ORIGINAL ARTICLE

RENAL FUNCTION AND OXIDATIVE STRESS IN DOXORUBICIN-TREATED WISTAR RATS: IMPLICATIONS FOR NEPHROTOXICITY

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Background: Doxorubicin is the most potent broad-spectrum anthracycline antibiotic. Despite having increased anti-tumour activity, it has been reported toxic to different organs including kidneys. This study was conducted to investigate nephrotoxic effects of Doxorubicin by assessing the renal functions parameters and oxidative stress in adult male Wistar rats. **Methods:** From September 2023 to February 2024, this quasi-experimental study was carried out in Department of Pharmacology, Isra University, Hyderabad. Twenty healthy male rats, weighing 180–220 grams, were divided into Group I (control) which received 10 mL distilled water I.P., and Group II (experimental) which received injections of doxorubicin (1.2 mg/Kg body weight) I.P. twice a week for 21 days. The animals were slaughtered; blood samples were taken for renal function tests, and oxidative stress markers. Histological analyses were carried out for nephrotoxicity. **Results:** Group II rats showed a significant decline in absolute kidney weight ($p<0.05$) accompanied by a significant decrease in animal weight. These rats also exhibited significantly higher ($p<0.05$) serum levels of renal and inflammatory indicators. Serum levels of superoxide dismutase, reduced glutathione, glutathione peroxidase and catalase in renal tissues were statistically substantially lower ($p<0.05$) in Group II, but malondialdehyde and Nitric oxide levels were significantly higher ($p<0.05$) in the same group. Histopathological analysis revealed renal tubule dilation and reduced number of renal corpuscles in Group II rats. **Conclusion:** Doxorubicin poses a significant damage to the renal tissues by imbalance between antioxidant and free oxygen radicals, which causes protein oxidation resulting in tissue damage.

Keywords: Doxorubicin, Histopathology, Nephrotoxicity

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INTRODUCTION

Kidneys are essential for homeostasis as they remove toxic substances from metabolism and control fluid volume and electrolyte balance.[1] Kidney injuries, whether acute or chronic, arise from various factors and are recognized as a significant public health issue. Recent studies show that chronic kidney disease (CKD) affects approximately 10% to 14% of the general population worldwide. The total burden of kidney related diseases has nearly doubled during the last several decades. Over 850 million individuals are suffering from various kidney related diseases. The disease creates a bigger problem for specific high-risk groups because about 33% of diabetic patients and 20% of hypertensive patients develop CKD which now ranks as the 9th most common cause of death world-wide.[2]

Several factors contribute to renal injury, including external chemicals, such as medication like doxorubicin (DXR), that have the potential to harm renal tissue. The kidney damage resulting from DXR shows itself through an acute decline in kidney function. This damage is not limited to sudden injury; the condition usually develops into a chronically damaged renal parenchyma. Whether the injury is acute or chronic, the underlying nephrotoxic mechanism eventually impairs the filtration process, resulting in accumulation of metabolic products such as creatinine and urea in blood.[3]

Doxorubicin is the most powerful and effective broad-spectrum anthracycline antibiotic. It has been used as an anti-cancer medication since 1960s to treat lymphomas and a wide range of solid cancers.[3] Despite having increased anti-tumour activity, DXR's toxicity to the heart, kidneys, lungs, testicles, and blood has limited its usage in chemotherapy. DXR is reported to work by obstructing the synthesis of macromolecules, inhibiting topoisomerase II, halting the growth of tumour cells in the G2 phase, causing apoptosis.[4,5] DXR creates oxidative stress through its ability to disrupt the balance between free radicals and antioxidants which leads to lipid peroxidation that damages tissue structures. Although the exact process remains unclear it generates an imbalance between free radicals and antioxidants, which leads to lipid peroxidation (LPO) macromolecules and iron-based damage of cell membranes and large molecular structures.[6,7] This study aimed to investigate the

DXR-induced nephrotoxic effects in adult Wistar rats by analysing renal biomarkers and oxidative damage of DXR by assessing the renal functions parameters and oxidative stress.

