Doxorubicin Induced Histomorphometric Changes in the Kidney of Albino Rats and Protective Role of Nigella Sativa

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Significance:
Doxorubicin adversely affects renal tissue but owing to its remarkable therapeutic role, it cannot be substituted, hence, the strategy is to explore a remedy to combat its organ toxicity. Present study was designed accordingly to investigate the protective effect of NS against DOX related renal toxicity.

ABSTRACT

Objective: Doxorubicin is presently a leading antineoplastic drug with promising efficacy. This study was designed to investigate the histological effects of doxorubicin toxicity on rat kidneys and how much protection is provided by Nigella Sativa.

Materials & Methods: A randomized controlled trial conducted on thirty adult male wistar rats divided randomly into three equal groups. Group A served as a control. Group B was injected with weekly intraperitoneal injections of doxorubicin at a dose of 3mg/kg b.w. Group C rats received doxorubicin along with nigella sativa at a dose of 1000mg/kg b.w. orally daily. At the end of these interventions, animals were sacrificed, and kidneys were removed for the purpose of histological staining. Renal glomerular and tubules related histopathological parameters were assessed qualitatively as mild, moderate & severe. Renal glomerular diameter was digitally measured by microscope. Ethical approval was taken from Ethical Committee, Jinnah Postgraduate Medical Centre (JPMC), Karachi. All the parameters were statistically analyzed.

Results: Group B rats' renal tissue was adversely affected by the drug showing marked necrosis of tubules and the glomeruli along with interstitial cells' infiltration. Glomerular diameter was also significantly decreased in group B as compared to group A. These histological features in group C rats' renal tissue were milder and glomerular diameter was close to that of group A.

Conclusion: According to our study doxorubicin treatment proved noxious for the renal tissue, both tubules and glomeruli, while nigella sativa significantly competed against these pathological alterations.

Introduction

Adriamycin or Doxorubicin (DOX) is presently the most widely used anticancer drug in combination regimens for a wide range of neoplasia including acute lymphoblastic leukemia, and lymphomas; breast, ovarian and lung carcinomas; and pediatric malignancies like Ewing's sarcoma and neuroblastoma. (1) Its antineoplastic mechanism is tridimensional; it intercalates into DNA molecule and ceases its replication, it inhibits topoisomerase II which is an essential enzyme for DNA replication, it generates oxygen free radicals which destroy nuclear base pairs and cell membrane and lastly it binds to membrane channels and halts intercellular transport. (2) DOX was formulated by hydroxylation of daunorubicin as later was highly cardiotoxic but soon it was revealed DOX has similar toxic effects with narrow therapeutic window. DOX cannot be abandoned as it is highly effective against the cancer cells and presently there is no alternate drug. Hence, its precautionary usage is advised in case of compromised vital organs and its safe formulations are being designed and experimented. (3) Nephrotoxic effects of DOX involve renal glomeruli, renal tubules and renal interstitium and has been reported in the recent literature. (4) Its cardiotoxic effects can be countered by chelating agents but presently there is no effective remedy for the prevention of DOX induced renal toxicity. (5)

Among the hospitalized patients with acute kidney injury (AKI), drugs are responsible for 19% - 26% of cases. Older subjects and the females are more susceptible owing to their reduced body muscle. Patients with poor hepatic status (hypo-albuminemia & cirrhosis) and hypovolemic conditions are unable to metabolize the drug hence develop AKI or chronic renal failure. (6,7) In the modern era anticancer therapy is more effective than the past but concurrently the risk of nephrotoxicity is mounting and need to be addressed.

Centuries old herbs and plant derived extracts have well-documented preventive and protective role against drug induced organ toxicities. One of such plants is Nigella sativa (NS), commonly named as 'Kalonji' or 'Black Seeds,' belongs to Ranunculaceae family of kingdom plantae. It is cultivated Southwest Asia, Southern Europe, and North Africa. (8) NS is source of essential amino acids, vitamins, minerals, poly unsaturated fatty acids (linoleic acid & oleic acid) and lastly the dithymoquinone constituent which is biologically active and responsible for its pharmacological properties including antidiabetic, antihypertensive, anticancer, antioxidative, antimicrobial and anti-inflammatory properties. (9,10) NS oil is recently recommended to blend with commercial oils to reduce the peroxide value (Oxidative Level Indicator) and enhance the nutritional value. (8)

Doxorubicin adversely affects renal tissue but owing to its remarkable therapeutic role, it cannot be substituted hence the strategy is to explore a remedy to combat its organ toxicity. Present study was designed accordingly to investigate the protective effect of NS against DOX related renal toxicity.

