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Reproductive Toxicity of Gemcitabine on Breeding and Fertility in Male Albino Rats

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ABSTRACT

Although chemotherapy for malignancies is highly effective, their related gonadotoxic side effects may severely impair fertility and cause gonadal toxicity in male patients.The aim of the present work was to investigate influence of Gemcitabine toxicity on reproductive system of albino male rats (breeding and fertility tests). Animal experimental study conducted in zoology department, College of science, King Saud University during period from June to October 2014 using albino rats (Rattusnorvegicus) (Wistar strain). Males were divided into four different groups (control" 0 mg/kg",7 mg/kg,14 mg/kg, and 21 mg/kg).Male fertility index, female fertility index, pregnancy index, number of pregnancies per pregnancy and total number of births decreased in all groups treated. Mean number of implantation sites per female and the implantation index also decreased significantly ($p \leq 0.05$) and the rate of paradise loss before implantation increased at both doses 14 and 21 mg / kg, while the rate of loss of fetuses after implantation also increased. The histological examination in both the testis and the epididymis showed that they were significantly affected at the level of the three doses, and the effects ranged between moderate and severe. Histological examination of testis segments at a dose of 7 mg / kg showed atrophy of spermatogenic epithelial degeneration in many seminal tubules in most animals. Fluid accumulation was observed in some cases and sperm retention. It is concluded that the drug can be considered highly toxic to the male reproductive system, and despite the severity of the observed effects, it has been recovered to a large extent.

KEY WORDS: FERTILITY-GEMCITABINE-RATS-SPERMS-TESTIS-TOXICITY.

INTRODUCTION

The incidence of cancers commonly diagnosed in the adolescent and young adult population, including Hodgkin and non-Hodgkin lymphoma, acute lymphocytic leukemia and testis cancers, is on the rise worldwide (Okada & Fujisawa, 2019, Chan, 2013), Siegel et al., 2016). Simultaneously, the latest combination chemotherapy treatments provide the safety and efficacy and have improved the survival rates of these patients to more than 75%–90%, making them more able to be fathers and form family. Even that chemotherapy and radiation therapy for malignancies are highly effective, their related gonadotoxic side effects possibly will severely impair fertility in agent- and dose-dependent manners and may cause impermanent or permanent gonadal toxicity in male patients (Wong et al., 2009). Where 24% of the cases suffered from persistent azoospermia or severe oligozoospermia. On the other hand, a n important question was presented about the efficacy of post-therapy spermatozoa for conception, either naturally or through assisted reproductive technologies, where, many of the survivors have a complete return of sperm production.

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Gemcitabine (GCB) is a pyrimidine antimetabolite exceedingly used in various solid tumors as a single treatment or as a component of multidrug plans (Okada & Fujisawa, 2019).

Most patients have a good tolerance for GCB, however, sometimes life-threatening complications occur. where, the main side effects were laboratory variations (mild proteinuria transaminase elevation, myelosuppression, and hematuria) (Chan, 2009, Siegel et al., 2016). The main factors raising GCB toxicity were; alcohol abuse, combination with platinum derivatives or taxanes, and liver and kidney diseases (Trost & Brannigan, 2012, Hryciuk et al., 2018). There is no obvious evidence about the dose amendment of GCB, and clinical decisions are mainly made depend on experimental bases. The aim of the present study was to investigate the influence of Gemcitabine toxicity on reproductive system of albino male rats (breeding and fertility tests).

MATERIAL AND METHODS

This animal experimental study was conducted in zoology department, College of science, King Saud University, Saudi Arabia. The study was conducted during the period from the beginning of June to the end of October 2014.

Animals: Sexually mature male and female albino rats (Rattus norvegicus) of the Wistar strain, with their ages ranged between 8-10 weeks and weights of 220-250 gm, were obtained from animal house, College of Pharmacy, King Saud University.

Drugs: Gemcitabine is available from the manufacturer in packages containing either 200 mg or 1 gram of salicetabine hydrochloride prepared with mannitol (200 mg and 1 g respectively) and sodium acetate (12.5 mg and 62.5 mg respectively) (Casciato, 2004, Eli Lilly and Company, 2007). It was dissolved in a 0.9% solution of sodium chloride and remained stable for 24 hours at room temperature. The drug was not cooled in the refrigerator after it was thawed, to avoid any crystallization, (Eli Lilly and Company, 2006, Mini et al., 2006).

