The Influence of Doxorubicin on Testicular Histology and Sperm Parameters in Male Wistar Rats

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ABSTRACT

Chemotherapy plays a vital role in cancer treatment, but its impact on normal tissues and organs, including male reproductive function, is a significant concern. This study aimed to assess the influence of Doxorubicin, a commonly used chemotherapeutic agent, on testicular histology and sperm parameters in male Wistar albino rats. Thirty healthy adult rats were randomly assigned to a control group and two experimental groups receiving doses of Doxorubicin. Testicular histology was evaluated using hematoxylin and eosin staining, while sperm parameters, including general motility, progressive motility, viability, acrosomal integrity, morphology integrity, and concentration, were assessed. The results demonstrated remarkable histopathological changes in the testicular tissue of the experimental groups compared to the control group. Doxorubicin administration significantly reduced sperm motility, viability, acrosomal integrity, morphology integrity, and concentration. The findings of this study align with previous research, further highlighting the harmful effects of Doxorubicin on testicular function and sperm quality.

Keywords: Doxorubicin, Testicular Histology, Sperm Parameters

Introduction:

Infertility is a significant global health concern affecting millions of couples worldwide since, according to the World Health Organization (WHO), infertility affects approximately 10-15% of couples, with male factors contributing to almost 50% of the cases (Al-amery et al., 2022). Various factors can contribute to male infertility, including genetic disorders, hormonal imbalances, infections, lifestyle choices, and exposure to certain medications. Among these medications, chemotherapeutic agents are crucial in cancer treatment but can harm reproductive health (Al-Mousaw et al., 2022).

Within the realm of male infertility, the impact of chemotherapeutic agents on reproductive health has garnered considerable attention. Among these agents, Doxorubicin, a widely used anthracycline antibiotic in cancer treatment, has been implicated in reproductive toxicity (A'laa Hassan Abdul Hussain et al., 2022). Doxorubicin, a chemotherapy medication used to treat cancer, is essential to any chemotherapy regimen for various malignancies (Chatterjee et al., 2010). The previous studies exhibit potent anti-cancer properties which inhibit the DNA replication and induce cell death in rapidly dividing cancer cells. Furthermore, the Doxorubicin has its therapeutic efficacy and it is associated with several adverse effects, including cardiotoxicity, nephrotoxicity, neurotoxicity, and reproductive toxicity(Moraes et al., 2012).

The previous research has contributed significantly to our understanding of the impact of Doxorubicin on various tissues. Their studies have demonstrated the profound consequences of doxorubicin administration on tissues such as the heart, liver, and kidneys (Kumral et al.,

2015). On the other hand study observed histological changes, oxidative stress, and inflammation in these vital organs following doxorubicin treatment (angomla et al., 2018).

Their findings indicated that doxorubicin administration resulted in cardiotoxicity, characterized by myocardial damage, impaired cardiac function, and the development of heart failure. Furthermore, their research revealed hepatotoxicity, manifesting as hepatocellular injury, fibrosis, and altered liver enzyme levels. Additionally, they identified nephrotoxicity as a significant consequence of doxorubicin treatment, with evidence of renal tubular damage, glomerular dysfunction, and impaired renal function. In addition to the heart, liver, and kidneys, (Manno et al., 2016) explored the effects of Doxorubicin on other tissues, such as the lungs, gastrointestinal tract, and bone marrow. Their studies revealed pulmonary toxicity, including pulmonary fibrosis and impaired lung function. They also observed gastrointestinal toxicity, presenting as mucosal damage and altered intestinal permeability (Sekiya & Imamura, 2008). Moreover, bone marrow toxicity was evident, with doxorubicin-induced myelosuppression and anaemia (Venkatesh et al., 2007).

Reproductive toxicity induced by Doxorubicin has received considerable attention due to its potential impact on male fertility, in which the testicular histology and sperm parameters, including motility, viability, acrosomal integrity, morphology, and concentration, serve as crucial male reproductive health indicators. Understanding the influence of Doxorubicin on these parameters can provide valuable insights into the mechanisms underlying its reproductive toxicity and help develop strategies to mitigate its adverse effects. Furthermore, elucidating the side effects of Doxorubicin on testicular histology and sperm parameters is crucial for both clinical and research purposes (Bustani et al., 2022; Bustani & Baiee, 2021; Mohan et al., 2021).

The investigation of the influence of Doxorubicin on testicular histology and sperm parameters is paramount in comprehending this chemotherapeutic agent's reproductive toxicity by shedding light on the adverse effects of Doxorubicin on male fertility; this study aims to contribute to developing strategies to mitigate its impact and improve the reproductive outcomes of cancer patients.

