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RESEARCH ARTICLE
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DOXORUBICIN INDUCED NEPHROTOXICITY VIA FREE RADICAL ANTIOXIDANT ACTIVATION IN WISTAR RATS

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Abstract
Anthracycline antibiotics are very effective chemotherapeutic agents. Doxorubicin has broad spectrum of activity against solid tumors and hematologic malignant tumors. The development of nephrotoxicity and cardiomyopathy has greatly limited the use as a highly potent and efficacious anti-neoplastic agent. Adriamycin induced injury appears to be a multifactorial, but free radical generation is one of the most important denominators to most of the proposed mechanisms. The present study aimed to determine the toxic effects of doxorubicin on wistar albino rats. Toxicity study of doxorubicin was observed on LD50 dose 20 mg/kg, respectively. Single Intra-peritoneal injection of doxorubicin was given to male wistar rats which were divided into 3 groups. Group I served as control, Group II treated with doxorubicin 20mg/kg, Group III treated with α-Tocopherol 380 IU/day. The body weights, morphological behavior changes, static movement and oxidant enzymes tests were checked. Single Intra-peritoneal injection of doxorubicin induced significant ($p \leq 0.01$-0.001) pathological changes in the health, including general weakness, morphological changes associated with bleeding, change in anti-oxidant enzyme levels and inflammation of the intestine. It was found that after the intraperitoneal administration of doxorubicin the levels of glutathione peroxidase was significantly reduced along the protein oxidation. It is concluded that after the administration of α-Tocopherol the serious effects produced by doxorubicin were minimized and protective effects was seen in rats.

Keywords
Doxorubicin, Nephrotoxicity, Protein Oxidation. α-Tocopherol

Introduction

Doxorubicin affects both of the cells normal and malignant causing apoptosis. The cytotoxicity of the drug is due to its ability to generate reactive oxygen species via two different mechanism (Quiles et al., 2002; Reszka et al., 2003). Aerobic i.e. the formation of semiquinone derivative which is a ROS in nature. While other is the oxidation reduction enzyme system (De Beer et al., 2001; Basser et al., 1993). Where oxygen ($-O_2$), Hydrogen peroxide (H_2O_2) and free Hydroxyl ($-OH$) radicals are formed (Liu et al., 2003). On the other hand, doxorubicin may have the tendency to interact with ferrous ion (Fe^{+3}) to yield an iron-doxorubicin complex which will involve in a sequence of chemical oxidation-reduction reactions to produce free radicals of both Hydrogen peroxide (H_2O_2) and Hydroxyl $-OH$ (Quiles et al., 2002). These free radicals are supposed to have a damaging role by provoking cells to produce H_2O_2 radicals which react with lipids and proteins forming lipid peroxides (LPO) which abolish the plasma membranes and protein of the cell leading to several pathological and cellular changes in body parts, e.g. heart, kidneys, liver and genital organs (Kocak et al., 2003; Klimtova et al., 2002; Mostafa et al., 2000; Candussio et al., 2002; Kang et al., 2002).

α -Tocopherol is the vital antioxidant in fatty forms. Tocopherols has been traditionally used for their potent anti-inflammatory effect. In this study we have checked the effect of α -Tocopherol against doxorubicin. The current experimental study was designed to evaluate the nephrotoxicity status in rats after the administration of doxorubicin drug at a dose of 20 mg/kg. Negative effects in the morphological and anti-oxidant enzyme system were also be observed in rats.

1. Material and Methods

2.1. Drugs and Chemicals

Adriamycin was purchased from (Pfizer) Pharmaceuticals; Other solvents and chemicals were of analytical grade taken from local vendors.

2.2. Animal Care

Wistar albino 30 male rats weighing 300-400 gm were used in the experimental study. These rats were housed 5 in number per cage. Animals were taken from Dow University of Health Sciences, OJHA campus facility of laboratory animal sciences and kept under normal and constant room temperature with 12 hours light and 12 hours dark cycle with an open food and water access. Rats were acclimatized for one week prior to the commencement of the experiment. SOP's and the guidelines were set and regulated by Animal Ethical committee, (Institutional Review Board, ref no. IRB-691/DUHS/Approval/2016/172).

