CONFERENCE PAPER

AN ASSESSMENT OF CARDIAC HISTOPATHOLOGICAL CHANGES IN DOXORUBICIN DOSE-DEPENDENT ANIMAL MODELS

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ABSTRACT

One effective anthracycline human chemotherapy drug that is frequently used to treat solid and haematological cancers is doxorubicin (DOX). The dose-dependent cardiotoxicity of some medications can result in irreversible heart failure, limiting their clinical utility. Understanding the pathophysiology and early detection of DOX-induced cardiac injury is made possible by animal models, especially rats, using acute models of DOX cardiotoxicity due to less time-consuming operations. The aim of this research is to determine a potential cardiotoxic DOX dose in gender-specific Wistar wild-type rats using light microscopy for evaluating morphological changes of the heart.

Adult Wistar rats (n=10), including males (n=5) and females (n=5), were treated with doxorubicin intraperitoneal injection in different doses (25 mg/kg, 30 mg/kg and 40 mg/kg) per male rat and female rat, respectively. Rats were sacrificed after 48 hours and 72 hours for the models of 25 mg/kg and 30 mg/kg, while the rats of the 40 mg/kg model group were sacrificed 24 hours after. The myocardium of the left ventricle is analysed using a light microscope under magnifications of ten and twenty times.

Male Wistar rats developed more pronounced morphological changes of the left ventricle compared to female Wistar rats, resulting in myocardial interstitial oedema and disorganisation of myocyte architecture.

Male Wistar wild-type rats develop a more aggressive form of acute cardiotoxicity caused by doxorubicin compared to female Wistar wild-type rats.

Keywords: Cardiotoxicity, doxorubicin, myocyte injury

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INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic derived from *Streptomyces peucetius*, has been widely used in oncology due to its broad-spectrum activity against various solid tumours and hematologic malignancies (Belger et al., 2023). Despite its efficacy, its clinical use is severely limited by a well-documented risk of cardiotoxicity, which can manifest acutely or chronically. Acute cardiotoxicity may present as arrhythmias or transient myocardial dysfunction within days of administration, while chronic effects may develop months to years later, potentially progressing to irreversible heart failure (Chatterjee et al, 2010). The mechanism of DOX-induced cardiotoxicity is multifactorial and includes the generation

of reactive oxygen species (ROS), disruption of mitochondrial function, interference with topoisomerase-II beta in cardiomyocytes, and apoptosis. The heart is particularly vulnerable to oxidative damage due to its relatively low levels of endogenous antioxidant enzymes compared to other organs (Rawat et al., 2021). In recent years, increasing attention has been given to sex-based differences in drug response and toxicity. Hormonal, genetic, and molecular differences between males and females can significantly influence the pharmacokinetics and pharmacodynamics of therapeutic agents. Yet, preclinical studies often neglect to analyse male and female responses separately. Understanding these differences in the context of DOX cardiotoxicity is crucial for developing targeted cardioprotective strategies and improving patient outcomes (Belger et al., 2023; Rawat et al., 2021). This study focuses on the early morphological effects of DOX-induced cardiotoxicity using an acute rat model. We hypothesise that male and female Wistar rats exhibit differing degrees of myocardial damage following DOX administration, potentially due to inherent biological differences. This work contributes to the growing body of literature emphasising the importance of sex as a biological variable in toxicological research.

MATERIALS AND METHODS

Animal Selection and Ethical Approval

Ten healthy adult Wistar rats comprising five males and five females, aged 8 to 10 weeks and weighing between 220 and 300 grams, were procured from an accredited animal facility. Animals were acclimatised for one week under controlled environmental conditions (temperature 22 ± 2°C, humidity 55 ± 10%, 12-hour light/dark cycle). All experimental protocols were approved by the Institutional Animal Care and Use Committee and conducted in compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Doxorubicin Administration

Rats were randomly divided into three experimental groups based on the dose of DOX administered: 25 mg/kg, 30 mg/kg, and 40 mg/kg. DOX was diluted in sterile saline and administered as a single intraperitoneal injection. The time points for sacrifice were set as follows: 72 hours post-injection for 25 mg/kg, 48 hours and 72 hours for 30 mg/kg, and 24 hours for 40 mg/kg. These intervals were selected based on preliminary studies and literature indicating the onset of acute cardiotoxicity within these windows.

