DOI: https://doi.org/10.53350/pjmhs22161230

ORIGINAL ARTICLE

Hepatotoxic Potential of Doxorubicin in Albino Rats

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ABSTRACT

Background: Doxorubicin is an effective chemotherapeutic drug, but its toxic potential limits its use.
Aim: To study the effects of doxorubicin on the relative tissue weight index and hepatic central vein diameter in the albino rats.
Study Design: Experimental study
Place and Duration of study: Study was conducted on 20 adult albino rats of both male and female gender in the animal house of Anatomy department, (PGMI) Lahore, Pakistan in April 2019.
Materials & Methods: Twenty, healthy adult albino rats of either sex, aging 6 to 8 weeks with 180-220 g of weight were randomly allotted to two groups A and B, each group having ten animals. Standard Rat feed and distilled water were given intraperitonially in the dose of 1.2mg/kg/body weight to the control group whereas doxorubicin was given intraperitoneally in the dose of 1.2mg/kg/body weight to the experimental group twice a week for 21 days. Body weight along with relative Tissue Weight Index were measured in gms at the end of the experiment. Diameter of central vein (μm) was assessed histologically.
Results: The mean weight of the liver decreased significantly in the experimental group. On the contrary, the relative tissue weight index in all groups were significantly different according to the One-way-ANOVA test. The increase in the diameter of the central vein in the drug administered group commenced on significant basis (p-value < 0.001)
Conclusion: Doxorubicin has toxic effect on the relative tissue weight index and hepatic central vein diameter in the albino rats
KeyWords: Doxorubicin, Liver weight, relative tissue weight index, Central vein diameter, Liver, Hepatoxicity, Albino rats

INTRODUCTION

Doxorubicin, an anthracycline compound-based antibiotic with anticancer property, is separated from the bacterium *Streptomyces peucetius*[1]. It is available in Pakistan by trade names Rubicin, Doxobin, Adrim, Doxorubicin and Doxocin in the form of injections in the dose of 10mg, 20mg and 50mg. Doxorubicin is utilized in blend with different treatment options to treat particular kinds of cancers like breast, bladder, lung, ovarian and stomach, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, along with acute lymphoblastic leukemia and acute myeloid leukemia[2,3]. It is likewise utilized alone and in amalgamation with different specific forms of thyroid cancer, soft tissue or bone sarcomas, neuroblastoma and Wilms tumor[1].

The mechanism of action of doxorubicin in the cell is by two methods: (i) DOX intercalates into DNA of cell and inhibits the topoisomerase-II-mediated DNA repair and (ii) release of free radicals which can damage cellular membrane[4,5]. The release of reactive oxygen species can lead to lipid peroxidation as well as damage to cellular membrane, mutation of DNA, genetic changes, oxidative trauma which initiates the pathway of cell death or apoptosis[5,6]. The effect of Doxorubicin is seen in histology of various organs like liver, pancreas, kidneys, heart, testes and stomach[7,8]. In liver, Doxorubicin treatment shows greater tendency of hepatic fibrosis shown by the presence of focal granulomatous lesions (cellular cell infiltrates) associated with raised levels of tissue AST, LDH and ALP exhibiting liver tissue injury[9]

The liver of rat can be divided into four lobes, which are left, middle, right and caudate. The three lobes including middle, right and caudate which divide into two sub-lobes, have one portal branch and hepatic vein whereas left lobe have two portal branches and three hepatic veins[10]. The middle lobe bears a profound indentation at the site of the attachment of the round ligament. The right lobe divides into two sub-lobes whereas the caudate lobe is further separated into two parts, the Spiegel lobe and the paracaval portion[11]. The hepatic lobes of rats are comparable with human because they have same portal and hepatic vascular system. This study was, therefore, carried out to observe the histological effects of doxorubicin on the relative tissue weight index and hepatic central vein in albino rats.

METHODOLOGY

Twenty, healthy adult albino rats of either sex, aging 6 to 8 weeks with 180-220 g of weight were purchased from Veterinary Research Institute, Lahore. The animals were randomly allotted to two groups A and B, each group having ten animals (Table 1). They were housed in iron cages, using paddy husk bedding in the animal house of Post Graduate Medical Institute (PGMI), Lahore. The rats were provided with ad libitum tap water and laboratory standard rat feed. They were allowed to acclimatize for 1 week before the commencement of the experiment. All practices were performed painlessly, in a clean environment, as per the rules for the human utilization of lab animals, recognized at Postgraduate Medical Institute, Lahore, and after obtaining ethical permission. Body weight along with Relative Tissue Weight Index were measured in gms with the help of electronic balance. Diameter of central vein (μm) was assessed histologically. This parameter was measured with the help of ocular micrometer.

