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Histological Changes in Renal Glomeruli, Proximal and Distal Convoluted Tubules of Adult Albino Rats due to Doxorubicin

Samina Munawar[1], Shagufta Nasreen[2], Asma Siddique[3], Qurrat-Ul-Ain[4], Alvia Batool[5], Attiya Mubarak Khalid[6], Fouzia Farzana[7]

1 *Senior Demonstrator, Department of Anatomy, Rahbar Medical College, Lahore Pakistan*
Conceived, Designed & did statistical analysis, Editing of the manuscript, Is responsible for integrity of research

2 *Assistant Professor, Department of Anatomy, PGMI/AMC, Lahore Pakistan*
Data collection & Manuscript writing

3 *Associate Professor, Department of Anatomy, Akhtar Saeed Medical & Dental College, Lahore Pakistan*
Data collection & Manuscript writing

4 *Professor, Department of Anatomy, Rahbar Medical College, Lahore Pakistan*
Review and final approval of manuscript

5 *Associate Professor, Department of Anatomy, FMH College of Medicine & Dentistry, Lahore Pakistan*
Data collection & Manuscript writing

6 *HOD, Department of Anatomy, Rahbar Medical College, Lahore Pakistan*
Review and final approval of manuscript

7 *Professor, Department of Anatomy, Continental Medical College, Lahore Pakistan*
Review and final approval of manuscript

CORRESPONDING AUTHOR
Dr. Samina Munawar
Senior Demonstrator, Department of Anatomy, Rahbar Medical College, Lahore Pakistan
Email: saminanaqvi12@gmail.com

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ABSTRACT

Objective: The aim of study is to determine the nephrotoxicity induced by Doxorubicin (DXR) in adult albino rats using histopathological technique. Oxidative stress is the main factor in DXR induced nephrotoxicity. **Study Design:** Laboratories based randomized control experimental trials. **Settings:** Post Graduate Medical Institute (PGMI) Animal House Experimental Research Laboratory Lahore. **Duration:** One year from February 2019 to February 2020. **Methods:** 20 albino rats were divided into two groups A&B, comprising 10 rats each. A was control group. Group B received DXR 1.2 mg/kg body weight intraperitoneally twice a week for 21 days. Animals sacrificed after 21days. Gross (weight of rats, weight of both right and left kidneys) and histological parameters (Quantitative; diameter of renal corpuscle, PCT, DCT Qualitative; vacuolization within PCT and DCT, glomerular and stromal vascular congestion, inflammatory cells infiltration) were observed. **Results:** Chemotherapy has been established as remarkably effective at treating many cancers in the modern cancer treatments. This treatment kills healthy cells as well as cancerous cells. DXR due to its oxidative property exerts toxic side effects on kidney which were analyzed by present study. The renal sections of rats treated with DXR for 21 days (Fig.2) had shown significant atrophic changes, including many shrunken renal corpuscles and degenerated renal tubules (PCT & DCT) with increased diameter. Stroma of the kidney appeared vacuolated with focal hemorrhages, inflammatory cells infiltrate and congested blood vessels. DXR lead to an imbalance between antioxidant and free oxygen radicals, which causes with protein oxidation and lipid peroxidation (LPO) resulting in tissue damage8. **Conclusion:** Gross change was decrease in the body weight and paired kidney weights which was statistically significant. Histopathological changes were glomerular and tubular diameter, degenerative changes, vacuolization within cells of PCT and DCT, inflammatory cell infiltration in stroma, glomerular and interstitial congestion. DXR leads to free radical formation, ion-dependent oxidative damage of biological macromolecules, protein oxidation and membrane LPO.

Keywords: *Doxorubicin, Oxidant, Anthracycline, Histopathology, Nephrotoxicity.*

INTRODUCTION

Doxorubicin (Adriamycin) is one of the most commonly used anti-cancer drug.[1] It is most potent and effective broad spectrum anthracycline antibiotic, widely used for treatment of a variety of many solid malignancies and lymphomas.[2] It is extracted from the streptomycin paucities" bacterium.[3,4]

Doxorubicin (DXR) works by interference with the synthesis of macromolecules covalent DNA binding and DNA cross linking, inhibition of topoisomerase II, arrest of tumor cell progression in G2 phase, induction of

apoptosis and generation of reactive oxygen radicals, which can lead to lipid peroxidation and membrane damage. Oxidative stress initiates apoptotic pathways of cell damages in rats.