MATERIAL AND METHODS

This quasi-experimental study was done in the Department of Pharmacology, Isra University, Hyderabad from Sep 2023 to Feb 2024 after approval from the Ethical Review Committee of Isra University (IU/RR-10-ERC-23/N/2023/295). In accordance with international guidelines for the care and use of laboratory animals and institutional regulations, the study involved 20 healthy adult male Wistar rats, aged 9–12 weeks and weighing 180–220 grams, with no visible deformities or abnormalities. The sample size was calculated to be 20, using G*Power 3.1 assuming α=0.05 and a large effect size (f>0.60), which is typical for DXR-induced organ injury.

The animals were kept in clean, well-ventilated cages with unrestricted access to balanced laboratory diet and water, with 12-hour light-dark cycle at a temperature of 22±2 °C for one week for acclimatization. The body weights of all animals were recorded, and they were randomly assigned into two equal groups (n=10).

Group I was labelled as control and group II as experimental. Group I animals received 1 mL distilled water intra-peritoneal for 21 days, and normal chow (twice a week), while group II rats received DXR intra-peritoneal at 1.2 mg/Kg body weight (twice a week) for 21 days, and normal chow.[8] After 4 hours of administration of last dose, all rats were weighed again.

The animals were subsequently sacrificed under anaesthesia with sodium pentobarbital (45 mg/Kg) and dissected. Blood samples were collected through cardiac puncture to assess serum creatinine, blood urea nitrogen (BUN), and C-reactive protein (CRP) levels. Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx), and nitric oxide (NO) were also assessed as markers for oxidative stress. All tests were performed on Roche/Hitachi diagnostic kits, whereas GPx was performed on the bioassay technology ELISA kit. The kidneys of all animals were removed immediately, weighed, and examined grossly for any morphological changes. The Relative Tissue Weight Index (RTWI) was calculated by employing following formula:[4]

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

The kidneys were sliced into small pieces of 3 mm and fixed in 10% neutral formalin for 48 hours. The tissue slices were processed for sectioning at a thickness of 4 µm. The sections were stained with Haematoxylin and Eosin (H&E) and examined under a light microscope at 100 magnification. Quantitative measurements, renal corpuscular diameter, and appearance of proximal and distal convoluted tubules were assessed using a stage micrometer. The qualitative parameters including vacuolization within PCT and DCT, glomerular and stromal vascular congestion, inflammatory cells infiltration were observed.

Data was analysed using SPSS-24. Qualitative variables were presented as frequency and percentage while Mean±SD was employed for quantitative variables. Student's *t*-test was used for analysis of quantitative data, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

Table-1 shows the mean body weights (pre- and post-experimental) along with kidney weight and relative tissue weight index of both group rats. A significant decline in body weight, kidney weight and RTWI ($p<0.05$) was observed in group II rats. (Table-1).

Table-2 is demonstrating the comparison of renal markers, i.e., serum creatinine and BUN, and inflammatory marker (CRP levels) between control and DXR treated group. A statistically significant rise ($p<0.05$) in S. creatinine, BUN and CRP concentrations were observed in group II rats treated with DXR alone compared with group I controls.

DXR induced group II showed the oxidative stress with significantly ($p<0.05$) lowered levels of GPx, GSH, SOD and catalase in renal tissues, while MDA and NO levels were significantly higher ($p<0.05$) compared with the control group. (Table-3).

