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Research Article

Effect of doxorubicin on Male Albino Rats' heart: Biochemical and Histological study

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Abstract

Background: Drug-induced cardiotoxicity especially the anticancer drugs can be a major cause for limitations of these drugs therapeutic usage. Doxorubicin is one of the most important chemotherapy, it treats many types of cancers but it has toxic effects on heart tissue as it causes oxidative stress, apoptosis and inflammation and this limits the therapeutic usage of doxorubicin as an anticancer drug. **Aim of the work:** To study the effect of doxorubicin on Albino Rats' heart. **Methods:** Thirty male albino rats were divided equally into two groups: C-group (control group) and B-group (doxorubicin exposure group). Measurement of malondialdehyde (MDA) in the heart tissue, an oxidative stress marker, and histological approaches were done. **Results:** B-group showed significant increase in MDA levels in cardiomyocytes compared to control group and had degenerative changes in the heart tissue. **Conclusion:** Doxorubicin had an apparent damaging effect on cardiac tissue structure by different mechanisms as oxidative stress, apoptosis and inflammation

Keywords: Cardiotoxicity, DOX, MDA.

Introduction

Drug-induced cardiotoxicity especially the anticancer drugs can be a major cause for limitations of these drugs therapeutic usage [1].

Although doxorubicin (DOX) is among the most potent anticancer medications, its therapeutic value is severely constrained by acute and chronic toxic side effects it causes to different organs [2], the toxic effects of doxorubicin affect many organs in the body mainly heart, kidney and liver[3].

Doxorubicin-induced cardiotoxicity is one of the major side effects of doxorubicin and can occur by several mechanisms including oxidative stress, free radical generation by inducing mitochondrial damage in cardiomyocytes [4]. Also, doxorubicin causes apoptosis and inflammation in cardiomyocytes [5].

Material and Methods

- **Animals:**

This study was conducted in Histology and Cell Biology Department of Minia University's Faculty of Medicine. Minia University's department of agriculture procured the rats from the animal house of the growth centre. The experiment included thirty adult male albino rats. Rats were 8-12 weeks old and their weights were approximately 150-180 gm.

All rats were pathogenically free. Rats had unrestricted access to food and drink in sterilised plastic cages and were given a regular laboratory diet. A clean, well-ventilated space was provided for them. The rats were kept in a controlled environment with a 12-hour light/12-hour dark cycle, with temperatures ranging from 24 to 30 degrees Celsius. Before the trial, the animals were acclimated for two

weeks. The ethics committee of Minia University's Faculty of Medicine ensured that the experiment adhered to all applicable regulations.

- **Experimental design:**

The animals were randomly divided into 2 groups (n=15 per group) as the following:
Control group (C-group):
Rats received single dose of 10mg/kg saline intraperitoneally.
Doxorubicin-group (B-group):
Rats received single dose of 10mg/kg doxorubicin intraperitoneally**[6]**.

- **Animal sacrifice & tissue collection:**

The experiment concluded with the decapitation of rats administered under mild halothane anaesthesia.

It didn't take long to extract the rats' hearts. The left ventricles were quickly fixed in a 10% buffered formalin solution for one day after dissection and rinsed with normal saline. They were then cleaned with tap water and processed to create paraffin slices for the histological investigation. For the biochemical analysis, we extracted samples from the left ventricles, which we then preserved at -80°C until we needed them to prepare the tissue homogenates.