Thus present study aims to investigate the influence of Doxorubicin on testicular histology and sperm parameters in male Wistar rats by evaluating the histological changes in the testes and assessing various sperm parameters, and we can gain a comprehensive understanding of the impact of Doxorubicin on male fertility.

Materials and Methods

This experimental design aimed to evaluate Doxorubicin's impact on testicular histology and sperm parameters in male Wistar albino rats. This research approach has been previously employed in studies conducted by Babaei et al. (2020), Mohan et al. (2021), and Venkatesh et al. (2007) (Babaei et al., 2020; Mohan et al., 2021; Venkatesh et al., 2007). By examining the effects of Doxorubicin on testicular histology and sperm parameters, this study aimed to provide insights into the potential reproductive implications of Doxorubicin treatment.

Doxorubicin

The Doxorubicin was purchased from Al-Dijla Company in Najaf, Iraq.

Animal and Experimental Design

In the present study, we used 30 healthy adult male Wistar albino rats weighing 180-200g purchased from animals housed in Tikrit. The rats were randomly divided into three groups, each comprising ten rats. The first group was the control group (C) which received no treatment. The second group received a weekly dose of 2.5 mg/kg body weight (BW) of Doxorubicin for eight weeks (D1). Doxorubicin was administered via intraperitoneal (i.p) injection and was dissolved in normal saline. The third group received a higher dose of 5 mg/kg BW of Doxorubicin per week for eight weeks (D2), also administered via i.p. injection and dissolved in normal saline. The dose indicated according to the previous studies

(Bennink et al., 2004; Swamy et al., 2011; Uchegbu et al., 1994).

Histopathological

The histopathological changes in the testes were evaluated following the methodology described by previous studies (Russell et al., 1993; Taib et al., 2013). At first, the testes from both groups were fixed in a ten percentage buffered formalin solution and underwent a standard histological procedure then staining was performed on all testicular sections by Hematoxylin and eosin (H&E), and morphological changes were observed under magnifications of 10X and 40X. For ultrastructural analysis, take small pieces (1 mm3) of the testes and treat them with a 2.5% glutaraldehyde solution in 0.1 N phosphate-buffered saline for 1 hour at room temperature. Subsequently, the samples were post-fixed with osmium tetraoxide for an additional hour. Finally, the Dehydration was carried out using sequential acetone concentrations of 70%, 90%, and 100% (twice) for 5 minutes each. The samples were then treated with a 1:1 ratio of acetone to resin for 5 minutes before being embedded in epoxy resin (Lara et al., 2018; Russell et al., 1993; Taib et al., 2013).

Sperm parameters

The assessment of sperm parameters included the evaluation of general and progressive motility, viability, acrosomal integrity percentage, and sperm concentration. To collect the spermatozoa, the tail of the epididymis was rinsed and incubated in two millilitres of normal saline at 37°C. It was then cut into approximately 200 pieces using anatomical micro-scissors to release the spermatozoa from the epididymal tubules (Alabedi et al., 2021). The motility of the spermatozoa was determined by placing ten microliters of the semen on a dry and warm slide, which was then covered with a coverslip. Microscopic observation was conducted to estimate the motility of the sperm (Khodair & Mohammed, 2022). Morphology, viability, and acrosomal integrity were assessed using an eosin nigrosine stain. This involved mixing 20 microliters of sperm with 20 microliters of the stain and evaluating the samples under a microscope.

Ethic:

In this study, all procedures, including animal husbandry, handling, and euthanasia, were conducted following the guidelines set forth by the Animal Ethics Committee of the University of Kufa, Najaf, Iraq.

The result and desiccation

According to Table -1, the outcomes of the result showed the value of significant differences in the sperm parameters among the different experimental groups. The control group (C) exhibited higher values for general motility (83 ± 1.2%), progressive motility (77 ± 0.75%), viability (85.5 ± 1.5%), acrosome defect (0.75 ± 0.15%), morphology integrity (97 ± 1%), and concentration (1.15 x 10^9). Comparatively, the group treated with a weekly dose of 2.5 mg/kg body weight of Doxorubicin for eight weeks (D1) displayed lower values for general motility (70 ± 2.5%), progressive motility (65 ± 1.5%), viability (67.5 ± 2.5%), acrosome defect (1.75 ± 0.55%), morphology integrity (93 ± 1.5%), and concentration (0.5 x 10^9). Furthermore, the group receiving a higher dose of 5 mg/kg body weight of Doxorubicin per week for eight weeks (D2) exhibited even lower values for general motility (50 ± 2.5%), progressive motility (45 ± 2.5%), viability (40.2 ± 3.5%), acrosome defect (2.15 ± 0.45%), morphology integrity (90 ± 1.5%), and concentration (0.45 x 10^9). Statistical analysis revealed significant differences among the groups for all the measured parameters. The lowercase letters (a, b, c) indicate significant differences. Group C (control) showed higher values compared to groups D1 and D2, suggesting that the administration of Doxorubicin harmed testicular histology and sperm parameters in the male Wistar albino rats.

