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Ameliorative Role of Grape Seed Extract (Vitis Vinifera) on Gemcitabine - Induced Testicular Damage in Rabbits

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ABSTRACT: Spermatogenesis is a highly conserved and regulated process and it is sensitive to fluctuations in the physical and chemical environment. Gemcitabine is a novel antimetabolic anticancer drug used frequently in the treatment of many cancers. Gemcitabine may disrupt spermatogenesis by targeting various testicular cell types. Grape seed extract is a natural product, recently identified as one of the most powerful antioxidants. In addition, it has been reported to exert anticarcinogenic effects.

This current work aimed to study the effect of gemcitabine on the testicular tissue of local adult rabbits and to evaluate the possible protective role of Grape seed extract.

A experimental study was performed on twenty seven adult male local rabbits, were divided into three groups, nine rabbit in each group as following: group (A): animals of control group received normal saline injection, group (B): animals of treated group received therapeutic dose of gemcitabine alone in a dose of 25 mg/kg body weight intraperitoneally once per week for six successive weeks and group (C): served as protective group and was concomitantly treated with grape seeds extract by oral gavages in a dose of 250 mg/kg body weight and gemcitabine in a dose of 25 mg/kg body weight intraperitoneally once per week for six successive weeks.

The results of the present investigation showed that gemcitabine toxicity produced significant structural changes in the testis of group B, gemcitabine treated group, in the form of multiple distortion of the seminiferous tubules, with cellular disorganization, testicular tubular cell degeneration, necrosis, tubular atrophy, desquamation and sloughing of the seminiferous epithelium which was accumulated in the centre of the seminiferous tubules in the form of degenerated tissue. Many seminiferous tubules showed widely spaced spermatogenic cells. Karyopyknosis, disappearance of interstitial cells of Leydig and necrotic Sertoli cells, progressive tubular and interstitial testicular damage together with spermatogenic arrest was an initial necrotic change induced by gemcitabine exposure. Disorganization of spermatogenic cells, an interruption in spermatogenesis process and the seminiferous tubules are devoid of sperms was also showed in this study.

Sections from the testes of animals received therapeutic dose of gemcitabine and treated by grape seed extract, group (C) were showing partial preservation of the normal structure of the seminiferous tubules. Most of them more or less resembled the normal structure.

It could be concluded that gemcitabine caused marked alterations in the structure of the seminiferous tubules of the rabbit testis that may be minimized by administration of grape seed extract. Our findings simply revealed that grape seeds extract treatment significantly inhibits testicular cell apoptosis, which suggests that apoptosis may be one of the underlying mechanisms by which grape seeds extract protects against gemcitabine induced defective spermatogenesis.

KEY WORDS: Adult male rabbits, Gemcitabine, grape seed extract, testis.

INTRODUCTION

Usage of chemical drugs is the most common way in treatment cancer patients. Thus, researchers are more interest to evaluate the safety of the anti-cancer drugs. Although, these drugs provide some chemical control on this disease, most of these drugs have toxic effect on the normal cells. Unfortunately, up till now, there are no ideal drugs that can damage cells of cancer only without being

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damage the normal ones. Generally, these drugs damage DNA molecule leading to secondary tumors appearance after chemotherapy ceasing [1].

In the present study, one of these new anti-cancer drugs Gemcitabine was selected.

Gemcitabine ,Gemzar® is belongs to a class of drugs called antineoplastic agent, antimetabolite (Pyrimidine Analog). Gemcitabine is a nucleoside analog that has been used as a chemo- therapeutic agent for more than 15 years. It was originally investigated for its antiviral effects, but it is now used as an anticancer therapy for various cancers [2, 3].

As a prodrug, gemcitabine is transformed into its active metabolites that work by replacing the building blocks of nucleic acids during DNA elongation, arresting tumour growth and promoting apoptosis of malignant cells [4].

