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histopathological effects of doxorubicin on liver and kidney of wistar albino rats

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ABSTRACT

Introduction: - Ideal anticancer drugs should eradicate cancer cells without harming normal tissues. Unfortunately, no currently available agents meet these criteria, and clinical use of these drugs involves a weighing of their benefits against their toxicity in search for a favorable therapeutic index.

Methods: - experimental animals were divided into 4 groups, control, low dose, therapeutic and high dose respectively. After giving respective doses and sacrificing the animals organs were taken and observed grossly and on under light microscopy.

Result: - microscopic changes were observed in all groups except control group. Low dose group showed less change while therapeutic group showed most of the changes which were observed by other authors. High dose group showed marked toxic changes in organs like hemorrhages and congestion.

Conclusion: - The present study showed that the toxicity pattern is almost same in low and therapeutic doses of doxorubicin, and this could be of immense value while treating the carcinoma patients while in high doses it cause severe toxicity on liver and kidneys.

KEYWORDS :

Introduction: Cancer is basically a disease of cells characterized by a shift in the control mechanisms that govern cell proliferation and differentiation. The problem of cancer is universal. In developed countries, cancer is ranked as the second commonest cause of death, while in developing countries; it is third next to infectious and cardiovascular diseases. Doxorubicin (DXR), also known as Adriamycin, an anthracycline compound, is one of such natural products. DXR was originally isolated from a colony of streptomyces in 1957 and was shown to have significant activity in patients with acute myeloid leukemia.1

Materials and methods: Albino rats (wistar strain) of either sex, weighing 125-160 grams were used for current experimental studies. They were procured from animal house, Government Medical College Jammu. The clearance for the use of animals for experimental purpose was obtained from Animal Institutional Ethical Committee constituted for the before purpose. Animals were housed in polypropylene cages (6/cage) with dust free rice husk as bedding material under laboratory conditions with controlled environment of temperature of 25±2℃ , humidity (16% ± 10%) and 12 hours light/dark cycle (16- 18) as per Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Indian guidelines. They were provided standard rodent chaw/feed and water ad-libitum. Subjecting them for experimentation, animals were given a week's time to acclimatize with laboratory conditions. Animals were fasted for 24 hours before experimentation.

The principal of Rationalization, Refinement and Reduction (3 "R's") was strictly followed while undertaking the following experiment.

Experimental Design: The animals were divided into 4 groups with each group consisting of 6 animals.

GROUP 1was administered weekly intraperitoneal injections of 3ml of sterile distilled water and it served as a healthy control.

GROUP 2 was administered a weekly low dose of doxorubicin (0.2 mg/kg b.w) intraperitoneal injection.

GROUP 3 was given a weekly therapeutic dose of doxorubicin (1mg /kg b.w) intraperitoneal injection.

GROUP 4 received a single intraperitoneal toxic dose of doxorubicin (20mg/kg b.w).

The above dosing schedules were adapted after going through studies conducted by **El-Sayyed H *et al.*[2], Sule A *et al.*[3] & Shivakumar P *et al.*[4]**

The animals were sacrificed after 48 hours of the administration of final dose of drugs as per the prescribed methods by CPCSEA.

After sacrificing the animals liver and kidneys were isolated and fixed in 10% buffered formalin solution and processed to prepare 5 micron thick paraffin sections. Paraffin sections were stained by using H&E/other relevant staining. Histological sections were examined by light microscopy to assess the degree of toxicity. The histopathological findings among the 4 groups were compared to achieve relevant conclusions.

Result: In control group NO 1:- no gross and microscopic changes were observed

In the *LOW DOSE GROUP* NO 2:- There were no gross changes present in any organ under study. Microscopically, LIVER showed mild hyperemia with the presence of lymphocytes with sinusoidal dilatation within hepatic cords (Fig. 1). In KIDNEY there was glomerular hyperaemia with focal areas of haemorrhage (Fig. 2).

In the *THERAPEUTIC DOSE GROUP* NO 3:- No gross change was seen in any of the organs under study. Histologically in the LIVER tissue, parenchymal extravasation of red blood cells was seen, hepatic sinusoids were markedly dilated, vascular congestion in portal areas was seen(Fig. 3). Inflammatory cells were present with the abnormal pattern of hepatic cords in the form of sinusoidal dilatation. In KIDNEY tubular necrosis was present with sloughing of tubular epithelium, Bowman's space was dilated while interstitial haemorrhage was also noticed in some areas(Fig. 4).

Fig 1: liver showing central vein congestion(CVC)(low dose 100X) .

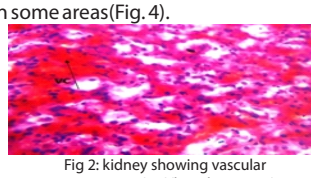

Fig 2: kidney showing vascular Congestion(VC)(low dose 400X).

In DOSE *the HIGH GROUP NO* 3:-No gross changes were detected in any of the organs under study except black discoloration of liver tissue. Under light microscopy, LIVER exhibited severe necrosis in the portal areas, hepatic sinusoids were markedly dilated(Fig. 5). Congestion and vasodilatation of central veins were also seen. In addition extensive areas of haemorrhages were also sighted with derangement of normal hepatic cord pattern.In KIDNEY, tubular necrosis was seen with sloughing of epithelium in the form of feathery degeneration. Interstitial haemorrhage was observed with congested cortical vessels. Widening of Bowman's space along with atrophy of glomeruli was seen (Fig.s 6).Tubular casts were also seen.