Table 1: Sperm Parameters in Different Experimental Groups

Experimental Group	*General Motility (%)*	*Progressive Motility (%)*	*Viability (%)*	*Acrosome Defect (%)*	*Morphology Integrity (%)*	*Concentration (x10^9)*
Control (C)	83 ± 1.2 (a)	77 ± 0.75 (a)	85.5 ± 1.5 (a)	0.75 ± 0.15 (a)	97 ± 1 (a)	1.15 (a)
D1	70 ± 2.5 (b)	65 ± 1.5 (b)	67.5 ± 2.5 (b)	1.75 ± 0.55 (b)	93 ± 1.5 (b)	0.5 (b)
D2	50 ± 2.5 (c)	45 ± 2.5 (c)	40.2 ± 3.5 (c)	2.15 ± 0.45 (c)	90 ± 1.5 (c)	0.45 (b)

Values are presented as mean ± standard deviation.
Significant differences were detected between the groups using lowercase letters (a, b, c, d,.)

The results of the present study indicate that the administration of Doxorubicin in male Wistar albino rats resulted in significant alterations in testicular histology and sperm parameters. These findings are consistent with previous studies (Akman et al., 2015; Levi et al., 2015; Sakai et al., 2018; Shati & Khalil, 2023), which reported similar detrimental effects of Doxorubicin on male reproductive health. The sperm parameters revealed a dose-dependent decrease in general motility, progressive motility, viability, acrosome integrity, morphology integrity, and concentration of sperm in the Doxorubicin-treated groups (D1 and D2) compared to the control group (C) since these findings agree with the observations made by Akman et al. (2015), who demonstrated a decline in sperm quality and quantity following Doxorubicin administration in rats. Similarly, Sakai et al. (2018) reported reduced sperm motility and viability associated with oxidative stress induced by Doxorubicin. The mechanism underlying the harmful effects of Doxorubicin on testicular histology and sperm parameters can be attributed to its cytotoxic properties. Doxorubicin induces oxidative stress, producing reactive oxygen species (ROS) and subsequent damage to sperm cells. The elevated levels of ROS can cause lipid peroxidation, DNA damage, and disruption of the mitochondrial membrane potential, ultimately impairing sperm motility, viability, and acrosomal integrity. Moreover, Doxorubicin has been shown to interfere with cell cycle progression and inhibit DNA synthesis, which can lead to reduced sperm production and concentration.

Furthermore, Doxorubicin exerts its cytotoxic effects by generating free radicals and disrupting cellular antioxidant defence systems. It can deplete endogenous antioxidants, such as glutathione, and impair the activity of antioxidant enzymes, including superoxide dismutase and catalase. The imbalance between oxidative stress and antioxidant defence mechanisms contributes to testicular damage and compromised sperm parameters.

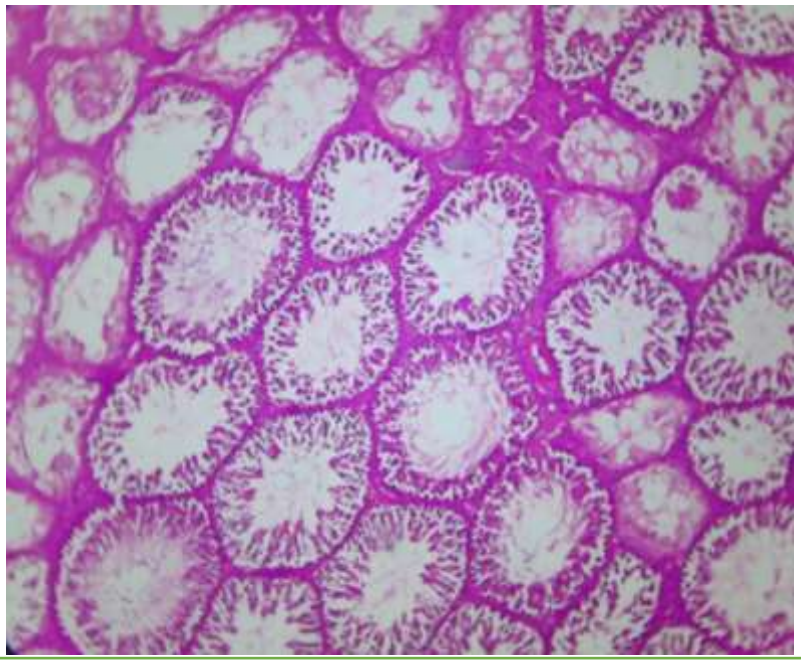

Figure -1 Histopathological section of the control group.