Progressive division and differentiation of spermatogonia in seminiferous tubules and production of spermatozoa constitutes spermatogenesis. Chemotherapeutic agents may disrupt spermatogenesis by targeting various testicular cell types (Leydig cells, Sertoli cells, and germ cells). Chemotherapeutic agents have been shown to have lethal effects on the actively dividing cells involved in spermatogenesis. During S- phase of the cell cycle, replicating deoxyribonucleic acid (DNA) is susceptible to damage. Purine and puramidine analogs get incorporated in DNA and prevent normal synthesis of genetic material and thereby altering normal cell division [5].

Abu Baker, 2009 investigated the toxic impact of gemcitabine on the histological structure of white mice testis and some embryonic organs that were treated with gemcitabine intraperitoneally [6].

The harmful effects of 14 chemotherapeutic drugs on spermatogenesis in the mouse have been evaluated by studies of testicular cell killing and morphological and genetic damage produced [7].

Most studies examining the reproductive effects of chemotherapeutic agents have documented an increase in the percentage of seminiferous tubules containing apoptotic germ cells [8].

Many findings suggest that identifying herbal medicines for the protection of antioxidants, and the treatment of toxicity and human disease is required. Among such natural products recently identified is the grape seed extract (GSE). The protective effects of GSE on the reproductive system have been demonstrated for various conditions. All these studies attribute the protective effects to its antioxidant and anti-apoptotic activities. Many papers published in recent decades have documented that, the putative beneficial effects of polyphenols are frequently related to their antioxidant activity. There in all these implying beneficial role of GSE [9].

Grape seed extract (GSE) is a natural extract from the seed of grape (Vitis vinifera). It is an important cultivated plant that has long been studied due to its positive effects on consumer health. The seeds of this plant contain a wide range of biologically active components that help to neutralize the adverse effects of free radicals. It is a rich source of one of the most beneficial groups of plant polyphenols, flavonoids, proanthocyanidins as well as biologically active dietary components [10]. Grape seed extract (GSE) is a natural product, recently identified as one of the most powerful antioxidants, which contains high levels of bioflavonoids, vitamin C and vitamin E. Grape seed extract (GSE) protects cells from damage by regulating cell oxidative damage [11, 12], reducing organ injury, improving the balance between oxidants and antioxidants, and reducing the release of inflammatory mediators [13]. In addition, GSE has been reported to exert anticarcinogenic effects[14] .

The protective effects of GSE on the reproductive system have been demonstrated for various conditions [15, 16, 17, 18, 19, 20]. From previous studies and researches, it has been found that most cytotoxic chemotherapeutic drugs have an effect on testicular tissues. There is paucity of literature regarding the effect of gemcitabine on testicular tissues. Its effects on their cells haven‘t been reported in literature to the best of our knowledge which motivates us to study the histopathological picture of the gemcitabine influences on these tissues.

In addition to that the studies dealing with protective role of antioxidant treatment with grape seed extract (GSE) on different chemotherapeutics -induced spermiotoxicity were mainly concerned with their clinical implications. The histological aspects seem to be overlooked.

MATERIALS AND METHODS

The present experiment was conducted on twenty sevsen healthy male rabbits, 4-5 months old of local breed, weighing between 1.5 – 2.5 kg, they were kept under controlled laboratory conditions for two weeks for acclimatization of animals to the laboratory

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environment. The animals were allowed unrestricted access to food and water. The rabbit was examined daily for possible behavioral and gross morphological or physical changes.

In the current study, the animals were divided into three equal number of experimental and control groups, A, B and C.

The concentration of gemcitabine dose and grape seed extract was selected based on previous studies[21,22,23,24].

Group (A): Animals of control group received normal saline injection, 1ml intraperitoneally once per week for six weeks to simulate the effect of injection.

Group (B): Animals of treated group received therapeutic dose injection of gemcitabine alone in a dose of **25** mg/kg body weight intraperitoneally once per week for six successive weeks[21,24].

Group (C): served as protective group and was concomitantly treated with grape seeds extract by oral gavages in a dose of **250** mg/kg body weight[22,23] and gemcitabine in a dose of **25** mg/kg body weight intraperitoneally once per week for six successive weeks.

In this group, the animals received a dose of grape seed extract, starting from the first day of the experiment for 6 consecutive days before and 6 consecutive days after the gemcitabine injection and continued daily for 6 weeks.