Experimental Design: Animals were dealt with and the various experiments and tests were designed in general according to the guidelines and standards used in estimating the toxicity of chemical compounds on the reproductive system (Clegg et al., 2008). Males were divided into four different groups (control" 0 mg/kg",7 mg/kg,14 mg/kg, and 21 mg/kg). Each group of the four groups included 20 rats, which were divided into two subgroups, each of which included 10 rats, so that the different tests were applied to the first subgroup immediately after the end of the treatment period (9 weeks) in order to find out the harm caused by the drug, while different tests were applied to the group. The second subset after one of the spermatogenesis cycles has passed, with the aim of knowing whether or not the reproductive system functions recover and return to a normal state.

The animals of each group were injected into the peritoneal cavity "IP" once a week for a period of 9 weeks. The control group was injected with a physiological solution, while the three treated groups were injected with a physiological solution in which the drug was dissolved according to the specified dose. During the injection period, the animals were monitored daily in order to follow up the appearance of disease signs resulting from drug toxicity or mortality, and their weights were recorded at the beginning of the experiment and then weekly. Immediately after the end of the injection period (10 males from each group) or after a complete sperm cycle equivalent to nine weeks (10 males - recovery group), then the males were mated with healthy females at a rate of (1: 2).

The males were sedated after the end of the treatment period and mating with ether, and then killed, and blood was collected from the arterial stem in the neck and left to coagulate at room temperature for an hour, then the serum was collected after centrifugation at 3000 rpm for 20 minutes and kept at -80 ° C. To measure testosterone concentration at a later date (Foster & Harris, 2005). After killing males and collecting blood, the abdomen was incised and the internal organs were fully exposed and examined, and any anatomical or pathological changes were recorded. The organs of the reproductive system consisting of the testes, epididymis, prostate gland, seminal vesicles, and associated clotting glands were removed, and the adipose tissue attached to them was trimmed and weighed all. The weight of the different organs was expressed as absolute weight and as relative weight to body weight, which was calculated according to the formula (member weight / body weight x 100) (Andrade et al., 2002, Yu et al., 2009). The left testis and left epididymis were used to assess the toxic effect of the drug on histopathology.

In order to determine the effect of treating male rats with the drug gemcitabine on their fertility and on the resulting offspring, they were mated immediately after the end of the treatment period with untreated females, at the rate of 2 females for each male. After that, half of them were killed on day 20 of pregnancy and the other half were left to complete the normal pregnancy period and give birth to their young, which were followed up until the end of the breastfeeding period, and the various indicators of male fertility, rates of fetal loss, birth survival, and the incidence of abnormalities, which in general give a complete picture of Condition and efficiency of the reproductive system after treatment with the drug.

Statistical Analysis: The obtained data were represented as mean ± standard error of mean ± SE and a significant level of P 0.05 and P 0.01 was adopted. For the statistical analysis of the data, both SPSS version 16 and SigmaStat version 3.5 were used. Data for the mating index, fertility

index for males and females, and pregnancy index were analyzed using chi-square, and in case of differences between groups.

RESULTS AND DISCUSSION

For groups treated with Gemcitabine and identified to study recovery from the toxic effects of the drug, the various external observations observed during the drug treatment period disappeared and the animals returned to normal. After the autopsy and examination of the internal organs, all were healthy except for atrophy and redness of the lung in one of the subjects of the higher dose and the fibrosis of some lung lobes in one of the members of the medium dose and one of the members of the lower dose. It was also noted that the testicles atrophied in one of the subjects in the third dose and their stature was soft compared to the normal state.