The cytotoxic effect of DXR on malignant cells and its toxic effects on various organs liver, kidney, heart, lung, testis and blood cells have been reported.[3] Damage to kidney by DXR is reported due to oxidative stress produced by free oxygen radicals.

METHODS

These laboratories based randomized control experimental trials were conducted at post graduate medical institute (PGMI) animal house experimental research laboratory from Feb 2019 to Feb 2020.

Dose of DXR: 1.2 mg/kg body weight intraperitoneally[6] purchased from paramedic (Pvt).

Twenty albino rats (age:6-8 weeks, weight: 180-220 gm) were divided into two groups, comprising ten rats each. (Random Number Generator)

Group A was labelled as control and group B as experimental. Group A received 1ml distilled water intraperitoneally twice a week for 21 days while group B received DXR intraperitoneally 1.2mg/ kg body weight twice a week for 21 days. Body weight was recorded on electronic scale.

All the rats were dissected 24 hours after the administration of last dose. They were sacrificed under chloroform anesthesia. The kidney was removed and sliced into small pieces (3mm). Fixed in 10% neutral formalin for 48 hours, then dehydrated in rising grades of alcohol washed in xylene and embedded in molten paraffin wax at melting 56C-58C. Paraffin blocks were prepared, solidified, refrigerated and fixed in the chunks of rotator microtome. 5um thick sections were obtained and stained with Hematoxylin & Eosin (H&E) staining and examined under light microscope. Gross and Histological parameters were observed.

Gross Parameters: (1) Body Weight: Each animal was weighed before and at the end of the study. Body weight was recorded on an electronic scale (Sartorius Precision Balance, Germany) (Table1). **(2) Paired kidney weight:** Weight of both right & left kidneys and then paired kidneys weight of each animal was recorded immediately after dissection from body. **(3) Relative Tissue Weight Index (RTWI):** Relative tissue weight Index was calculated from the following formula;

$$RTWI = \frac{\text{Paired weight (g) of kidneys}}{\text{Animal body weight (g)}} \times 100$$

Histological Parameters: (1) Quantitative parameters: Under H&E, diameters of renal corpuscular, PCT and DCT were studied by using stage micrometer. **(2) Qualitative parameters:** Vacuolization within PCT and DCT, glomerular and stromal vascular congestion, inflammatory cells infiltration) were observed.

RESULTS

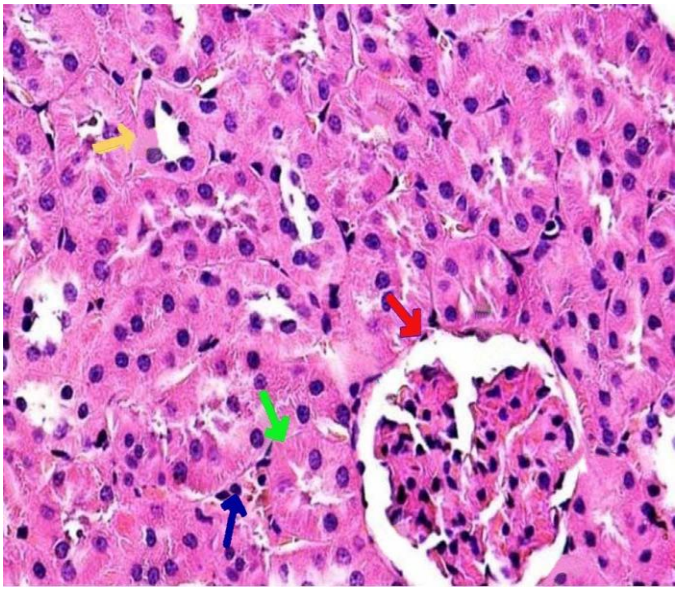
Weight of Animals: The animals were weighed at the start and end of experiment and examined to assess the state of their health; they remained healthy throughout the experimental period. One-way ANOVA test was applied to compare the initial body weight and final body weight among groups. There was a statistically significant difference in mean body weights among groups at the end of the experiment (Table 1). Pair wise comparison of mean final body weight among groups. Pair wise comparison of paired kidney weight and relative tissue weight index among groups (Table 1).

