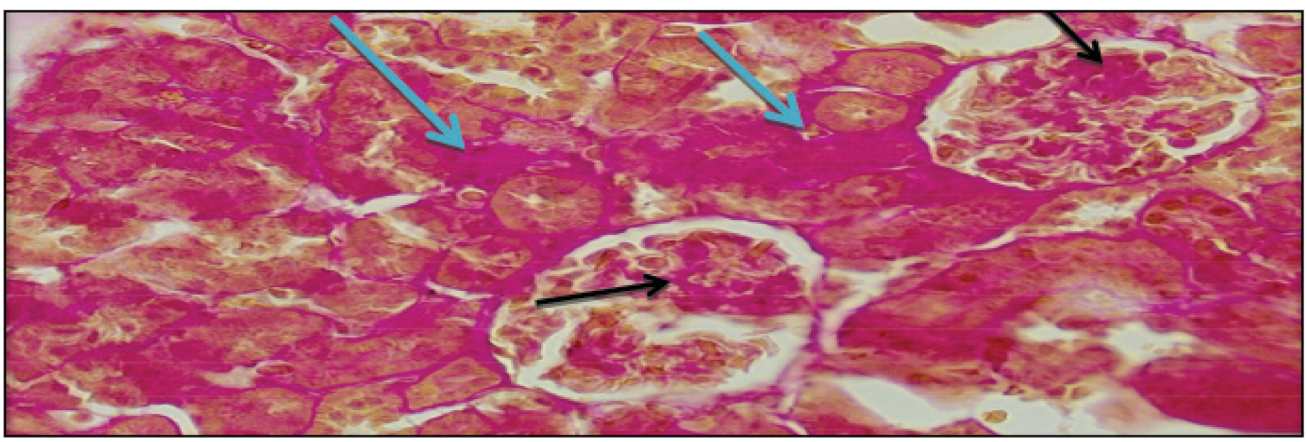

Figure 3: histological section of kidney rat tissue of group B showed the sclerotic changes and fibrosis in the glomerular tuft (segmental glomerulosclerosis) (black arrow), collagen fibers proliferation in the interstitial tissue (blue arrow). (OrcinVanGeison X400).

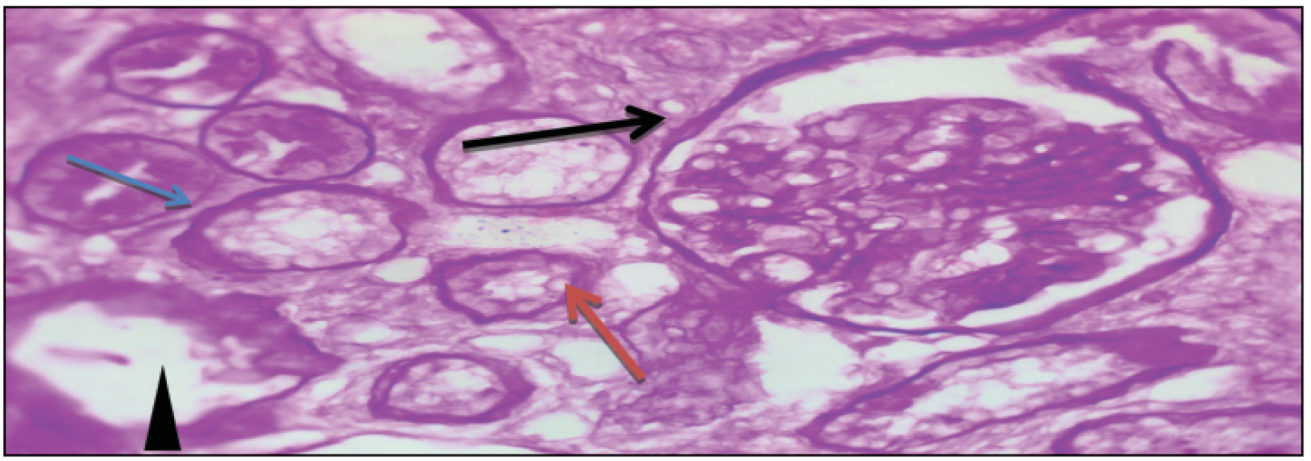

Figure 4: histological section of kidney rat tissue of group B showed thickening of the glomerular basement membrane (black arrow), thickening of the tubular basement membrane (blue arrow), vacuolation of tubular epithelial cells(red arrow) cystic changes in the tubule(arrow head).(PAS X600).