Tissue Collection

At the respective time points, rats were anesthetized using a combination of ketamine (80 mg/kg) via intraperitoneal injection. Once deep anesthesia was confirmed, rats were euthanised by cervical dislocation. Hearts were immediately excised, rinsed in cold phosphate-buffered saline (PBS), and fixed in 10% neutral-buffered formalin for 72 hours.

Histological Processing and Staining

Fixed heart tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome and mounted on glass slides. Hematoxylin and eosin (H&E) staining was performed for general histological evaluation. Sections were examined using a light microscope at magnifications of 10× and 20×.

Morphological Assessment

Histological analysis focused on identifying specific features of myocardial injury, including interstitial oedema, cytoplasmic vacuolization, myofibrillar disarray, nuclear morphology, necrosis, and inflammatory cell infiltration. Each parameter was semi-qualitatively scored with a value of 1 (yes) and 0 (no) and then summed up to a mean value by two independent pathology researchers. Average scores for each rat were calculated and compared between sexes and across doses.

RESULTS

Overview of Morphological Findings

Histological examination revealed dose-dependent myocardial damage in all experimental groups. The extent and severity of damage were more pronounced in male rats across all doses. Common alterations observed included myocardial fibre disorganisation, interstitial oedema, cytoplasmic fragmentation, and focal necrosis.

25 mg/kg Group (48 hours and 72 hours)

At the lowest dose, male rats exhibited mild interstitial oedema and occasional vacuolization of cardiomyocytes with partial change in morphology of nuclei after both periods (Figure 1). In contrast, female rats showed minimal structural disruption, with mostly preserved myofibrillar integrity. No significant inflammatory infiltration, haemorrhage or necrosis was noted in either sex (Figure 2).

30 mg/kg Group (48 hours and 72 hours)

Histological changes became more pronounced at this intermediate dose, but morphological changes remained the same in both periods, i.e. after 48 hours and 72 hours. Male rats demonstrated highly expressed severe interstitial oedema, moderate myocardial disorganisation, cytoplasmic vacuolization with hyperemia (Figure 3). Female rats exhibited mild to moderate interstitial oedema, mild myocardial disorganisation and mild cytoplasmic vacuolization with hyperemia.

40 mg/kg Group (24 hours)

Rats exposed to the highest dose showed severe cardiac pathology. In males, widespread necrosis, interstitial oedema with haemorrhage, and inflammatory cell infiltration were evident, together with changes in nuclear morphology. Myocardial fibres were disorganised and fragmented (Figure 4). Female rats also showed signs of damage, much less interstitial oedema, but with lesser severity of myocyte disorganisation and with mild focal necrosis changes (Figure 5). Nuclear morphology for both sexes in this group was changed.