Table 1 shows the different effects of the drug gemcitabine on the different indicators of mating and fertility and on the offspring resulting from mating the drug-treated males with untreated females. It is evident from the data of this table that the mating index was not affected by the drug-treated males, as it recorded values equal to that of the control group. As for the male fertility index, it decreased in all groups treated with gemcitabine, and in the 7, 14 and 21 mg / kg doses treated groups, 70%, 70% and 66.67% were recorded, respectively, compared to 90% in the control group. It is evident from these data, as well, that there is a significant decrease in the female fertility index and the pregnancy index. Where the female fertility index decreased, and at doses 7, 14 and 21 mg / kg, the percentage was 55%, 50% and 50%, respectively, compared to 90% in the control group, and the pregnancy index at the same doses scored 68,75% and 55,55. % And 56,25%, respectively, compared to 90% in the control group. The number of pregnancies per pregnancy also decreased at all doses, but this decrease was not significant only in the 14 mg / kg group (p 0.05).

Table 1. The effect of gemcitabine on different mating indicators, autopsy results of pregnant females, and birth data immediately after the end of treatment (Mean ± SE).

Parameters	Dose			
	Control	7 mg	14 mg	21 mg
Male mating index	100	100	100	100
Male fertility index	90	70	70	66.7
Female fertility index	90	55*	50**	50**
Pregnancy index	90	68.75	55.55*	56.25*
Caesarean section data				
Litter size/dam	11.1 ± 1.34	7.71 ± 2.31	5.71 ± 1.06*	8.67 ± 2.17
Implantation sites/dam	13.3 ± 0.52	10.29 ± 1.79	8.86 ± 1.86*	8.83 ± 2.19*
Dead fetuses/litter	0	0	0.14 ± 0.14	0
Resorptions/litter	2.2 ± 1.33	2.57 ± 1.41	3.14 ± 1.39	0.17 ± 0.17
Corpora lutea/dam	14.5 ± 0.7	15.43 ± 0.87	13.71 ± 0.81	15.83 ± 1.01
Implantation index	92.52 ± 2.76	70.33 ± 13.36	62.46 ± 10.74*	58.82 ± 15.41*
% Preimplantation loss/litter	7.48 ± 2.76	29.67 ± 13.36	37.54 ± 10.74*	41.18 ± 15.41*
% Postimplantaion loss/litter	16.05 ± 9.48	26.21 ± 14.92	36.5 ± 13.64	1.52 ± 1.52
Fetal body weights (g)	4.00 ± 0.17	4.33 ± 0.3	4.33 ± 0.25	4.46 ± 0.2
natural delivery data				
Total no. of delivered pups	95	42	38	29
Live pups delivered/litter	10.56 ± 0.96	8.4 ± 2.16	9.5 ± 2.33	9.67 ± 1.33
Live birth index	92.9 ± 4.14	100	96.43 ± 3.57	100
pups body weights at PND 0(g)	5.72 ± 0.12	6.61 ± 0.44	5.89 ± 0.25	6.50 ± 1.04
4-days survival index	95.9 ± 2.18	75.00 ± 25.0	80.72 ± 11.04	100
pups body weights at PND 4(g)	9.11 ± 0.41	9.24 ± 0.39	9.40 ± 1.64	9.82 ± 0.78
weaning index	90.87 ± 6.27	92.86 ± 7.14	100	100
pups body weights at PND 21(g)	38.44 ± 1.43	41.95 ± 2.54	44.08 ± 7.13	41.14 ± 3.03
Sex ratio (% males/litter)	52.7 ± 4.3	58.5 ± 2.3	43.85 ± 2.7	49.0 ± 3.9
Externally malformed fetuses/litter	0	0	0	0

* Significantly different from control (p ≤ 0.05).PND (Postnatal Day).

The mean number of implantation sites per female also decreased significantly (p ≤ 0.05) at both doses 14 and 21 mg / kg. There was no significant change in the number of dead or absorbed embryos per pregnancy. Regarding the implantation index, it was significantly decreased (p 0.05) at each of the 14 and 21 mg / kg doses, and the

rate of paradise loss before implantation for these two groups also increased significantly (p 0.05), while the rate of loss of fetuses after implantation also increased. No significant increase was recorded. There was no effect on the different birth indicators nor on the proportion of males in each pregnancy, and no external abnormalities were recorded in any of the pregnancies or births, but the total number of births was significantly reduced in the drug-treated groups compared to the control group. (Table 1)

Regarding the drug recovery group, it is noticed from Table 2 that all indicators affected by the drug return to levels equal to or close to the control group levels, and no significant differences were observed on any of these indicators or measurements except the number of live births at the dose of 14 mg / kg. (Table 2)

Table 2. The effect of gemcitabine on different mating indicators and birth data in the recovery group after a complete spermatogenic cycle after cessation of treatment with the drug (Mean ± SE).