Materials and Methods

This randomized controlled trial was conducted at Jinnah Postgraduate Medical Centre (JPMC) Karachi after approval from the Institutional Research Ethics Committee. Nigella Sativa seeds were dried and grinded to extract the powder which was stored in the refrigerator till use. Rat dose of NS was determined by

the published data. (11) DOX was obtained from Pfizer Pharma in powdered form and its sterile solution was prepared by dissolving 50gm of powder in 25ml of normal saline. (12)

30 male albino rats weighing 180–250gm and age 90-120 days were procured from Animal Care Center of JPMC Karachi and kept under optimal conditions. After two weeks of acclimatization the animals were randomly divided into three groups of 10 animals each. Group A served as a control. Group B received five weekly intraperitoneal doses of DOX at a dose of 3mg/kg body weight. (13) Group C received five weekly intraperitoneal doses of DOX at a dose of 3mg/kg body weight and aqueous suspension of powdered NS 1000mg/kg body weight orally daily for five weeks.

At the end of these interventions all the animals were sacrificed under chloroform anesthesia and the kidneys were removed from each animal which were fixed in 10% formalin for 72 hours and about 5mm thick tissue pieces were placed in separate tissue cassettes for processing in automatic tissue processor. Paraffin blocks were prepared and 4µm thick sections were cut through rotatory microtome and placed over albumenized slides. After air drying slides were stained through hematoxylin and eosin (H&E) and Periodic acid Schiff (PAS) stains according to the instructions given in literature. (14)

For calculating mean diameter of the group, five fields from each of the ten slides were observed from each animal. Diameter of three oval glomeruli per field was digitally recorded through Nikon Eclipse 50i microscope. Qualitative parameters include necrosis of glomeruli and tubules, loss of brush border, tubular vacuolations and tubular cast. Three random non-overlapping microscopic areas of a slide were selected for recording the pathological findings of each animal's kidney.

The severity of these findings was graded semi-quantitatively as: Score 0 (normal)for no pathological finding; Score + (mild) for 10% to 25% of the examined fields with histological alterations; Score ++ (moderate) for 25% to 50% of the examined fields with histological alterations and Score +++ (severe)for more than 50% of the examined fields with histological alterations. (15)

Ethical approval was taken from Ethical Committee, Jinnah Postgraduate Medical Centre (JPMC), Karachi. Data was analyzed by using SPSS (Statistical package for social sciences) software version 21. Mean & standard deviation was calculated for quantitative parameter and for comparison ANOVA and Post hoc tukey's was applied. Frequency & percentages were calculated for qualitative variables and for comparison chi square test or fisher exact test was applied.

Results:

In control group A, normal renal histology of rat kidney was observed as shown in figure 2. Renal tissue of toxic group B was adversely affected by the drug (figure 3). Corticomedullary architecture was intact while renal corpuscles had widened Bowman's space, shrunken and necrosed glomeruli with irregular capillary tuft. Renal tubules were disrupted owing to significant loss of brush border, desquamation of its cells and presence of luminal cast. Nuclei of few tubular cells were condensed indicating pyknosis and cell death. Interstitium was significantly filled with inflammatory cells. When PAS stained sections were observed, Glomerular and tubular basement membranes were found to be intact. These pathological features of tissue damage were reverted in kidney sections from group C (figure 4). Renal corpuscles showed normal capillary tuft and Bowman's capsule and necrotic changes improved as compared to the toxic group B (figure 1). Proximal and distal tubules were slightly dilated, and brush border loss was less evident as compared to group B. Interstitial edema and inflammatory cells population also decreased in group C.