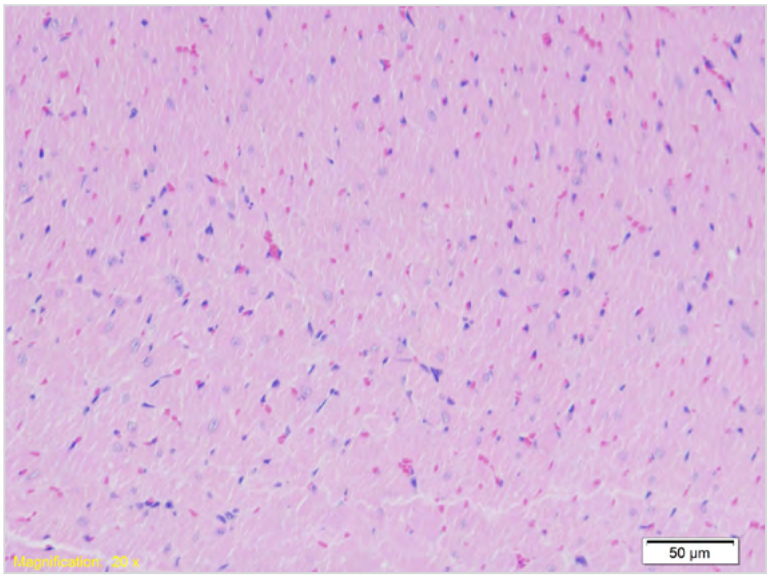

Figure 1 Representative photomicrograph of a male rat in the 25 mg/kg group

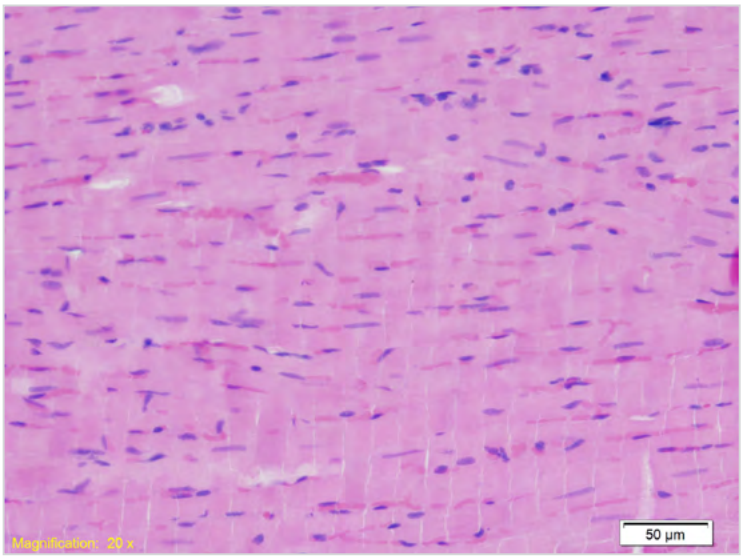

Figure 2 Representative photomicrograph of a female rat in the 25 mg/kg group

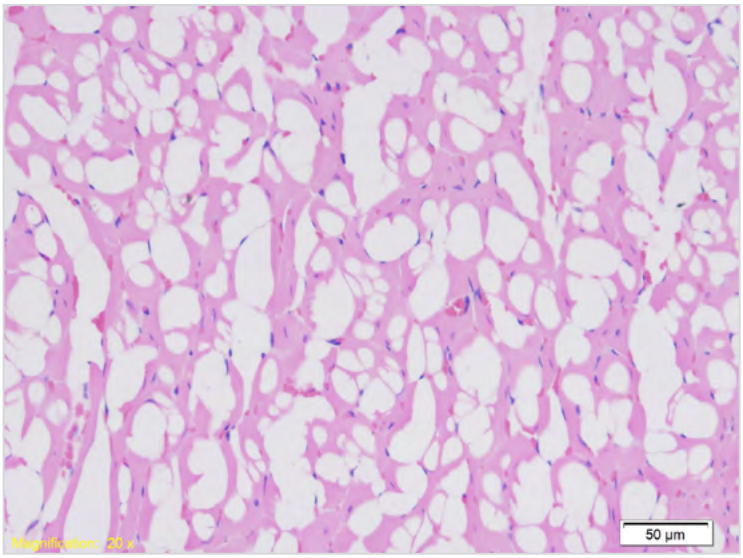

Figure 3 Representative photomicrograph of a male rat in the 30 mg/kg group

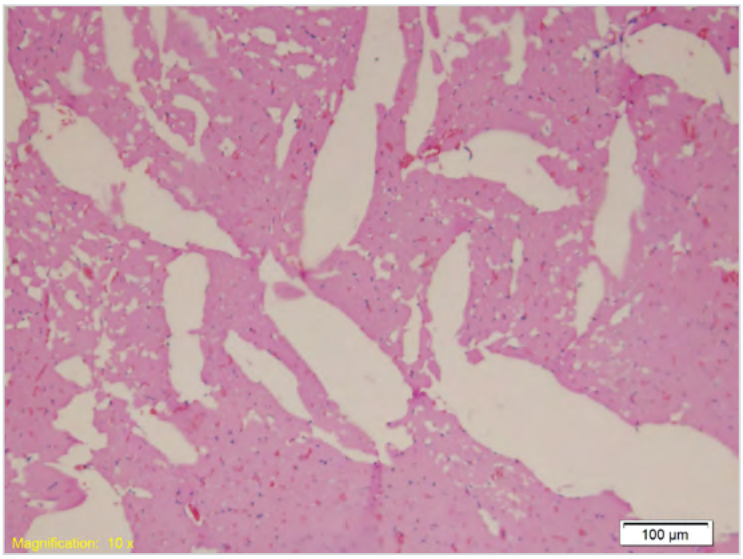

Figure 4 Representative photomicrograph of a male rat in the 40 mg/kg group

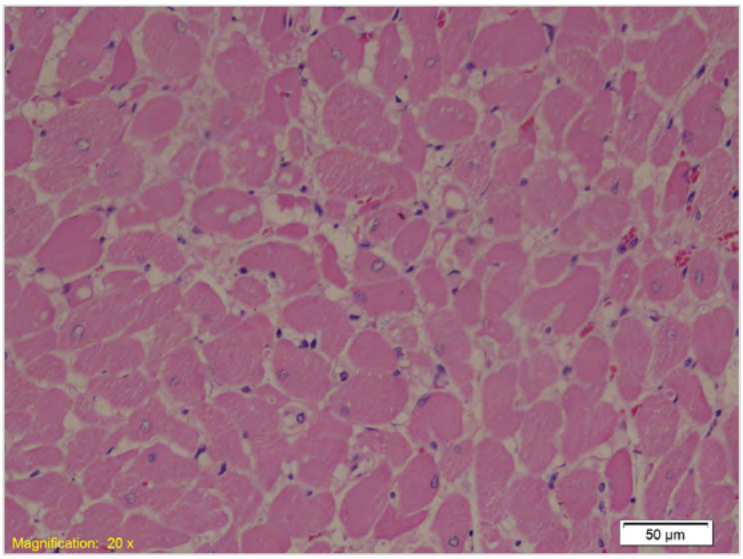

Figure 5 Representative photomicrograph of a female rat in the 40 mg/kg group

DISCUSSION AND CONCLUSION

Our study reveals clear sex-based differences in the morphological manifestations of acute DOX-induced cardiotoxicity in Wistar rats. Male rats consistently displayed more severe myocardial damage than their female counterparts, particularly at higher DOX doses. These findings suggest that biological sex may be a critical factor in modulating the heart's response to chemotherapeutic injury. One plausible explanation lies in hormonal influences. Estrogen has been reported to exert cardioprotective effects through several mechanisms, including enhancement of mitochondrial function, reduction of oxidative stress, and upregulation of anti-apoptotic pathways. Male rats, with lower circulating estrogen levels, may thus be more vulnerable to the mitochondrial and oxidative damage induced by DOX (Rattanasopa et al., 2019). Several mechanisms have been implicated in DOX-induced cardiotoxicity, with oxidative stress, ROS generation, and apoptosis being the most extensively documented. Beyond