Parameters	Dose			
	Control	7 mg	14 mg	21 mg
Male mating index	100	100	100	100
Male fertility index	80	80	75	80
Female fertility index	80	80	75	80
Pregnancy index natural delivery data	80	80	75	80
Total no. of delivered pups	106	89	59	98
Live pups delivered/litter	13.25 ± 0.53	11.13 ± 0.72	9.83 ± 1.4*	12.25 ± 0.59
Live birth index	100	100	100	100
pups body weights at PND 0(g)	5.63 ± 0.11	6.15 ± 0.21	6.16 ± 0.35	5.42 ± 0.13
4-days survival index	98.33 ± 1.09	98.96 ± 1.04	100	100
pups body weights at PND 4(g)	8.28 ± 0.27	8.82 ± 0.35	9.44 ± 0.81	8.15 ± 0.48
weaning index	73.44 ± 4.38	65.40 ± 9.60	72.32 ± 9.90	55.36 ± 5.36
pups body weights at PND 21(g)	41.67 ± 1.18	41.86 ± 2.08	45.82 ± 2.79	41.01 ± 5.60
Sex ratio (% males/litter)	48.39 ± 5.44	46.89 ± 7.84	54.07 ± 4.78	56.59 ± 6.99
Externally malformed fetuses/litter	0	0	0	0

*Significantly different from control ($p \leq 0.01$).
PND (Postnatal Day)

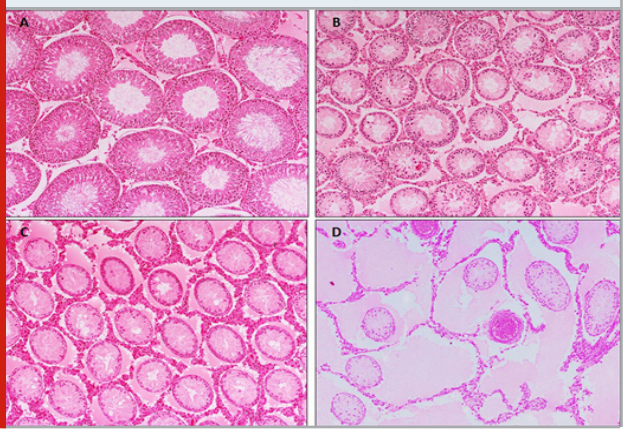
The histological examination of the testis and epididymis segments in the control group showed in the dissected animals immediately after the end of the treatment, as well as that of the morgue after the passage of time after the cessation of the treatment of normal tissue composition. All types of germ cells appeared naturally organized inside the seminiferous tubules, and Leydig cells appeared distributed in the tissue areas between the seminiferous tubules with the blood vessels. In the epididymis, the tubules appeared naturally in terms of cellular composition of tubule walls, interstitial tissues, and sperm content of the tube lumen. As for the animals treated with the drug, the histological examination of the histological sectors in both the testis and the epididymis showed that they were significantly affected by the treatment with the drug at the level of the three doses, and the effects ranged between moderate and severe between the members of the single treatment group and between the three doses. Also, these effects continued, to varying degrees, even after some time had passed since treatment with the drug was discontinued in animals of the recovery group from the effect of the drug.

Histological examination of testis segments at a dose of 7 mg / kg showed atrophy of spermatogenic epithelial degeneration in a large number of seminal tubules in most animals. Most of the histological changes observed in this group can be summarized as follows: Some seminiferous tubules appeared irregular epithelia, missing some types of germ cells. Presence of seminal tubules devoid of all cells except for Sertoli cells and very few spermatogonia. The lumen of some seminiferous tubules contained cellular necrotic materials, and residues of a darker pigment, Eosinophilic debris. Some seminiferous tubules contained cells with pyknotic nuclei and evidence of these cells' degradation. Separation of spermatocytes and spermatids from the epithelialization of the seminiferous tubules as a sign of the onset of the process of germ cell sloughing or exfoliation and the formation of cell masses within the lumen of the tube. Vacuoles form in Vacuolation of sertoli cells. Retention of elongated spermatids (Step 19 Step 19) occurs at the lumen of the tubules and near the basement membrane of the seminiferous tubules in stages IX - XII. The presence of elongated spermatids in an irregular position and scattered unlike the normal state.