The collected samples of the testes, included in this study, were run through paraffin embedding technique to get paraffin blocks. Histological serial sections were cut from the testis of each group. Serial sections 5 µm thickness were cut and mounted on glass slide, and then stained with ordinary Hematoxylin and Eosin (H & E) stain[25].

RESULTS

Clinical observations:

The following observations were noticed 60 minutes after injection in the rabbits of group (B) which were injected with therapeutic dose of Gemcitabine: Excessive sleep, loss of appetite, fearless behavior, decrease movement, rapid breathing, occasional trembling, diarrhea and spasms and finally we were noticed some animals death after second and \ or third doses of drug. The dead animals were replaced by additional rabbits, injecting the same dose of gemcitabine for the desired duration.

Mild to moderate clinical signs were noticed in rabbits of group (C), such as loss of appetite, animals were appeared lazy and fearless behavior. No clinical signs were seen in control group (A). There was no mortality among the animals of control group (A) and group (C), grape seeds extract+ Gemcitabine.

Histological and histopathological findings:

Control group (A):

The histological descriptions for the slides stained by Haematoxylin and Eosin (H&E) stain of control group (A) were noticed that the tunica albuginea capsule send numerous septae that divided the testicular parenchyma into numerous lobules. Each lobule contained the seminiferous tubules and the richly vascularized interstitial spaces **(Fig.1a).**

The seminiferous tubules appeared rounded or oval in shape. They were lined by a complex multilayered or stratified epithelium showing spermatogenic cells at different stages of developments (spermatogonia, primary and secondary spermatocytes, and spermatids).

These cells were supporting by large plentiful cytoplasmic cells, known as the Sertoli cells.

The seminiferous epithelium was resting upon obvious thin basement membrane occupying most of the tubular thickness **(Fig. 1b).**

The center of the seminiferous tubule lumen contained numerous spermatozoa which appeared as small oval to elongated cells and had long tails which project into the lumen.

As recorded in most of the domestic animals, and within the interlobular septae, the interstitial or Leydig cells were organized in clusters or dispersed as small polygonal or pyramidal shaped cells with round nucleus and acidophilic cytoplasm. Blood capillaries were seen in the interstitial spaces, in between the seminiferous tubules. Outside the basal membrane and within the interlobular septae, continuous sheet of single layered flattened polygonal myoid or peritubular cells had the appearance of smooth muscle cells, with flat and elongated nuclei **(Fig. 1c).**

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Treated group (B):

Sections from the testes of treated animals with gemcitabine, group (B) showed disturbance in the normal architecture with evidence of structural changes than those in the control group (A).

There was disorganization of the seminiferous tubules. Numerous seminiferous tubules were sloughed and atrophic. Abnormal spermatogenic cell types in damaged seminiferous tubules were also observed in the testicular sections of the gemcitabine treated group (B).

The interlobular septae were appeared slightly congested **(Fig.2).**

Most tubules were appeared with distorted and necrotic spermatocytes, reduction in the numbers of spermatogonia, primary and secondary spermatocytes. The epithelium was reduced to single layer in several of the tubules.

The primary spermatocytes were appeared more densely, distorted, irregular in shape where they appeared oval, flat or polygonal and rarely rounded.

The lumen of the tubule was filled with desquamated secondary spermatocytes. While the spermatids and spermatozoa were completely absent, therefore the lumen of most tubules appeared empty.

Spermatogenic activity was halted. Intraepithelial bleb noted. The spermatogenic cells were detached from the basement membrane. The basement membrane was appeared decaying or irregularly shape **(Fig.3).**

High magnification of the seminiferous tubules treated with gemcitabine (B group) were showing degenerated spermatogenic epithelium and loss of the releasing spermatozoa.