these primary pathways, additional contributing factors include mitochondrial dysfunction, dysregulation of iron homeostasis, disruption of intracellular Ca^{2+} balance, impaired autophagy, enhanced nitric oxide production, activation of inflammatory mediators, and altered expression of genes and proteins involved in apoptotic signalling (Chatterjee et al., 2010; Osataphan et al., 2020). DOX has also been shown to suppress DNA methyltransferase 1 (DNMT1) activity, resulting in reduced global DNA methylation. This epigenetic alteration is associated with dysregulation of mitochondrial regulatory genes such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1), and mitochondrial transcription factor A (TFAM) in cardiac tissue. Furthermore, Dox exposure modulates microRNA expression profiles and perturbs histone deacetylase (HDAC) activity, further contributing to its cardiotoxic effects (Rawat et al., 2021; Rattanasopa et al., 2019 Osataphan et al., 2020). Additionally, sex-specific gene expression profiles in cardiac tissue could contribute to differential responses. Previous studies have shown that genes involved in detoxification, inflammation, and cell death pathways are expressed at different levels in male versus female myocardium. These molecular differences may affect the rate and extent of DOX-induced injury (Agostinucci et al.,2023; Dulf et al., 2023). Our findings are consistent with human studies reporting that male patients are more likely to develop anthracycline-related cardiac complications, particularly in pediatric oncology. This underlines the importance of including