Methods:

Biochemical study:

Biochemical analysis was done at the Pharmacology Department, Faculty of Medicine, Minia University. according to the manufacturer's instructions**.**

***Measurement of malondialdehyde (MDA):**
MDA is a marker for oxidative stress [7, 8] , tissue samples were obtained and clorometric assay kits were used to measure the tissue levels of MDA [9]**.**

Histological study:

1) For light microscopic examination:

a) The Paraffin Technique[10]:

1- After a day of room temperature fixation in 10% neutral-buffered formalin, the muscle specimens were removed.
2- The samples underwent dehydration in a graded alcohol series after correct fixation.
3- Submerged in paraffin wax and cleaned with xylene.
4- Then slice using a microtome.
To facilitate further staining, slices measuring 5– 5 μm were adhered to glass slides.

b) Hematoxylin and Eosin (H&E) staining[10]
Technique:

1- In order to confirm histological alterations, we stained some sections that were placed on deparaffinized glass slides with H&E.
2- After removing the wax, the sections were stained with hematoxylin for 7 minutes and then thoroughly rinsed under running water.
3- The next step was to soak the parts in eosin for three minutes. Any excess stain was then rinsed out with water.
4- Different grades of alcohol were used to dry the parts.
5- Xylene flushed it out.
6- The specimens were thereafter placed on glass slides and covered to allow light microscopy to examine them for the purpose of the general histological analysis research.
Result: The cytoplasm appeared red to pink while the nuclei took a blue color.

Results

1) Laboratory findings

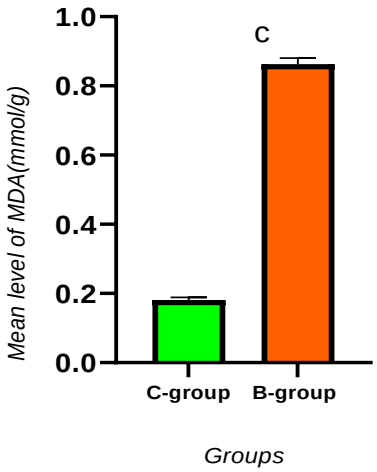
- **Malondialdehyde (MDA) levels in cardiac tissues (mmol/g):**

B-group showed a significant increase in the MDA levels compared to C-group (P<0.0001).

Table 1: The mean levels of heart tissue MDA (nmol/g) in the studied groups:

Groups	Mean±SEM	P-value
C- group	0.1816± 0.00323	
B-group	0.00772 ± 0.8632	0.0001 $^{C*}<$

SEM: standard error of mean, ^{C}: vs C-group; *: significant at p<0.05.



Bar chart 1: The mean tissue levels of MDA (mmol/g) in studied groups (n=8)
(Significant: c vs control group).

2) Histological Results

- **Hematoxylin and Eosin results:**

➢ **C-group:**

Longitudinal section of rat's left ventricle showed closely packed sheets of cardiac muscle fibers with normal histological structure in the form of branching acidophilic striated fibers with oval vesicular nuclei located centrally. Fibers were joined together by intercalated discs. Elongated nuclei of fibroblasts were observed in the inter fiber spaces (Figure 1).

➢ **B-group:**

Longitudinal section in rat's left ventricle mostly revealed disturbed normal structure of cardiomyocytes in the form of degenerated cardiac muscle fibers with pale acidophilic cytoplasm and others showed strong acidophilic homogenous cytoplasm with pyknotic deeply stained nuclei. Fragmented muscle fibers were also observed (Figure 2).