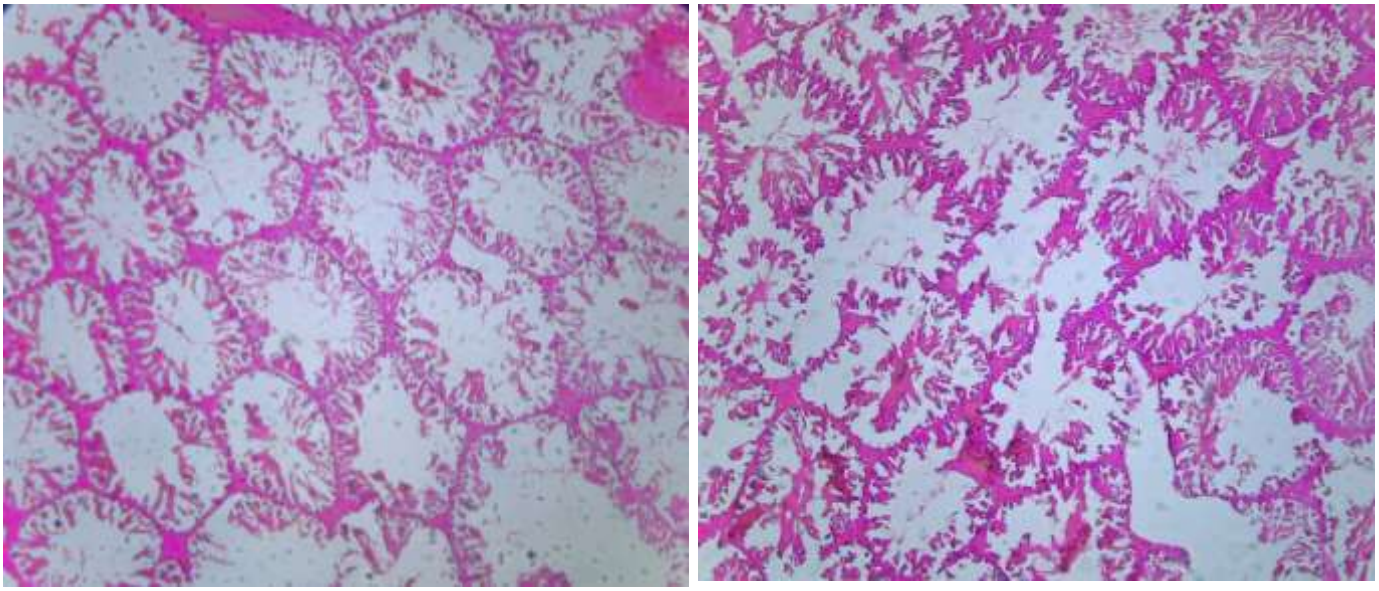

Figure -2 Histopathological section of the 2.5 mg/kg body weight (BW) of Doxorubicin for eight weeks.