Mean glomerular diameter (µm) and standard deviation of group A, B & C was 71.09 ± 7.01, 49.77 ± 3.99 and 67.50 ± 8.80 respectively. According to Tukey's post hoc test the inter group differences were statistically significant (p value < 0.01).

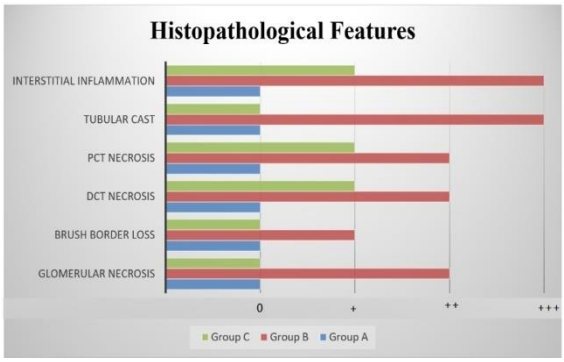

Figure 1**:** Comparison of histo-pathological features of kidney among group A, B & C.

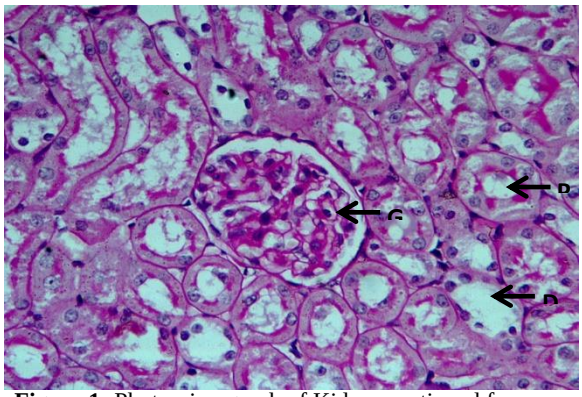

Figure 1: Photomicrograph of Kidney sectioned from Figure 2: Group A Normal Histology showing glomeruli (G), proximal tubules (P) & distal tubules (D). PAS,40X.

Discussion

NS is being prescribed in the form of grinded powder and oil for wide range of miscellaneous issues like infertility, indigestion, and traumatic brain disorders but the medics are unaware of standard dosage, formulations &pharmacological effects. (16)

Doxorubicin treated rats' kidney revealed significant glomerular necrosis evident by distorted microscopic anatomy and decreased glomerular diameter as compared to that of control group. To demonstrate DOX induced renal impairment Xie et al performed "Micro CT imaging and structural analysis" of

glomeruli and observed that number of glomeruli and their nuclei are decreased butvariation of glomerular size was different among right and left kidney. They explained DOX increases glomerular size at an early stage and vice versa at an advanced stage. (17)

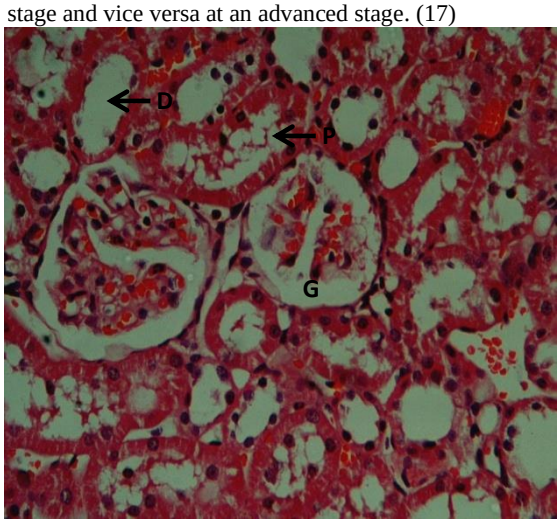

Figure 3: Photomicrograph of Kidney sectioned from toxic group B showing Proximal and distal tubules (PT & DT) with brush border loss and cellular desquamation along with a necrotic glomeruli (G). H&E, 40X.