At a dose of 14 and 21 mg / kg, the atrophy of a very large number of the seminiferous tubules was observed, and in some cases the atrophy of all the seminiferous tubules

in the transverse sections examined. In most cases, these tubes contained only Sertoli cells or a few spermatogonia, and in some cases there were some sperm cells. Among the most prominent histological changes observed, in addition to the above, are the following: They form Giant multinucleated cells. In some cases, hyperplasia was observed in Leydig cells, where their number was observed to increase at a rate more than normal. Dilated interstitial tissue spaces due to the accumulation of an amount of fluid in some cases. Edema. Too large shrinkage of some seminiferous tubules. The basement membrane of the seminiferous tubules is thickened. In some cases, at a dose of 14 mg / kg, hematopoietic infiltration was observed in the interstitial tissue, where red blood cells were observed outside the vessels, and in one of the members of this group heavy bleeding occurred, and the interstitial tissue was observed which was filled with red blood cells. Regarding the treatment group, the histopathology was observed in most of the cases examined in the three doses to a position very close to the normal structure, with a small number of seminal tubes containing only Sertoli cells and a few spermatogonia. However, there remained a small number at different doses in which the seminiferous tubules did not recover, as they were seen in a state of semi- or partial atrophy. Fluid accumulation was observed in some cases and sperm retention (Figure 1).

Figure 1: Sections of testis of a rats from the four groups.
A: Section of testis of a rat from the control group shows a group of seminiferous tubules at different stages of the seminiferous epithelial cycle (H&E, x 100).
B: Section of testis of a rat at dose of 7 mg shows most of the seminal tubules atrophied (H&E, x 100).
C: Section of testis of a rat at dose of 14 mg shows atrophy included all the seminiferous tubules, with only the spermatogenic cells remaining in most of them. (H&E, x 100).
D: Section of testis of a rat at dose of 21 mg shows a significant expansion of the interfacial area occurred as a result of fluid accumulation(edema). All the seminiferous tubules were atrophied and their cavities blocked due to the extension of the Sertoli cell growths. (H&E, x 100).



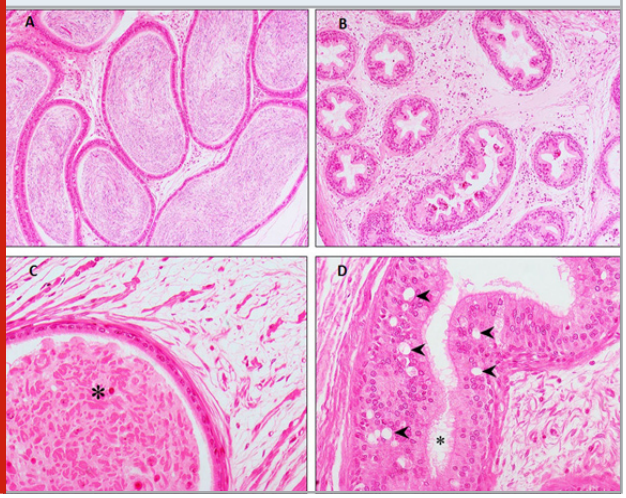
Histopathological examination of epididymal segments showed the effect of treatment with the drug gemcitabine, which caused significant damage to the epididymal tubes, especially in the lining epithelial cells. The tail of the epididymis was the most affected part, while the head of the epididymis was not significantly affected, and its epithelial cells appeared almost similar to their counterparts in the control group except that they contained few or no sperms and an increased number of damaged cellular remnants within them. The tissue damage seen in the tail of the epididymis can be summarized as follows: Thickened tubular epithelium, which is composed of several layers of Stratified epithelium, and sometimes the cells appear to be very high. Absent or low sperm count inside the epididymal tubes.