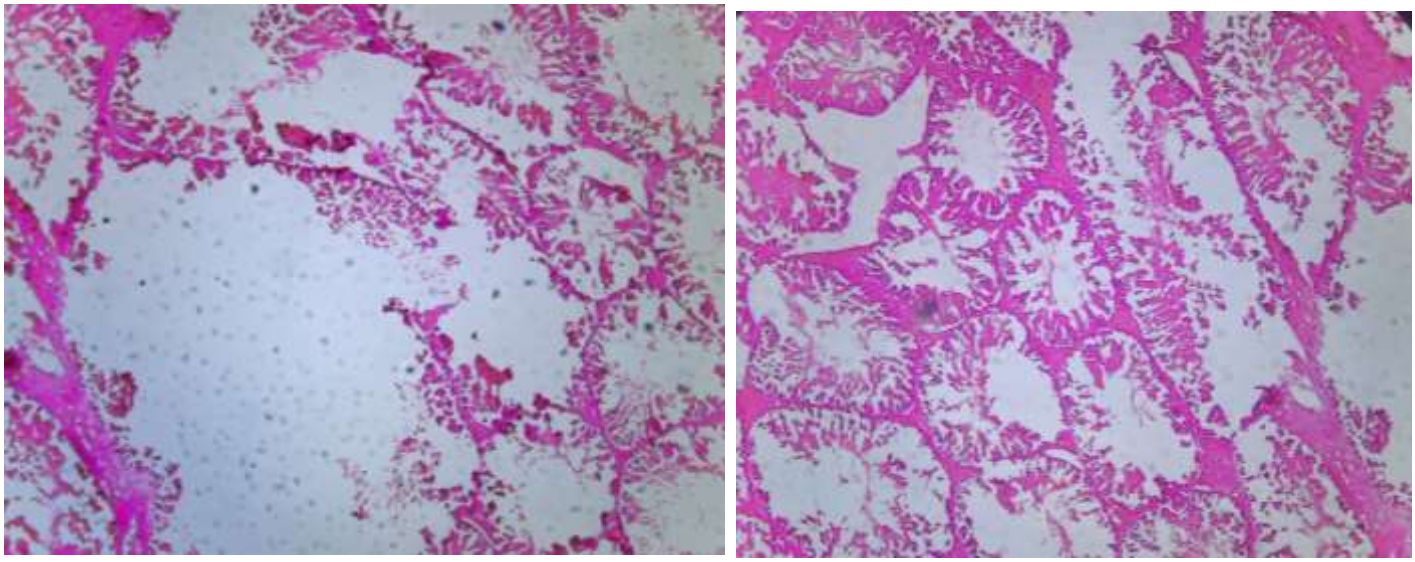

Figure -3 Histopathological section of the 5 mg/kg body weight (BW) of Doxorubicin for eight weeks.

Chemotherapy treatments have a well-established role in managing various types of cancers but these treatments can effectively target cancer cells, they often have adverse effects on normal tissues and organs. Specifically, in the context of male reproductive health, chemotherapeutic agents have been shown to have detrimental effects on testicular histology and sperm parameters(Al-amery et al., 2022; ALIBRAHEEMI et al., 2021).

The Figure1- shows that histopathological examination of the testes from the control group revealed well-defined stages of spermatogenesis, demonstrating the normal progression of sperm cell development. The lamina propria and lumen were visible in the histological section. Hematoxylin and eosin (H&E) stain was used to enhance the visualization of tissue structures. The section was captured and observed under a magnification of 20×, allowing for a detailed examination of the testicular histology. Furthermore, the figure depicts the histological analysis of the testis section in the control group, demonstrating the absence of any histological changes associated with the degenerative effects of Doxorubicin.

Furthermore, the present study's findings provide evidence of Doxorubicin's impact on testicular tissue, as observed in Figures 2 and 3. Compared to the control group (Figure 1), the histopathological sections of animals in groups 2 and 3 (Figure 2 and 3) clearly demonstrate the detrimental effects of Doxorubicin. The adverse effects of Doxorubicin on spermatozoa and germ cells are prominently displayed in Figures 3 and 2, indicating testicular degeneration resulting from the administration of both 2.5 mg/kg and 5 mg/kg body weight (BW) of Doxorubicin for eight weeks.

The findings of the present study regarding the testicular degeneration caused by Doxorubicin align with previous research conducted by (Abdelaziz et al., 2019; Renu & Gopalakrishnan, 2019; Shivakumar et al., 2012). These studies also reported adverse effects of Doxorubicin on testicular tissue and sperm parameters. Regarding histopathological changes, our study (Figures 2 and 3) and the previous studies consistently demonstrate the impact of Doxorubicin on the testicular tissue. This includes alterations in the architecture of the testes, disruption of spermatogenesis, and degeneration of germ cells. The histopathological sections clearly illustrate the damaging effects of Doxorubicin on the testicular structure.

Furthermore, when examining the effects on sperm parameters, our study's results (Figures 3 and 2) are consistent with previous findings. These studies demonstrate that Doxorubicin administration significantly declines sperm motility, viability, acrosome integrity, morphology integrity, and concentration. The adverse effects on sperm quality and quantity indicate impaired reproductive function caused by Doxorubicin treatment. Our study's results align with the previous research, reinforcing the understanding that Doxorubicin has detrimental effects on testicular histology and sperm parameters.

Conclusion

This study examined the effects of Doxorubicin, a commonly used chemotherapeutic agent, on testicular histology and sperm parameters in male Wistar albino rats since the result exhibits significant histological changes and alterations in the testicular tissue of rats exposed to Doxorubicin, indicating the occurrence of testicular degeneration and the sperm test showed it causes significantly reduced sperm motility, viability, acrosomal integrity, morphology integrity, and concentration. Identifying these adverse effects highlights the importance of implementing protective measures to mitigate these impacts and safeguard male fertility.

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