There were sloughing in seminiferous tubules and numerous intraepithelial empty spaces (vacuolations) and cellular detachment, which accumulated in the center of the seminiferous tubules. Pyknotic nuclei of the spermatogenic cell were seen. The peritubular basement membrane was thickened with numerous flat myoid cells. Necrotic and pyknotic lesions in Sertoli cells were also noticed **(Fig.4).**

In many of the seminiferous tubules there were accumulation of fibers had been observed in their lumen, indicated that sloughing of the seminiferous epithelium which was accumulated in the centre of the seminiferous tubules form of atrophy and degenerated tissue **(Fig.5).**

The vacuolization and distorted in testicular tissues was observed in many sections exposed to therapeutic doses of gemcitabine. Disappearance of interstitial cells of Leydig, necrotic Sertoli cells, pyknotic lesions in spermatogonia and the seminiferous tubules are devoid of sperms **(Fig.6).**

Protective group (C):

Sections from the testes of animals received therapeutic dose of gemcitabine and treated by grape seed extract, group (C) were showing partial preservation of the normal structure of the seminiferous tubules. Most of them were more or less resembling the normal structure. Mild congestion persisted in the interstitial tissue of the interlobular septae had been observed **(Fig.7).**

There was an improvement in the seminiferous tubule structure, with mild cytoplasmic vacuolation of few spermatogenic cells. The interstitial space was wide and cells of Leydig were seen seemingly normal. The basement membrane appeared normal unseparated. **(Fig.8).**

High magnification photomicrograph illustrates cross section of the seminiferous tubules of group (C) was exhibited limited histological changes as slightly blood congested in the center of their tubules, but their spermatognic cells appeared in active form which the spermatogonia were small spherical cells with round nucleus and condensed chromatin, resting upon the thin basement membrane and were quiet able to divided. **(Fig.9).** In some seminiferous tubule, Sertoli cells were exhibited significant reduction in number with apparently normal structure. The myoid cells were also appeared in normal architectures.

DISCUSSION

Gemcitabine is one the newer nucleoside analogs that has a broad spectrum of anti–tumoral activitiy in various solid tumor models. It is one of the anti-metabolite drugs group.

This group consists of three divisions; parapholic acid, para-purine and para-pyrimidine.

The para-pyrimidine that characterized by its efficient on inhibiting the vital formation of the nuclides pyrimidine or resembling their normal metabolism, thus they interfere with the DNA synthesis or on its function[26].

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Gemcitabine being a deoxycytidine analog requires an intracytoplasmic phosphorylation to convert to active gemcitabine-nucleotides, gemcitabine 2',2'-difluoro 2'-deoxycytidine diphosphate and gemcitabine 2',2'-difluoro 2'-deoxycytidine triphosphate, which in turn inhibit DNA synthesis. This possibly explains the arrest of the spermatogonial cells in the early stages of meiosis where DNA synthesis has to occur; and to a lesser extent to the formation of relatively unstained crescents surrounding the chromatin, perceived as perinuclear halo in the histology[27].

Gemcitabine exerts its activity primarily by inducing cell cycle arrest and cell death[28].

As expected, gemcitabine had some adverse effects in animals. The leading symptom shown in group of animals treated intrapretonially by treated dose of gemcitabine was diarrhea, decrease in the amount of feed consumed and loss of skeletal muscles compared to the control group, which could in turn be ascribed to drug induced toxicity, psychological pressures and the necrotizing effects of the drug on the digestive system[29]. Furthermore, the drug also affects the mucous lining of the gastro-intestinal tract[30]. Our results corroborate the findings of previous researchers; Germoush 2009[31]; Rickenbacher et al. 2011[32] and Hailana et al., 2018[33].

In the present study the histological descriptions for the slides stained by Haematoxylin and Eosin stain of control group (A group) were noticed that the testicular parenchyma of the adult male rabbits were composed of packed, well organized oval or round seminiferous tubules and separated by narrow interlobular septae that contained clusters of interstitial cells and blood vessels. These results are in agreement with those of Jayachitra et al., 2019[34].

These seminiferous tubules were surrounded by thin and regular abasement membrane formed of connective tissue fibers and myoid cells and lined by stratified germinal epithelium including many layers of spermatogenic cells and Sertoli cells. The same results were reported by Jarrar 2011[35].