The presence of remnants of immature germ cells, abnormal germ cells, and largely necrotic cells in the tube lumen was observed. Significantly reduced diameter of the epididymal tubes. The presence of a relatively large amount of connective tissue that surrounds the epididymal tubes, especially fibroblasts, and the space between the tubes seemed to be wider than in the normal case. Significant damage to the epithelium and the emergence of gaps sometimes. Cribriform changes. Abnormal material in the lumens of the epididymal tubes. White blood cells appear near some tubes as an indicator of Chronic inflammation.

In the recovery group, the histological structure of the epididymis returned to its normal position in most animals, especially the epithelialization of the epididymal tubes, and only an increase in the percentage of cellular remnants was observed in some cases, as well as the absence of spermatozoa in animals in which the testicles had not regained their normal structure. In some cases, these tubes appeared to be heavily filled with material stained by the PAS stain, and sometimes the epithelium was seen to be folded and with many vacuoles (Figure 2).

The current study aimed to investigate the toxic side effects of one of the most prominent relatively new anti-cancer drugs on the reproductive system of male rats, as an animal model through which similar effects can be predicted on humans in the absence of any information or studies dealing with that. It is known that the process of spermatogenesis is similar to a large extent in many of its characteristics between humans and experimental animals, especially mice and rats (Meistrich, 2013).The mating index is a reliable measure of normal sexual behavior and the presence of sexual desire, and it also provides indirect information related to the function and state of the hypothalamic-pituitary-epiphysis axis (Holson et al., 2006). This indicator can be affected by many factors, including physical damage, acute intoxication, or changes in the neuroendocrine-gonadal axis that affect sexual desire or hormonal balance (Parker, 2006).

Figure 2: Sections of epididymis of rats from the four groups
A: Section of a rat epididymis of the control group. Note the normal structure of the epithelial layer of the epididymal tubes, the filling of the epididymal tubes with sperm and the normal volume of the interstitial tissue. (H&E, x 100).
B: Section of a rat epididymis at dose of 7 mg. Note the significant contraction of the epididymal tubes, the absence of sperm, the fold of the epithelium, and the expansion of the interstitial space. (H&E, x 100).
C: Section of a rat epididymis at dose of 14 mg. Note that the epididymal tube lumen is filled with abnormal contents and dissolved eosin-stained cellular residues (the star). (H&E, x 400).
D: Section of a rat epididymis at dose of 21 mg. Note the formation of vacuoles in the epithelium of the epididymal tubes (arrowheads), their detachment and pushing towards the tube lumen (the star) causing near closure. Note also that the tube is empty of sperm. (H&E, x 400).



The results of the current study indicated that there was no change in the mating index due to the treatment with the drug gemcitabine, and this indicates that the drug did not inflict any harm on sexual behavior as a result of not affecting the hormonal neurogenic gynecological axis. The Male Fertility Index measures their ability to produce sperm that can induce pregnancy in females. Because the occurrence of mating does not necessarily imply the occurrence of pregnancy, this indicator provides additional and valuable information. Fertility index can be affected by many factors like those that affect the mating index, in addition to several other factors, including the process of sperm maturation, transfer through the reproductive tracts, and their ability to fertilize eggs. The pregnancy index measures the ability of females to reach pregnancy (Parker, 2006).

Pregnancy success is considered the most effective measure of an animal's ability to produce gametes, mate and produce live offspring (Dixon, 1986). This indicator is affected by the same factors that affect the fertility index in males (Parker, 2006). The results of the current study showed that the treatment of males with the drug Gemcitabine caused a significant reduction in both indicators of fertility and pregnancy compared to the control group.

A 90% reduction in the sperm available for ejaculation in rats and other laboratory animals by surgery does not affect fertility (Amann, 1986), and if chemical treatment leads to the same decrease in the number of sperm ejaculated during mating, the changes in reproductive function will not be Sufficient to cause a significant decrease in fertility or pregnancy size ((MEISTRICH, 1982); (Keller, 2006)). However, despite the observed decrease in the fertility index at all doses used in the current study, the number of sperms in the epididymis did not decrease in the drug-treated groups below the limit affecting fertility except in the group treated with the large dose, which confirms that this decrease in fertility is a result of Basically, the quality of the sperms available for ejaculation is low, as indicated by the results of estimating their motility and the percentage of their abnormalities. This is supported by the results of the study conducted on 5-azcytidine.