Table 1: Comparison of initial body weight and final body weight, paired kidney weight & relative tissue weight index among groups

Parameters	Group A	Group B	p-value#
Initial body weight (gm)	176.8 ± 8.9	182.8 ± 7.4	0.246
Final body weight (gm)	197.0 ± 5.2	168.0 ± 5.1	< 0.001
Paired kidney weight	1.72 ± 0.06	1.37 ± 0.03	< 0.001*
Relative tissue weight index	0.88 ± 0.04	0.82 ± 0.03	0.002

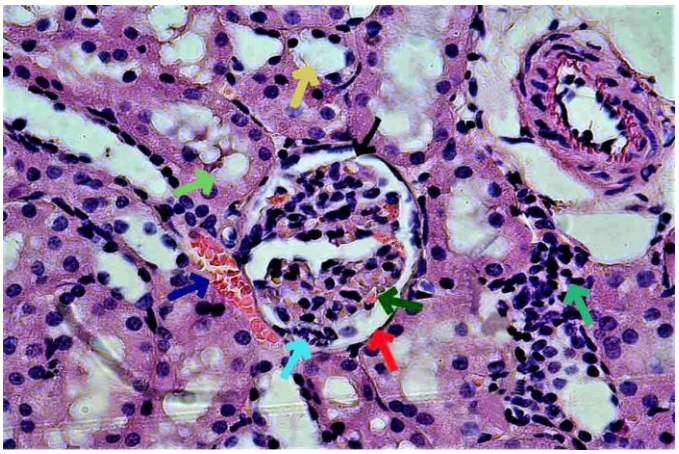
*#One way ANOVA, *p- value ≤ 0.05 is considered statistically significant*

Figure 1: Photomicrograph of cortex of kidney (group A)



Showing normal looking renal corpuscle (red arrow), proximal convoluted tubules (light green arrow), distal convoluted tubules (yellow arrow) and stroma (blue arrow) H&E stained 400X.

Figure 2: Photomicrograph of cortex of kidney (group B)



showing renal corpuscle (red arrow) with glomerulus congestion (dark green arrow) and inflammatory cell infiltration (aqua blue arrow). Cytoplasmic vacuolization in PCT (light green arrow) and DCT (yellow arrow). Stromal congestion (blue arrow) and inflammatory cell infiltration (aqua green arrow). H&E stained 400X.

Histological Parameter under Hematoxylin and Eosin (H&E) Staining Quantitative Parameters: The mean diameter of renal corpuscle, PCT and DCT in all groups was calculated. One-way ANOVA test was applied to compare diameters of renal corpuscle, PCT and DCT among two groups. It was found that the mean diameter of renal corpuscle, PCT and DCT in all groups were significantly different (*p*-value < 0.001), (Table 2, Fig 2). Pair wise comparison of diameter of renal corpuscle PCT and DCT among two groups which was considered statistically significant.

Qualitative Parameters:

Cytoplasmic Vacuolization: Fisher's exact test showed that there was an association between cytoplasmic vacuolization of PCT and groups. Cytoplasmic vacuolization of PCT and DCT in all rats of group A was absent. In group B, Cytoplasmic vacuolization was present in all rats. (Table 2, Fig 2).

Glomerular and Stromal Vascular Congestion: Fisher's exact test showed that there was an association between glomerular vascular congestion and groups. Glomerular vascular congestion was absent in all rats of group A. In group B, Fisher's exact test showed that there was an association between stromal vascular congestion and groups. Stromal vascular congestion in all rats of group A was absent. In group B, stromal vascular congestion present in all the animals. (Table 2, Fig 2).

Inflammatory cells Infiltrate: Fisher's exact test showed that there was an association between inflammatory cells infiltrate and groups. Inflammatory cells infiltrate in all rats of group A was absent. In group B, inflammatory cells infiltration was present in all the animals. (Table 2, Fig 2).

Table 2: Histological Parameters

Quantitative Parameters			
Parameters	**Group A**	**Group B**	**p-value#**
Diameter of renal corpuscular (µm)	85.5 ± 3.1	74.0 ± 2.9	< 0.001*
Diameter of PCT (µm)	34.8 ± 4.1	45.1 ± 4.5	
Diameter of DCT (µm)	32.1 ± 3.0	40.6 ± 5.5	
Qualitative Parameters			
Cytoplasmic Vacuolization	**Group A n (%)**	**Group B n (%)**	**p-value**
PCT	0 (0.0%)	10 (100.0%)	< 0.001*
DCT	0 (0.0%)	10 (100.0%)	
Glomerular Vascular Congestion	**Group A n (%)**	**Group B n (%)**	
Present	0 (0.0%)	10 (100.0%)	
Stromal Vascular Congestion	**Group A n (%)**	**Group B n (%)**	
Present	0 (0.0%)	10 (100.0%)	
Inflammatory Cells Infiltrate	**Group A n (%)**	**Group B n (%)**	
Present	0 (0.0%)	10 (100.0%)	