Table-1: Body weight (pre- and post-), kidney and relative tissue weight index of both study groups

	Group I	Group II	*p*
Pre-body weight (g)	188.3±7.81	191.5±8.11	0.381
Post-body weight (g)	192.2±6.30	173.2±5.81	0.000
Kidney weight (g)	1.69±0.07	1.41±0.04	0.000
RTWI (%)	0.88±0.04	0.82±0.03	0.001

Table-2: Comparison of serum renal markers and CRP levels between both groups

	Group I	Group II	*p*
S. Creatinine (mg/dL)	0.43±0.03	0.79±0.08	0.000
BUN (mg/dL)	22.41±2.50	51.20±2.91	0.000
C-Reactive Protein (mg/dL)	0.11±0.09	0.83±0.31	0.000

Table-3: Comparison of oxidative stress markers between both groups

	Group I	Group II	*p*
GPx (U/mg protein)	9.89±1.30	6.78±0.71	0.000
GSH (µmol/g tissue)	1.16±0.01	0.31±0.02	0.000
SOD (U/mg protein)	13.17±0.61	10.37±0.91	0.0 00
Catalase (µ/mg protein)	19.38±1.1	15.67±1.4	0.000
MDA (nmol/mg/protein)	0.71±0.05	2.18±0.18	0.000
NO (µmol/g/protein)	0.13±0.02	0.63±0.01	0.000

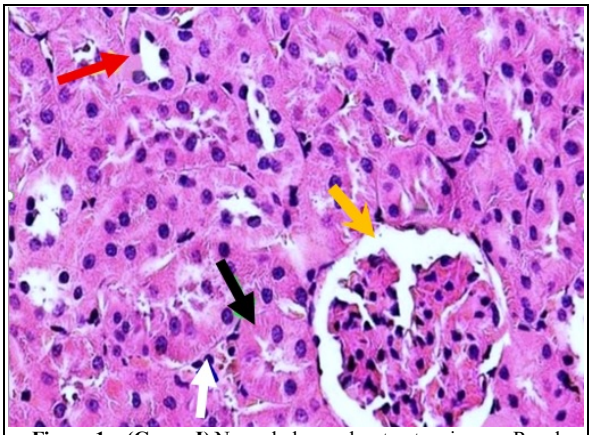

Figure-1a: (Group I) Normal glomerular structure is seen. Renal corpuscle (Yellow arrow). Proximal convoluted tubules (Black arrow). Distal convoluted tubules (Red arrow). Stroma (White arrow)

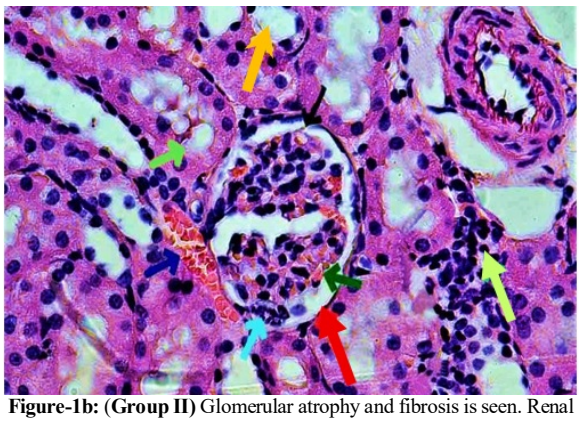

Figure-1b: (Group II) Glomerular atrophy and fibrosis is seen. Renal corpuscle (red arrow) with glomerulus congestion (dark green arrow) and inflammatory cell infiltration (aqua blue arrow). Cytoplasmic vacuolization in PCT (light green arrow) and DCT (yellow arrow). Stromal congestion (blue arrow) and inflammatory cell infiltration (aqua green arrow)

Figure-1a & 1b: Photomicrograph of renal tissue of control and DXR treated groups

The cytoplasmic vacuolization in PCT and DCT were observed along with the vascular congestion of glomerulus and stroma as well as infiltration of inflammatory cells was demonstrated in group II renal tissues compared with group I tissues. There was statistically significant (p<0.05) decline in renal corpuscles diameter of group II (73.1±3.0 µm) compared with group I (84.8±2.9 µm). There was a significant (p<0.05) increase in diameter of PCT and DCT of group II (45.5±3.7 µm and 41.3±5.2 µm) compared with group I (33.7±4.2 µm and 32.5±2.9 µm) respectively.