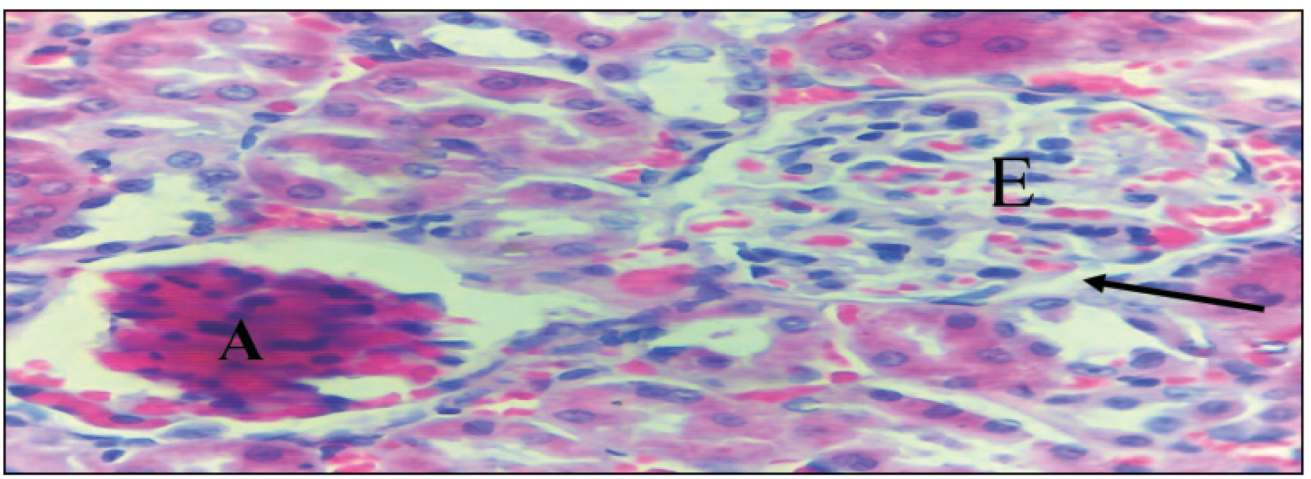

figure 5: histological section of kidney rat tissue of follow up animals showed expansion of the glomerular tuft (E), obliteration of the urinary space (arrow) and Atrophy of the glomeruli(A) (H& E X600).

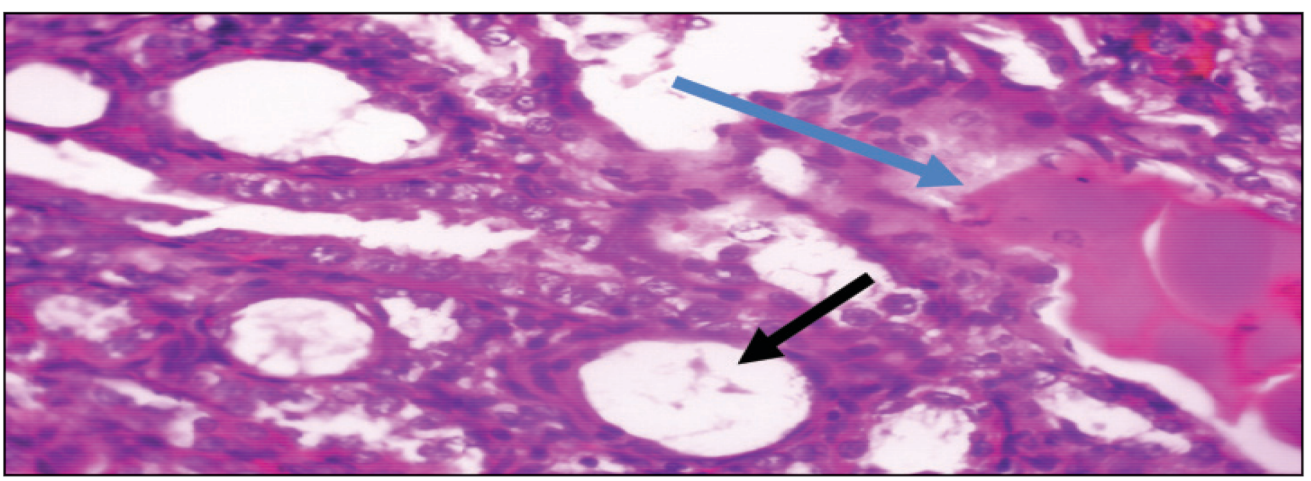

figure 6: histological section of kidney rat tissue of follow up animals showed cystic dilatation of the convoluted tubules lined by flat epithelial cells (black arrow)and protein cast (blue arrow) (H &E X400).

Reference:

1. *Dirk Lebrecht, Bernhard Setzer, Rolf Rohrbach et al., Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis.Nephrol Dial Transplant., 2004;19(2): 329–336. IVSL.*

2. *Minami M, Matsumoto S, Horiuchi H. Cardiovascular side-effects of modern cancer therapy. Circ. J., 2010; 74(9): 1779-1786.*

3. *Fadillioğlu E. , Erdoğan H. , Söğüt S. et al., "Protective effects of erdosteine against doxorubicin-induced cardiomyopathy in rats"Journal of Applied Toxicology, 2003; 23(1):pp. 71–74.*

4. *Karaman A. , Fadillioglu E. , Turkmen E. et al., "Protective effects of leflunomide against ischemia-reperfusion injury of the rat liver "Pediatric Surgery International, 2006; 22(5):pp. 428–434. IVSL.*

5. *Liu LL, Li QX, Xia L, et al., Differential effects of dihydropyridine calcium antagonists on doxorubicin-induced nephrotoxicity in rats. Toxicology, 2007; 231, 81-90.*