In rats treated at a dose of 4 mg / kg for 11 weeks, the drug reduced the number of sperms in the epididymis to more than 90%. However, these animals were still able to mate and fertilize eggs. Most of the resulting embryos were abnormal as was proven by examination on the second day of the occurrence of pregnancy, and they also died before implantation (Doerksen & Trasler, 1996). Loss before implantation represents the number of eggs that were not fertilized or that was fertilized but was lost before implantation. It was evident from the results of the current study that the treatment of male rats caused an increase in the pre-implantation loss rate at the level of all the doses used, although the statistical analysis did not indicate its significance except at the medium and large doses. Any defect in the genome coming from the father as a result of exposure to chemicals may have severe consequences on the vitality and growth of the embryos (Kelly et al., 2003).

Interpretation of the loss prior to implantation may require information on the extent to which the agent induces mutations (Zenick, H. and Clegg, 1989). In order to determine the cause of the increased loss before implantation, additional studies must be conducted, including direct examination of fertilized eggs and early embryos. It must also be realized that the loss of embryos before and after implantation occurs naturally in untreated rodents as is the case with treatment, which contributes to the natural variation between the number of births per pregnancy (Parker, 2006).The number of embryos per pregnancy and the number of live births are influenced by the number of eggs available for fertilization, the fertilization rate, the implantation rate, the percentage of implanted embryos that survive the due date, and sperm measurements such as movement and number (Holson et al., 2006). The weight of the newborns after birth and throughout the growth period, as well as their survival rates, depend on their weight at birth, on

gender, on the natural formation of the individual, on the number of births per pregnancy, and on the ability of the newborn to breastfeed. Any defect in these indicators may indicate the effect of the toxic agent on one of these factors (Parker, 2006).

Determination of sex in mammals depends on the male through fertilization of the egg with a sperm that carries either of the Y or X chromosomes. Therefore, an effect on the production of a specific type of them or in its transmission through the reproductive tracts or in its ability to fertilize may result in a change in the sex ratio. There are also influences that may cause selective loss of one of the sexes, or they may have an effect on the external appearance by interfering with the process of growth of the reproductive system and thus lead to a change in the sex ratio in the births or the production of births bearing the characteristics of both sexes (Parker, 2006).

The tissue structure of the testis is the most sensitive indicator for detecting reproductive toxicity (Parker, 2006). It was observed in this study the occurrence of significant tissue damage in both organs as a result of treatment with the drug gemcitabine. Histological manifestations of the damage caused by anti-cancer drugs are characterized by depletion of germ cells, and most tissue sections show complete loss of them, as the seminiferous tubules appear to be devoid of all germ cells except for Sertoli cells, and sometime there can be a few sporadic spermatogonia, sperm cells and spermatogonia. The seminiferous tubules appear atrophied while the Leydig cells remain normal in appearance (Schilsky et al., 1980).

In addition to the manifestations of previous tissue damage, the histological observations recorded in this study also included a failure in sperm release and retention in the later stages of the spermatic epithelial cycle, the appearance of vacuoles in Sertoli cells, the formation of multinucleated giant cells, and the occurrence of hemorrhage in the interstitial tissues. The accumulation of fluid in the interfacial tissue. The histological composition of the epididymis was also affected, and the epithelium lining its tubes was bent and thicker than normal. Sometimes these cells separated and headed into the tube lumen. The accumulation of foreign substances positive for the periodic acid-Schiff stain was observed that filled the cavities of the epididymal tubes.These histopathological observations are consistent with the tissue damage caused by anticancer drugs that has been reported by several studies (Kelly et al., 2003, Oakes et al., 2007).

Regardless of the initial site of damage, most testotoxins will cause germ cell lysis and a decrease in their number. If the effect is severe or lasts for a long time, the end result will be a seminiferous tubule containing only Sertoli cells. Although Sertoli cells are very sensitive to dysfunction, they are exceptionally resistant to cell death (Creasy, 2001). The formation of vacuoles in Sertoli cells is one of the most prominent morphological responses to damage, and optical microscopy does not provide an opportunity to determine whether these vacuoles originate within them or between adjacent cells (Russell et al., 1991). The vacuole formation is followed by germ cell lysis and its irregularity or detachment, and the normal separation of the cells indicates the primary effect on the intercellular connections between the germ cells and Sertoli cells. Despite the severe effect, Sertoli cells remain intact and line the partially or completely empty tubes (Creasy, 2001).