Table 1 Showing details of the Animal Groups, Route of administration, Experimental Intervention and dosage

Groups	Labelling of groups	No. of rats in each group (n)	Specifications
A	Control	10	Standard Rat feed and distilled water given intraperitonially in the dose of 1.2mg/kg/body weight twice a week for 21 days.
B	Experimental	10	Doxorubicin given intraperitoneally in the dose of 1.2mg/kg /body weight twice a week for 21 days (12).

RESULTS

The weight of the animals was measured at the beginning as well as at the culmination of the experiment. To have the statistical comparison of the initial and final body weights, One-way-ANOVA test was employed. It revealed that the average body weight of animals was not significantly dissimilar among groups at the start of the experiment, while on the contrary, there was a statistically substantial variance in average weight of the body among groups

just as before the finish of the experimentation (p-value = 0.858 and 0.048 respectively) (Figure 1).

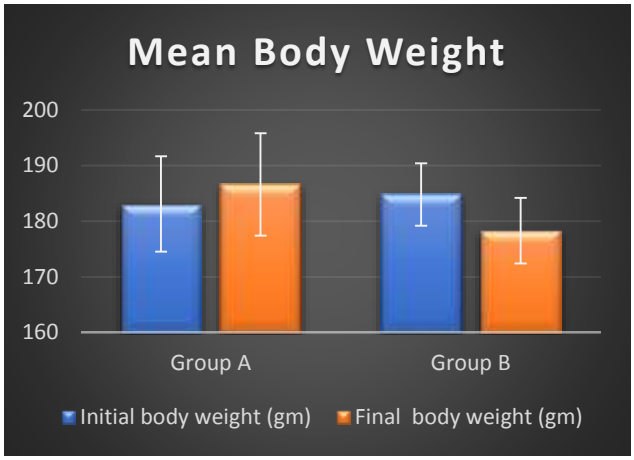

Figure 1 Comparison of the initial body weight and the final body weight among groups. Error bars showing standard deviation.

The average weight of liver and relative tissue weight index in all groups was observed and analyzed. The mean liver weight along with the relative tissue weight index in all groups were significantly different according to the One-way-ANOVA (p-value < 0.001; p-value < 0.001 respectively) (Table. 2)

Table 2: Comparison of paired kidney weight and relative tissue weight index among groups:

Parameters	Group A	Group B	p-value#
Liver weight (gm)	5.2 ± 0.6	7.4 ± 1.2	< 0.001*
Relative tissue weight index	2.86 ± 0.31	4.15 ± 0.65	< 0.001*

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

The mean diameter of central vein in all groups was measured. One way ANOVA was applied and analyzed in order to compare the diameter of central vein amongst groups. The diameter of central vein on average scale was uncovered to be significantly different in all groups with the p-value < 0.001. (Figure 2)

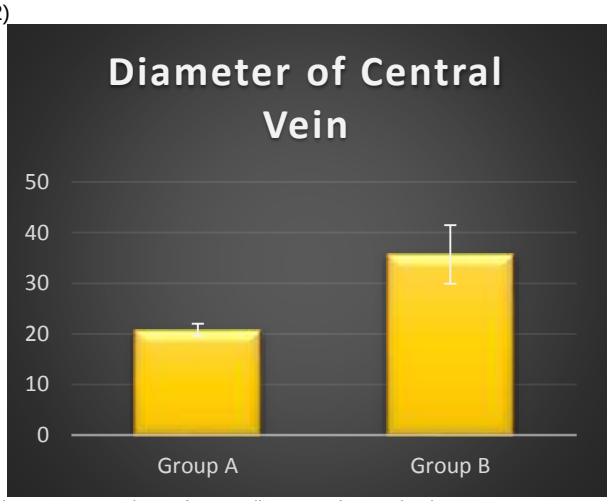

Figure 2 Comparison of mean diameter of central vein among groups. Error bars showing standard deviation

DISCUSSION

Incidence of cancer is increasing gradually as a result of multiple etiologies[13]. The cancer treatment modalities are surgery, radiotherapy, and systemic treatment including chemotherapy and immunotherapy[14]. Doxorubicin is an anthracycline (McGowen et al.,2017) which is used in the treatment of soft tissue carcinomas, bone sarcomas, thyroid cancer, carcinomas of ovary, urinary bladder, breast cancer, leukemias on acute basis like acute myeloblastic leukemia, acute lymphoblastic leukemia and Hodgkin's and Non-Hodgkin's lymphoma and small cell lung cancer[15].