6. *De Beer EL, Bottone AE, Voest EE. Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. Eur J Pharmacol., 2001; 9:415(1):1-11.*

7. *Gewirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics Adriamycin and daunorubicin. Biochem Pharmacol, 1999; 57: 727-741.*

8. *Perego P, Corna E, De Cesare M, et al., Role of apoptosis and apoptosis-related genes in cellular response and antitumor efficacy of anthracyclines.Curr Med Chem., 2001; 8: 31-37.*

9. *Vincent WS lee and David CH Harris. Adriamycin nephropathy: A model of focal segmental glomerulosclerosis. Nephrology, 2011; 16(1): 30-38. IVSL.*

10. *Nayira A. AbdElbaky, Lamiaa N. Hammad, Tarek A. Atia. Protective Effect of Simvastatin against Adriamycin-Induced Nephrotoxicity in Rats; Biochemical and Histological Study. The Egyptian Journal of Hospital Medicine, 2006; Vol. 25: 725 – 739. IVSL.*

11. *Noboru Manab , Akiko Kinoshita , Misuzu Yamaguchi , et al., Changes in quantitative Profile of Extracellular Matrix Components in the Kidneys of Rats with Adriamycin – induced Nephropathy. J. Vet. Med. Sci. 2001; 63(2) : 125-133.*

12. *Yang wang, Yi ping wang, Yuet-ching Tay, et al., Progressive Adriamycin nephropathy in mice: Sequence of histological and immunohistochemical events. Kidney International. 2000; Vol. 58: 1797–1804.*

13. *Stevens A and Lowe J. Histology Gower medical publishing. London. New York. Cited by Ebaid H, Dhkil MA, Danfour MA, Tohamy A, Gabry MS (2007) Piroxicam-induced hepatic and renal histopathollogical changes in mice. Libyan. J. Med., 1997; 2: 1-16.*

14. *Bertani T, Remuzzi G. Epithelial cell damage, in Glomerular Injury: 300 years after Morgagni, edited by Bertani T, Remuzzi G, Milano, Wichtig Editore, 1983; p. 163.*

15. *Bertani T, Rocchi G, Sacchi G. Adriamycin-induced glomerulosclerosis in the rat. Am J Kidney Dis., 1986; 7: 12–19.*

16. *Bertani T, Zoja C, Abbate M, et al., Age-related nephropathy and proteinuria in rats with intact kidneys exposed to diets with different protein content. Lab Invest. 1989; 60:196-204.*

17. *Bertani T, Cutillo F, Zoja C, et al., Tubulo-interstitial lesions mediate renal damage in Adriamycin glomerulopathy. Kidney Int., 1986; 30:488 -496.*

18. *Lovett DH, Ryan JL, Sterzel RB. Stimulation of rat mesangial cell proliferation by macrophage interleukin I. Immunol. 1983; 131:2830-2836.*

19. *Grond J. Schilti-iuis MS. Koudstaal J, Elema JD. Mesangial function and glomerular sclerosis in rats after unilateral nephrectomy .Kidney mt. 1982; 22:338-343.*

20. *Grond J, Koudstaal J, Elema JD. Mesangial function and glomerular sclerosis in rats with amino nucleoside nephrosis. Kidney In!, 1985; 27:405-410.*

21. *Bevilacqija MP, Pober JS, Wheeler ME, et al., Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorph nuclear leukocytes monocytes and related leukocyte cell lines .J Clin. Invest. 1985; 76:2003-2008.*

22. *Shultz PJ, Dicorleto PE, Silver BJ, et al., Mesangial cells express PDGF mRNAs and proliferate in response to PDGF. Am J Physiol. 1988; 255: 674-684.*

23. *Nikolic-Paterson DJ, Lan HY, Hill PA, et al., Macrophages in renal injury. Kidney Int. 1994; 45(Suppl 45):S79–S82.*

24. *Emad Kudhair Abbas. Histopathological changes caused by Doxorubicin in laboratory mice (MusMusculus L). J Pak Med Assoc. 2011; 61: (11), pages 1108-1110.*

25. *Chen, C.H., H. Lin, Y.H. Hsu, Y.M. Sue, T.H. et al.,The protective effect of prostacyclin on adriamycin-induced apoptosis in rat renal tubular cells. Eur. J. Pharmacol., 2006; 529: 8-15.*

26. *Lee WM (2003). Drug – induced hepatotoxicity. N. Engl. J. med. 349:474-485.*

27. *Basit Javaid, Jean l. Olson, Timothy W. Meyer. Glomerular Injury and Tubular Loss in Adriamycin Nephrosis. American Society of Nephrology JASN. 2001; 12 (7) 1391-1400.*

28. *Bertani T, Poggi A, Pozzoni R et al. (1982). Adriamycin-induced nephrotic syndrome in rats: sequence of pathologic events. Lab Invest . 46: 16–23.*

29. *Scholey JW, Miller PL, Rennke HG, et al., (1989). Effect of converting enzyme inhibition on the course of Adriamycin induced nephropathy. Kidney Int . 36: 816–822.*