The formation of vacuoles in Sertoli cells and failure to release sperm are all indications that Sertoli cells have been malfunctioning as a result of treatment with the drug gemcitabine. It is not possible, from the results of the current study, to know whether this defect was due to the direct effect of the drug or if it was the result of germ cell degeneration, which in turn caused a dysfunction in Sertoli cells. In general, Sertoli cells in adult animals are not affected by most anticancer drugs because they do not divide (Trottmann et al., 2007), and this may suggest the second possibility as a cause of impaired function.The most important characteristic of cytotoxicity specialized in a type of germ cell is the rapid programmed cell death of this type of cell and the infected cells are ingested by Sertoli cells, leaving the tubes free of it.

This early event is followed by a rapid inhibition in the growth of the generations following the affected generation during the rest of the spermatogenesis process. The death of a specific type of germ cell will eventually leave the seminal tube empty of cells except for Sertoli cells and germ cells that precede the target cells with the toxic effect, and this gives the impression that the process of sperm formation has stopped, and in fact the unaffected cells continue to grow but It is killed as soon as it reaches the target stage with the toxin (Creasy, 2001). This may explain the emergence of some seminal tubules in rats treated with gemcitabine devoid of certain types of germ cells, especially spermatocytes and round and elongated spermatids.

The volume of interstitial fluid increases in many cases, such as obstruction of lymphatic drainage, damage to the epithelial lining of blood vessels as in the case of exposure to cadmium, or as a secondary result of decreased spermatogenesis and shrinkage of the seminal tubules, and this damage is usually associated with an increase in testicular weight (Creasy, 2001). This is consistent with the observations observed in the current study, as the tissue samples in which an accumulation of fluid was recorded in the interstitial tissues was observed to have a high weight compared to the rest of the members of the group to which it belongs. It led to this kind of tissue damage. Histological examination did not indicate that the Leydig cells were affected by the drug, as no change in their phenotype was observed from the control group (Lanning et al., 2002). The results of the current study, regarding the unaffectedness of Ledge cells, agree with what is known about the

latter in terms of their resistance to anti-cancer drugs (Fosså & Magelssen, 2004) and the reason for this is due to the rate of its slow division (Puscheck et al., 2004).

Histological examination of testicular tissue in medium dose animals showed severe hemorrhage in one of their subjects, which led to widespread proliferation of blood cells in the obvious tissues outside the blood vessels (Eli Lilly and Company, 2007), and there are several reports indicating the occurrence of vascular toxicity associated with the drug in a number of cases (Muñoz et al., 2002). The recording of this drug's toxic effects on the blood vessels may explain the hemorrhage that was recorded in this study, as it is believed that it may cause damage to the epithelial cells lining the small blood vessels spreading in the interstitial tissue, which led to the influx of blood cells to this tissue and their spread around the seminiferous tubules.

CONCLUSION

The results of the current study showed the possibility that there was no effect of the drug gemcitabine on the hormonal axon of the reproductive system, as it was evident from the fact that none of the testosterone concentration in the blood, the mating behavior, or the main testosterone producing Leydig cells were affected in the drug-treated groups compared to the control group. From this, it can be concluded that the significant damage caused by the drug to the germ cells, the histological composition of the epididymis, the quantitative and qualitative sperm measurements, and the fertility indicators are mainly due to the direct effect of the drug on the germ cells themselves.Although the largest dose used in the current study represents only one-tenth of the corresponding therapeutic dose in humans (1200 mg / m2), it caused significant damage to the tissue structure of the testicle, and it also caused the quantitative and qualitative measurements of sperm to be greatly reduced. Which ultimately reduced fertility in males treated with the drug. Accordingly, the drug can be considered highly toxic to the male reproductive system, and despite the severity of the observed effects, it has been recovered to a large extent.

Conflict of Interest: Authors declares no conflicts of interests to disclose.

Ethical Clearance Statement: The Current Research Work Was Ethically Approved by the Institutional Review Board (IRB) of Taibah University, Madinah, Saudi Arabia.

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