The spermatogenic cells included: spermatogonia, primary & secondary spermatocytes, spermatids and spermatozoa. The spaces between the seminiferous tubules were filled with connective tissue containing groups of Leydig cells, blood vessels and collagen fibers. Our result is consistent with Eurell and Frappier 2006 [36].

The results of the present investigation showed that gemcitabine toxicity produced significant structural changes in the testis of group B, gemcitabine treated group, in the form of multiple distortion of the seminiferous tubules, with cellular disorganization, testicular tubular cell degeneration, necrosis, tubular atrophy, desquamation and sloughing of the seminiferous epithelium which was accumulated in the centre of the seminiferous tubules in the form of degenerated tissue. Many seminiferous tubules showed widely spaced spermatogenic cells. Karyopyknosis, progressive tubular and interstitial testicular damage together with spermatogenic arrest was an initial necrotic change induced by gemcitabine exposure.

These alterations might indicate susceptibility of the seminiferous epithelium to gemcitabine toxicity with possible consequences on the intercellular junction between the strata of the germinal cells. These results were compatible with that of other investigators who reported

a similar finding; Lee et al., 1999[37]; El-Shahat et al., 2009[38]; Ceribasi et al., 2010[39]; Kamel et al., 2011[40]; El-Refaiy et al., 2013[41]; Mohamed et al., 2014[42]; Battan et al., 2015[43]; Viveka et al., 2015 [44]; Alkhedaide et al., 2016[23]; El-Beltagi et al., 2017[22]; El Gharabawy et al., 2019[45].

In the current work, hematoxylin and eosin stained sections of groups (B) revealed that the testes of the adult male rabbits were showing the detachment of germ cells from Sertoli cells and being sloughed in the lumen of the seminiferous tubules as seen in the present work might be resulted from the loss of adhesion between spermatogenic cells preventing their further maturation [46].

In addition, the loss of cell cohesiveness may be attributed to destruction of the cellular processes of Sertoli cells that fill the spaces between the germ cells leading to exfoliation of the spermatogenic cells into the lumen of the seminiferous tubules[47].

Evidence has emerged from several studies suggesting that Sertoli cell damage was due to disruption of tight junctions in blood testis barrier[47].

Such changes as separation of the basement membrane of spermatogonia and Sertoli cell is in fact signs of change before the apoptosis. In fact, the connection between Sertoli cells and germ cells is to increase lifetime germ cells. Newton et al., 1993 showed that in vitro intercellular connections are done through cadherin germ cells are increased lifetime germ cells[48].

Sertoli cells play an important role in normal spermatogenesis and in different ways have contact with Spermatogenic cells. Also Sertoli cells have receptors for FSH[49] and testosterone[50] that the role of these hormones than the Sertoli cells function shows.

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On the other hand, the detachment of spermatogenic cells from the basement membrane will indicate an altered interaction with basement membrane. Similar kinds of alterations are also observed after use of many anticancer drugs especially reported with mitomycin C[51].

The results of the present investigation may indicate that gemcitabine impairs spermatocytes differentiation due to cell injury or suppression of testosterone which plays an important role in the regulation of spermatogonial differentiation.

Spermatocytes / Sertoli cells cytoskeleton is testosterone dependent where the junction between spermatogenic cells is dispersed in the absence of testosterone. Low testosterone level might be due to Leydig cells damage[52].

Distorted and necrotic spermatocytes, reduction in the numbers of spermatogonia, primary and secondary spermatocytes was shown in our investigation. In support of this finding there are some reports showing that anticancer drugs could induced apoptosis in germinal epithelium[53,54].

On the other hand, apoptosis has a critical role on the removal of damaged spermatogonial cells to prevent the formation of abnormal sperms[55]. It is also shown that spermatocytes that fail to complete their mitotic division are removed by apoptosis[56]. It appears that gemcitabine as a chemotherapeutic agent induces apoptosis on spermatogenic cells.

Apoptosis is a physiological process that contributes to keeping the cell number in testicular tissue and helps to remove damaged cells, but excessive apoptosis could cause destruction of male reproductive function[57].