Comparison of the mean body weight of animals with a statistically significant difference in the mean body weight among groups at the end of the experiment. This difference can be related to the mean weight in group B, which at the end of the experiment being significantly lower as compared to shown by group A. Comparable findings were documented by El-Sayed, Mansour[16] that showed that the mean weight of Doxorubicin-treated rats was significantly less in comparison with the control. Weight reduction might be due to oxidative stress and inflammatory response caused by DOX[16]. These unsettling influences of decrease in liver weight may be ascribed to the generation of oxidative radicals, damage to DNA and its repair, apoptosis, local tissue necrosis, dysfunction of mitochondria and subsequent halting of cycle of hepatic cell division[7,16]. It might very well be because of toxicity of gastrointestinal tract with subsequent decrease in hunger along with decrease in the utilization and absorption of food. Chemotherapy`s direct effect on tubular structure of kidneys causes decline in reabsorption of water and more sodium loss in the tubular fluid following polyuria, dehydration and slump in body weight[4].

Results were similar to those published by Favreau-Lessard, Blaszyk[17] and Timm, Perera[8].

The mean liver weight and the relative tissue weight index in all groups when observed and compared in this study revealed substantial difference in all groups.

It was concluded that the relative tissue weight index of liver in group B was significantly higher in comparison with the group A. Parallel findings were given in the experimental work by Yao, Wu[18], Aikemu, Amat[19], Afsar, Razak[20] concluding the damage of liver manifested by increase of liver weight index. Contrary to this, a study was done to observe the protective consequences of Ber on endurance and toxicity in general by DOX generated acute toxicity in rats. There was analysis that DOX administration caused the reduction of body weight in comparison with the control group and there was no significant difference in the kidney and liver indexes amongst the experimental groups[21]. The increase in liver weight index of doxorubicin-given group has comparable findings with the control group when antiproliferative activity of Acacia hydaspica R. was studied[4].

The mechanism by which DOX has caused hepatotoxicity might be due to generation of reactive oxygen species, hampering of enzymatic activity of topoisomerase II which catalyzes a transient breakage and reunion of double-stranded DNA, together with raise in the levels of superoxide, catalase and glutathione peroxides enzyme in liver tissue which are part of antioxidant enzyme system. In addition, the decline in apoptotic responses, extrinsic and intrinsic, mediated by Fas and Bax are related with damage to the liver[6].

The mean of diameter of the central vein in all groups was calculated and the diameter of central vein among groups were compared. It was found that the mean diameter of central vein in all groups were significantly different with p-value < 0.001, representing that the diameter of central vein of experimental group B was significantly higher when compared with group A. The DOX induced decrease in hepatocyte division and necrosis of lobular hepatocytes around central vein. The subsequent fibrosis contracted the parenchyma around the central lobule leading to the increase in its diameter[4,9]

Same findings were found in studies conducted by Chen, Zhang[21]. Unusual consequences of resveratrol on the dose dependent doxorubicin generated hepatotoxicity along with cardiotoxicity was studied by Al-Saleem, Jumaa[22] and Podyacheva, Kushnareva[23] which showed fibers of dense

connective tissue around the central vein which causes increase in the diameter of the central vein.

CONCLUSION

From the foregoing results, it is clear that doxorubicin`s toxic potential deteriorates the relative tissue weight index of the liver and increases the diameter of the central hepatic lobule.

Acknowledgements: I'm really grateful to the staff of Anatomy Department of Postgraduate Medical Institute, Lahore for all their support and facilitation.

Conflict of Interests: The authors declare that they have no conflict of interests.

Financial Disclosure: None

Author's Contribution: AS: Principal investigator, topic selection, Data analysis and interpretation**. MBB:** Co-investigator, drafting the manuscript, statistical analysis. **AMK:** Co-investigator, revision of manuscript for important intellectual content**. FFB:** Co-investigator, revision of manuscript for important intellectual content**. MN:** Co-investigator, drafting the manuscript. **MMA:** Co-investigator, drafting the manuscript.

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