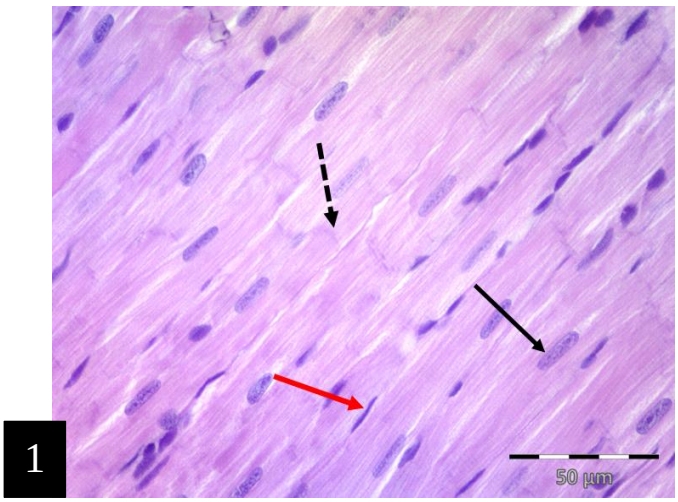


Figure 1: A representative photomicrograph of longitudinal section from rat's left ventricle from the control group showing: branched cardiac muscle fibers with striated acidophilic cytoplasm and central oval vesicular nuclei (black arrow) connected by intercalated disc (dotted arrow). Notice the elongated darker nuclei of fibroblasts (red arrow) in the inter fiber spaces.
H&E; x 400, scale bar=50 µm.

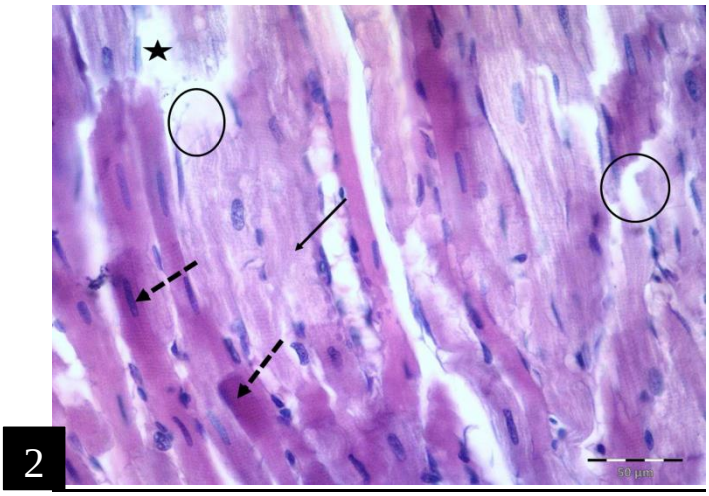


Figure 2: A representative photomicrograph of longitudinal section in rat's left ventricle from the Dx group showing: degenerated cardiac muscle fibers (circles) with pale acidophilic cytoplasm (thin black arrow), other fibers with hypereosinophilic homogenous cytoplasm and pyknotic deeply stained nuclei nuclei (dotted arrows), Notice fragmented muscle fibers (star).
H&E; x400, scale bar= 50µm

Discussion

Doxorubicin is the most widely used anthracycline, many cancers are treated with it as acute leukaemia, lymphomas, ovarian, testicular, lung, thyroid, breast cancer and many others [11].

The toxicity of doxorubicin to many body organs ,especially the heart, restricts its therapeutic usage despite its efficacy in cancer treatement[12].

Oxidative stress, apoptosis, inflammation, and mitochondrial dysfunction are widely believed to be the common mechanisms of the cardiac toxicity caused by doxorubicin[13].

Regarding the oxidative stress, the current study analyzed the tissue level of Malondialdehyde (MDA) which is a marker for oxidative stress[14]. This analysis in our study showed a significant increase in the mean value of MDA in the B-group compared to the C- group, these results were consistent with findings of [8] who demonstrated that doxorubicin could cause an increase in the level of MDA in cardiomyocytes.

This was explained by[15] as proposed that doxorubicin caused oxidative stress in cardiomyocytes through decreasing their endogenous antioxidants as GSH and producing free radicals that contributed to lipid peroxidation of cellular membranes of cardiomyocytes. The ability of doxorubicin to generate free radicals was revealed by[16] as reported that doxorubicin had strong affinity to cardiolipin in the inner mitochondrial membrane so could disrupt the electron transport chain of mitochondria producing an increase in reactive oxygen species (ROS).

In addition, it was proposed by[17] that doxorubicin enhanced the activity of nitric oxide synthase and NADPH oxidase which are important ROS sources.

Conclusion

From this study we can conclude that, the doxorubicin chemotherapy could induce cardiotoxicity by several mechanisms mostly by inducing oxidative stress.

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