Fig 5: liver showing diffuse sinusoidal dilation (high dose 400X)

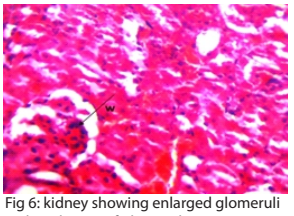

Fig 6: kidney showing enlarged glomeruli with widening of glomerular space (W)andcongestion of convoluted tubules(high dose 400x)

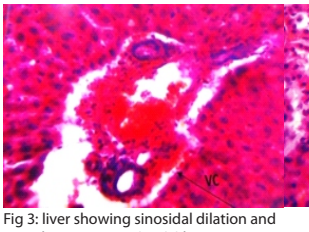

Fig 3: liver showing sinosidal dilation and vascular congestion(VC) (therapeutic dose 400X).

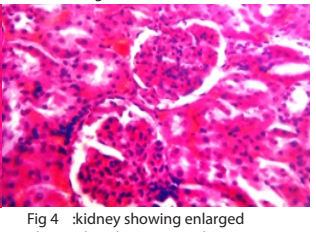

Fig 4 :kidney showing enlarged glomeruli with prominent bowman space(therapeutic dose 400X).

Discussion : The purpose of the present study was to evaluate at our best the toxicity profile of the drug doxorubicin histologically in Wistar albino rats in order to suggest whether to modify the present therapeutic dosage of this drug for avoiding its toxicities on major organs like liver and kidney. In the control group which was given normal saline only, no gross or microscopical changes were seen in all organs under study. In the low dose group, no gross changes were seen in any of the organs. Microscopically, in the liver there was hyperemia with focal inflammation. There was increased number of inflammatory cells in the form of numerous leucocytes in the parenchyma with predominant lymphocytic infiltrations. There was mild widening of the sinusoidal capillaries. These changes were in contrast with the observations by **El-Sayyed H *et al.*,[2]** in which doxorubicin in the same dose (0.2 mg/kg i/p) showed massive hepatotoxicity including dissolution of hepatic cords, focal inflammation and necrotic tissues. This could be probably because of the schedule of drug administration which was on alternate days for total period of 20 days. In their study, **Sundaram MK & Sangavai [5]** showed that the doxorubicin at both the low and therapeutic dose caused same features of focally collected cellular granulomatous lesions and this is in accordance with our present study.In the therapeutic dose group there was focal inflammation and necrosis with parenchymal extravasation of red blood cells. There was moderate dissolution and degeneration of hepatic cords with mild fusion of hepatocytes. Moderate dilatation of hepatic sinusoids was seen. However in a study done by **Raskovic A *et al.*, [6]** in which the doxorubicin was given in the same therapeutic dose, there was hyperemia without any other changes as observed in our study. **Taher MT *et al.*, [7]** showed that doxorubicin caused marked changes in rat liver, which occur even in low doses of 0.2 mg/kg in the form of vacuolation of the hepatocytes with widening of the sinusoidal capillaries, in addition to congestion and vasodilatation of the central veins. Microscopic observation in the current study was in concurrence with it. At therapeutic dose of 1 mg/kg body weight there was cloudy swelling, loss of striations and multifocal hemorrhage in cardiac tissue. Moderate hypertrophy of myocardium was seen with extensive areas of hemorrhage. These findings were quite similar to the study done by **Mettler FP *et al.*, [8]**.

The kidney tissue, there was no gross change at both low and therapeutic doses. On light microscopy, at low dose, there was glomerular hyperemia with mild focal glomerular necrosis. Perivascular leucocytic aggregation was also seen. There was mild medullary fibroblastic proliferation with slight glomerular basement membrane thickening and moderate dilatation of capillaries and urinary spaces. Kidneys showed no gross changes at high doses of doxorubicin but in liver dark discoloration was seen in some cases. Under light microscopy, liver revealed infiltration of inflammatory cells among hepatocytes with focal clusters of necrotic hepatocytes. Many hepatic sinusoids were dilated with marked congestion and vasodilatation of the central venules. These observations are in agreement with most of the studies conducted as stated by Rasha AR and **Abdella EM [9], Saber AS and Samah MA[10]** . On light microscopy the kidney tissue revealed atrophy of glomeruli with tubular necrosis. Variability of shape and size of tubular epithelia was seen in the lumen. There was congestion of cortical vessels. Marked interstitial hemorrhages were also seen along with tubular casts. These observations were in accordance with most of the authors including **Rasha AR and Abdella EM [9], Azza AK *et al.*,[11] Syed JH *et al.*,[12]**

Summary: The present study was done for the purpose of studying the histopathological effects of doxorubicin on liver and kidneys of wistar albino rats, so that appropriate dose adjustments and combination chemotherapy regimens can be suggested for cancer chemotherapy. It is therefore concluded from the present study that the toxicity pattern is almost same in low and therapeutic doses of doxorubicin, and this could be of immense value while treating the carcinoma patients while in high doses it cause severe toxicity on liver and kidneys.

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