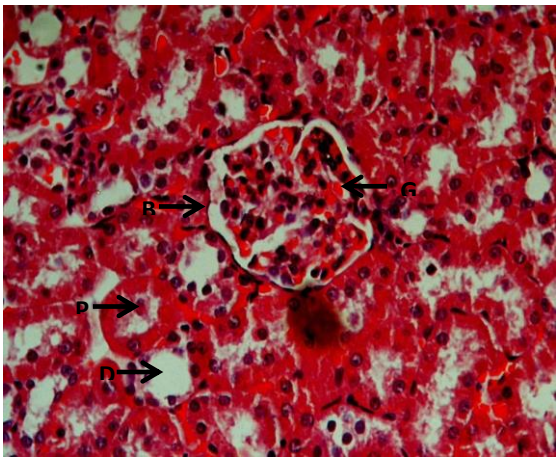

Figure 4: Photomicrograph of kidney sectioned from treatment group C, showing histological features recovery with Glomeruli (G), Bowman's capsule (BC), Proximal convoluted tubules (PCT) and Distal convoluted tubules (DCT). H&E, 40X.

DOX treatment adversely affected renal tubules both PCT and DCT. Tubules were dilated and the lumen was filled with cellular debris (cast) and desquamated cells. These pathological alterations were marked statistically significant against the control. DOX also affected the renal interstitium with significant inflammatory cells' infiltration which advocate its capability of initiating a cellular inflammatory response. (18)

Hosseinzadeh et al also observed glomerulo-tubular atrophy and inflammatory cells infiltration after DOX treatment in rats at 2.5mg/kg dose twice weekly for two weeks. Simultaneously they detected elevated inflammatory markers (TNFα- & IL-1β), elevated oxidative stress markers (malondialdehyde & nitric oxide) and lowered levels of antioxidants (catalase, superoxide dismutase and glutathione peroxide) among DOX treated animals. (19) Bilgic &Armagan also reported that single toxic dose of DOX adversely affects microscopic structure of both renal tubules and renal glomeruli and correlated these findings with biochemical markers (serum urea & creatinine) and oxidative stress. (20) Antimicrobial anticancer drugs cause acute tubular disease by direct tubular cell injury according to most recent drug induced AKI classification. (6) One possible tissue injury mechanism for these vacuolations or cellular swelling is failure of ion transport pump leading to hydrolytic changes. (21)

Microscopic findings of renal tissue specimen from group C was suggestive of substantial recovery. NS treatment prevented glomerular necrosis that was severe in the DOX intoxicated group. This finding was supported by the quantitative parameter i.e. mean glomerular diameter which was raised significantly in group C as compared to the toxic group.

NS treatment also shifted the proximal and distal tubular distortion from moderate to milder degree. Similarly, tubular cast was significantly and brush border loss was non-significantly reduced in group C. NS treatment reduced the concentration of inflammatory cells from renal interstitium that was severe with DOX intoxication which depicts its anti-inflammatory properties.

Contrary to our observations, Hadjzadeh et al., described NS effect as a nephro-protective agent is not significant as it reverted cisplatin induced histological features of glomeruli and tubules but chemical parameters were worsened. This may be because of difference of NS dose and the toxic agent. (22)

Hasan et al worked on acetaminophen induced nephrotoxicity resulting in glomerular shrinkage, tubular distortion and tubular dilatation and concluded that both aqueous and ethanolic extract of NS can normalize these pathological alterations. (23) This protection against drug toxicity is brought about by its antioxidative activity and prostaglandins' synthesis which improves renal perfusion by vasodilation. (24)

Concluding remarks of a recent meta-analysis stated that NS has definitive hepato-reno-protective role if the dose and duration of drug intake is optimal. (25) Bashandy et al., studied hepatotoxic effects of DOX and its protection by NS the end note cited that percentage change from oxidants to antioxidants was significantly higher in NS treated rats. (26)

Conclusion

Our study demonstrates that doxorubicin treatment significantly disrupts renal tubular and renal glomerular microarchitecture of rat's kidney. It also endorses recruitment of inflammatory cells throughout the renal interstium. NS treatment effectively relapsed these pathological effects and may act as a potential renoprotective agent against DOX toxicity.

Conflict of interest: Authors do not have any conflict of interest to declare.

Disclosure: None

Human/Animal Rights: No human or animal rights are violated during this study.

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