DISCUSSION

Doxorubicin (DXR), an anthracycline antibiotic has been used for the treatment of cancer since 1969. Despite, its increase anti-tumor efficacy, DXR's use in chemotherapy has been largely restricted due to its cardiac, renal, pulmonary, testicular and hematological toxicity.[7] DXR lead to an imbalance between antioxidant and free oxygen radicals, which causes with protein oxidation and lipid peroxidation (LPO) resulting in tissue damage8. Although the exact mechanism of DXR induced nephrotoxicity remains unknown, it is believed that the toxicity may be mediated through the radical formation, ion-dependent oxidative damage of biological macromolecules, protein oxidation and membrane LPO[8].

A comparison of mean animal final body weight with mean initial body weight in treated groups showed a significant difference (Table 2). A gradual normal increase in body weight in the control group (A) was observed. Statistically significant percentage weight loss in DXR treated group B (P-value<0.001) was noted. Similar results were demonstrated by work done by Chen *et al*[9] attributing DXR induced oxidative stress. They reported that DXR treated rats had significantly smaller body weight gain as compared to control rats.

Decreased in mean paired kidney weight of rats treated with DXR (Table 2) was in accordance with the available data from study done by[8,9,10] which revealed significant decrease (p<0.001) in mean paired kidney weight of adult rats treated with DXR due to atrophic and degenerative changes. The renal sections of rats treated with DXR for 21 days had shown significant atrophic changes, including many shrunken renal corpuscles and degenerated renal tubules (PCT & DCT) with decreased

diameter as well having disrupted basement membrane, discontinuous brush border of PCT, stroma of the kidney appeared vacuolated with focal hemorrhages, inflammatory cells infiltrate was present. In addition, blood vessels were congested.[9]

A significant decrease in the diameter of the renal corpuscle due to glomerular degeneration and vacuolation in the DXR treated group. [9] Oxidative stress, a major cause of DXR induced damage of renal corpuscle as demonstrated earlier by Khan *et al*.[10] Statistically significant increase in diameter of PCT and diameter of DCT in DXR treated group B was analogous to a study done by Sami *et al*.[8]

In current study, renal sections of rats treated with DXR 1.2 mg/kg twice a week for 21 days showed significant histological changes in PCT and DCT with degenerated cells (Fig2.). This supported the[11,12] who assigned renal pathologies due to production of oxygen derived free radicals and reactive oxygen species (ROS) leading to oxidative damage. These substances are harmful to biological systems as they react with protein, DNA and lipids causing cellular damage as reported earlier by.[13,14] Vacuolization within cells of PCT and DCT (Fig.2) might be the signs of renal toxicity and cell degeneration in DXR treated group (B) similarly observed by Al-Karawi *et al*.[13]

Congestion of blood vessels with stagnant blood cells & disrupted endothelium causing hemorrhage within renal stroma in DXR treated group B (Fig.2) may be due to the prevention of prostaglandin synthesis which could have regulated blood flow. Stromal inflammatory cell infiltrate in the present work was mainly the lymphocytes (mononuclear leukocytes) in DXR treated group (Figure 2). The migration of leucocytes towards the inflammatory site is called chemotaxis which is response of body tissue facing any injurious impact13,14. Lymphocyte is predominant in intoxication, viral and protozoal diseases, and macrophages are signs of chronic inflammation. Inflammatory cell infiltration.[13,14]

CONCLUSION

Gross change was decrease in the body weight and paired kidney weights which was statistically significant. Histopathological changes were glomerular and decreased tubular diameter, degenerative changes, vacuolization within cells of PCT and DCT, inflammatory cell infiltration in stroma, glomerular and interstitial congestion. DXR leads to free radical formation, ion-dependent oxidative damage of biological macromolecules, protein oxidation and membrane LPO.

LIMITATIONS

This study had a small sample size and several additional limitations.

SUGGESTIONS / RECOMMENDATIONS

On this topic, more larger scale studies are recommended.

CONFLICT OF INTEREST / DISCLOSURE

There is no conflict of interest

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