2.3. Experimental Design

After acclimatization, the animals were split into 3 equal groups i.e. each group contained 10 rats. Group I served as a control. Group II was treated with Doxorubicin (Dox) intraperitoneally at a dose of 20 mg/kg to induce nephrotoxicity (Sharifi-Rad et al., 2020). Group III was treated orally with α -Tocopherol 380 IU/day for 10 days before being subject to doxorubicin (Al-Sowayan et al., 2012). After completion of the study, the rats were sacrificed under Pentothal Sodium anesthesia 50mg/kg I.P injection as per their weight. The kidneys were excised. Serum was separated by centrifugation at 2000 rpm for 15min by using BHG Hermle Z230 Centrifuge machine. The Chemical Kits were used

(Diagnostica Merck, Germany) for biochemical analysis. For protein analysis kidney tissues were placed in liquefied nitrogen and also kept at -70◦C for gradual processing. All the samples were weighed and homogenized in (10% w/v) with 0.15M KCL after this, centrifuged at 10,000 rpm for 1.5h. For all assay's supernatant was used.

1.4. Catalase activity

Catalase activity was determined by spectrophotometric assay of H_2O_2 to form its stable complex with ammonium molybdate (Hadwan et al., 2016).

1.5. Protein oxidation activity

Protein oxidation was simply estimated by following method prescribed by Levine et al. with some modifications. Protein content was estimated in a homogenate, tissue supernatant and samples according to the technique mentioned elsewhere.

The protein carbonyl content was exhibited as nmole/mg protein. Lowry method using BSA as a standard was applied to determine the protein content (Hadwan et al., 2016).

2.6. Behavioral Measurements

Treated animals were observed with a naked eye during the whole experiments for their unbalanced and static movement and recorded as per animal. Percentages were taken as per total animals encountered in the experiments.

2.7. Weight Variation

The weight of both treated and control animals were taken from the start till completion of the study. The weights were used to analyze and establish the role of doxorubicin whether it reduces the weight of the rats in the course of the study.

2.8. Statistical Analysis

All the data were entered and evaluated by statistical software, IBM SPSS Statistics data editor version 21.0. One–way ANOVA has been used to evaluate the values. Percentages were calculated by measuring number of experimental demonstrated morphological alterations. 95% confidence level was statistically significant.

3. Results

3.1. Anti-oxidant Enzyme

The biochemical outcomes of kidney tissue are described in table 1. The and Catalase activity were lesser in the Doxorubicin group as compared to control groups. While control group showed the normal range of the catalase. The treated group with doxorubicin revealed the higher values of protein oxidation (PO) as compared to the control group. The catalase values were higher in α-Tocopherol treated animals along protein oxidation status which was higher in doxorubicin group as compared to α-Tocopherol.

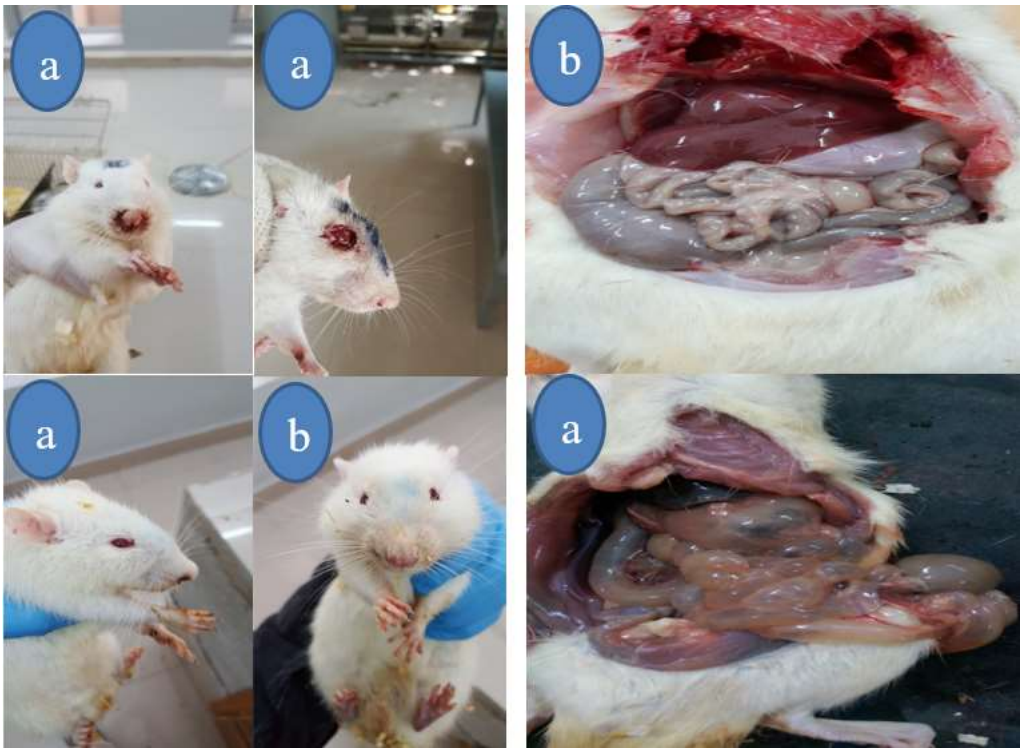
Groups	Catalase (U/mg Protein)	Protein Oxidation (nmole/mg Protein)
Control	4.50±0.44	7.61±0.34
Doxorubicin (Dox) 20 mg/kg	1.12±0.11*	24.5±8.82*
α-Tocopherol 380 IU/Day + (Dox)	5.11±1.10*	6.01±1.11*