both sexes in preclinical research and adopting sex-specific analyses in drug toxicity studies (Camilli et al., 2024). However, due to the fact that this is a pilot study about the determination of sex-related morphological changes in rat hearts caused by doxorubicin administration, the study is limited by its small sample size and lack of a control group, which could have provided a baseline for comparison. Future research should involve larger cohorts and explore the underlying molecular mechanisms driving sex differences in DOX cardiotoxicity. In conclusion, this study demonstrates that male Wistar rats exhibit more severe morphological damage than females in an acute model of DOX-induced cardiotoxicity. These results highlight the necessity of considering sex as a biological variable in preclinical toxicological studies. Recognising and understanding such differences may inform the development of tailored cardioprotective strategies and improve risk stratification in patients undergoing anthracycline-based chemotherapy.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception: RJ, ELS, MK, AF; Design: RJ, ELS, MK, AF; Supervision: RJ, ELS, MK, AF; Materials: RJ, ELS, MK, AF; Data Collection and/or Processing: RJ, ELS, MK, AF; Analysis and/or Interpretation: RJ, ELS, MK, AF; Literature Search: RJ, ELS, MK, AF; Writing – Original Draft: RJ, ELS, MK, AF; Critical Review: MK, AF

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PROCJENA HISTOPATOLOŠKIH PROMJENA NA SRCU U DOZNO-OVISNIM MODELIMA ŽIVOTINJA TRETIRANIM DOKSORUBICINOM

SAŽETAK

Jedan od djelotvornih antraciklinskih hemoterapijskih lijekova koji se često koristi za liječenje solidnih i hematoloških karcinoma kod ljudi je doksorubicin (DOX). Kardiotoksičnost nekih lijekova koja zavisi od doze može dovesti do nepovratnog zatajenja srca, što ograničava njihovu kliničku primjenu. Razumijevanje patofiziologije i rana detekcija srčanog oštećenja izazvanog DOX-om omogućeno je kroz modele na životinjama, posebno na štakorima, koristeći akutne modele DOX kardiotoksičnosti zbog manje vremenski zahtjevnih procedura. Cilj ovog istraživanja je utvrditi potencijalnu kardiotoksičnu dozu DOX-a kod spolno specifičnih Wistar štakora divljeg tipa korištenjem svjetlosne mikroskopije za procjenu morfoloških promjena na srcu.

Odrasli Wistar štakori (n=10), uključujući mužjake (n=5) i ženke (n=5), tretirani su intraperitonealnom injekcijom doksorubicina u različitim dozama (25 mg/kg, 30 mg/kg i 40 mg/kg) po mužjaku i ženki. Štakori su žrtvovani nakon 48 sati i 72 sata za modele sa 25 mg/kg i 30 mg/kg, dok su pacovi iz grupe s dozom od 40 mg/kg žrtvovani nakon 24 sata. Miokard lijeve komore analiziran je pomoću svjetlosne mikroskopije pri uvećanjima od deset i četrdeset puta.

Mužjaci Wistar štakori razvili su izraženije morfološke promjene lijeve komore u poređenju sa ženkama, što je rezultiralo intersticijskim edemom miokarda i dezorganizacijom arhitekture miocita.

Mužjaci Wistar pacova divljeg tipa razvijaju agresivniji oblik akutne kardiotoksičnosti izazvane doksorubicinom u poređenju sa ženkama Wistar pacova divljeg tipa.

Ključne riječi: Doksorubicin, kardiotoksičnost, oštećenje miocita