DISCUSSION

DXR is an effective treatment for various solid tumours; however, it can significantly impair kidney function, similar to other anti-tumour medications. DXR accumulates in the glomerulus, leading to substantial kidney damage. The mechanisms through which DXR causes glomerular toxicity remain poorly understood.[9,10]

This study discovered substantial alteration following DXR administration, which disrupted the BUN, Creatinine, and markedly affected renal histological damage. A comparison of mean animal post-experimental body weight with mean pre-experimental body weight in both groups showed a significant difference. A gradual normal increase in body weight in the control group I was observed. Statistically significant weight loss in DXR treated group II (p<0.05) was noted. Similar results were demonstrated by Munawar *et al*[8] and Chen *et al*[11] reported that rats had significant decline in body weight after induction with DXR compared with the control group.

A significant decrease (p<0.05) in mean absolute kidney weight and RTWI of rats treated with DXR compared to the control group was observed. Consistent with the results from the studies by Chen *et al*[11] Sami *et al*[12] and Khan *et al*[13] the current study demonstrated a significant decrease in kidney weight and RTWI following DXR administration. This may be due to adverse effects of DXR causing the atrophic and degenerative changes resulting in damage to the kidneys.

In our study, we showed that DXR treatment led to a substantial increase in MDA levels, accompanied by significant decreases in GSH content and the activities of GPx and SOD. These results align with findings reported by Liu *et al*[4] and Khan *et al*[13].

Histopathological analysis of renal sections of both group rats was done in this study to demonstrate the impact of DXR on renal tissues compared with the control rats. It has been observed that DXR treated rats for 21 days had shown significant atrophic changes in the glomerulus including many shrunken renal corpuscles and degenerated renal tubules (PCT and DCT) with decreased diameter as well having disrupted basement membrane, discontinuous brush border of PCT, stroma of the kidney appeared vacuolated with focal haemorrhages, and inflammatory cells infiltrate was present. In addition, blood vessels were congested. A significant decrease in the diameter of the renal corpuscle due to glomerular degeneration and vacuolation in the DXR treated group. Chen *et al*[10] and Afsar *et al*[14] also reported the similar findings which they have demonstrated under microscopic examination in their studies.

In the current study, renal sections of rats treated with DXR 1.2 mg/Kg twice a week for 21 days showed statistically significant histological changes including the increase in diameter of PCT and diameter of DCT with degenerated cells in DXR treated group II was analogous to a study done by, Liu *et al*[4], Munawar *et al*[8] and Sami *et al*[12], who assigned renal pathologies due to production of oxygen derived free radicals and

reactive oxygen species (ROS) leading to oxidative damage. This may be due to the fact that oxidative stress resulting in the free radicals resulting from the DXR induction. These substances are harmful to biological systems as they react with protein, DNA and lipids causing cellular damage. Moreover, vacuolization within cells of PCT and DCT might be the signs of renal toxicity and cell degeneration in DXR treated group II. These findings are supported by those observed by Al-Karawi *et al*[4] Al-Karawi *et al*[15].

Furthermore, our histopathological findings also revealed the congestion of blood vessels with stagnant blood cells & disrupted endothelium causing haemorrhage within renal stroma in DXR treated group II. This 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. The migration of leucocytes towards the inflammatory site is called chemotaxis which is response of body tissue facing any injurious impact which were also reported by Afsar *et al*, and Al-Karawi *et al*, in their studies.[14,15]

With strengths there are limitations in this study as the present student observed only DXR toxic effects on renal system and not on other organ system due to lack of funding and time constraints. Moreover, no high or low dose was used to compare the dose effects in this study.

CONCLUSION

DXR induces a significant renal tissue injury by precipitating an imbalance between antioxidant defenses and free oxygen radicals. This oxidative stress leads to protein oxidation, ultimately resulting in progressive tissue damage.

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Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

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