3.2. Morphological Parameters

Table 2 showed that the toxicity of doxorubicin was which directly proportionate with the dose i.e. 20 mg/kg. Constant monitoring of rats confirmed that the treated group with doxorubicin exhibited general weakness in activities and imbalanced movements with some morphological changes. However, the treated animals with α-Tocopherol showed marked reduction in bleeding and weakness was much improved.

Morphological Parameters	% change in control	% change in Doxorubicin	% change in α-Tocopherol
Eye Bleeding	0	10	02
Nose Bleeding	0	10	04
Mouth Bleeding	0	08	02
Red Urine	0	09	0
Unbalanced Movement	0	08	02
General Weakness	0	10	0

Figure 1. A comparison between the eye and nose/mouth bleeding and inflammation of the intestinal tract in doxorubicin treated rats, but no such bleeding and inflammation in was observed in α-Tocopherol group. Note the difference in both the groups (a & b).



3.3. Weight Variation

Groups	Mean weight ± SEM Start of Experiment	Mean weight ± SEM End of Experiment
Control	352±5.2***	357.5±3.8***
Doxorubicin	324.6±2.7	271.8±4.5
α-Tocopherol	270±4.4***	269±4.7***

Table 3. showed the weight differences in rat treated with 20 mg/kg doxorubicin and α-Tocopherol group only between the beginning and end of experiments. (*): $p \leq 0.01$.

4. Discussion

The eye bleeding, nose and mouth was the utmost obvious signs in doxorubicin treated animals. This could be interrelated that doxorubicin may cause impairment in the blood system which eventually leading to thrombocytopenia. Blood platelets are a vital factor in blood clotting leading to bleeding. However, the doxorubicin is an inhibitor to bone marrow causing myelosuppression to produce blood components (Frank et al., 1991). The doxorubicin could also facilitate blood leakage through the endothelial cells of the blood capillaries leading to red nose, eyes (Windholz et al., 1976).

A rapid decrease in the mean weight of rats treated with doxorubicin as compared to control group was observed. The outcomes of the current study are in coherence with earlier researches which elaborated most chemical drugs used in treatment of cancer leading to reduction in weight and loss of appetite anorexia, constipation, dysphagia, dyspesia and gastrointestinalis (Mitchell et al., 1992; Melichar et al., 2001). The doxorubicin could crumble the epithelia of the elementary canal and inflammation of mucosa mucositis which combinedly refer to the lethal and toxic effects of doxorubicin leading to weight loss (Herman et al., 1997; Pearlman et al., 2003).

After the administration of the doxorubicin injection, the levels of catalase and protein oxidation were disturbed it confirms the result of the previous research's (Mohan et al., 2010). A significant decrease in the arithmetic means of enzyme between the control and treated rat. while doxorubicin group shown significant increase in the protein oxidation in comparison with control only.

α-Tocopherol supplementation has countered the negatives symptoms like nose bleeding, weight loss and weakness. So, it can be concluded from the above study that α-Tocopherol administration has replenish the rats and scavenges the free radicals.

It is so concluded that following the doxorubicin injection at a LD_{50} dose i.e. 20 mg/kg the deteriorated effects were produced in the kidney. These results, thus confirm the that doxorubicin generates free radical which is a main reason behind its serious toxicities. Therefore, it creates a need to supplement with a strong anti-oxidant agent which scavenges the free radical and protect the certain organs like heart, liver and kidneys. Further research is desirable to explore the molecular mechanism of doxorubicin.

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Author contributions:
Ali Asgher: Data collection, analysis and interpretation, writing the article
Sumbul Shamim: Concept and Design, Overall Responsibility
Rubina Gulzar: analysis and interpretation
Sumreen Begum: analysis and interpretation
Uzma Shakeel: statistical analysis
Nazia Ahmed: critical revision of the article
Nadia Naeem: Final review
Muhammad Arshad: Analysis

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