The increased rate of the incidence of apoptosis in gemcitabine-treated animal testes as a result of inhibition or disruption of cells' DNA synthesis may be the mechanism by which this drug induces toxicity in the animal body. In addition the oxidative stress in the testes has already been considered as potent inducers of cell apoptosis[58].

Oxidative stress, a state related to increased cellular damage triggered by oxygen and oxygen-derived free radicals known as reactive oxygen species (ROS), has been identified as one of the
many mediators of male infertility by causing sperm dysfunction. During this process, augmented production of reactive oxygen species overwhelms the body's antioxidant defenses[59].

The present results demonstrated pyknotic nuclei in some spermatogonia. Such findings are in agreement with that reported by El-Beltagi et al., 2017 on the effect of gibberellic acid on the seminiferous tubules of testis of adult albino rat[22].

Kroemer et al., 2009 attributed the nuclear pyknosis to be a feature of apoptosis[59].

Otherwise, Kumar et al., 2013 reported that pyknosis is a pattern of nuclear changes related to cell necrosis and characterized by nuclear shrinkage with increased basophilia as its DNA condenses into a solid shrunken mass[60].

Moreover, the results of the current study showed pyknosis of the nuclei of the spermatogenic series which was similar to the findings of other authors who studied the toxic effects of cadmium on different organ systems of rodents and revealed pyknotic nuclear changes, particularly in the testis. Furthermore, many seminiferous tubules showed marked atrophy with degeneration and loss of the seminiferous epithelium[41].

In the present study, the spermatogenic cells have been reduced to single layer, showing complete halt of spermatogenesis. In most of the tubules studied the spermatogenetic halt was evident by reduced epithelial cell height and lack of sperms in the lumen. Injection of imatinib mesylate to mice gives similar results, in less than 2 weeks[61]. Decrease in epithelial height reported after injection of doxorubicin to mice[62].

Decreased in the thickness of germinal epithelium noticed in our study, is partly due to defect in spermatogonia and cease their division. Some researchers reported it was due to a reduction in mitosis and DNA changes following treatment with chemotherapeutic drugs[63].

In this work, parts seminiferous tubules showed many vacuoles of variable size in between the germinal epithelium. It was reported that atrophy of the seminiferous tubules was manifested by intraepithelial vacuoles. These atrophied tubules were lined by single layer of germ cells and Sertoli cells[64]. Appearance of intraepithelial vacuolations may be due to intraepithelial edema and altered intercellular connections, due to acute cytological toxicity of the drug used. Similar intraepithelial vacuolations are reported in mice treated with Neem tree extract[65], Brahmi leaves[66] and diaza cholesterol dihydrochloride[67]. Occasional such intraepithelial vacuolations are observed in the current study.

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As regards the vacuolated cytoplasm of spermatogenic cells, this could be attributed to lipid peroxidation with consequent damage to the cell membrane caused by gemcitabine, as well as membranes of the cell organelles with subsequent increase in their permeability[68].

In the present study, some seminiferous tubules showed irregular thick basement membrane and thin myoid cells with flat dense nuclei**.** This thickening might be attributed to increase in the amount of collagenous fibers that could result from either over production of collagen fibers by fibroblasts or decreased rate of collagen phagocytosis[69]. Any change in the proportion of collagen fibers and myoid cells may prevent the appropriate release of spermatozoa from the germinal epithelium into the lumen[70].

Disorganization of spermatogenic cells, an interruption in spermatogenesis process and the seminiferous tubules are devoid of sperms was also showed in this study. Such findings are in agreement with that reported by Abu Baker , 2009 on mice testes[6].

Our result also was demonstrated the disappearance of interstitial cells of Leydig and necrotic Sertoli cells in the rabbits treated with gemcitabine. These results are in accordance with that found by Boekelheide, 2005[71] and Blanco et al., 2009[72] in mice exposed to low doses of cadmium.

Leydig cell atrophy can be responsible for the reduction in serum testosterone levels. Therefore, changes in the seminiferous tubules, which were observed in the current histopathological study, may result from hormonal alterations induced by gemcitabine and may not be a direct effect of the drug.

Widening of the intercellular spaces of the seminiferous tubules was observed in this study with accumulation of fibers had been observed in their lumen, indicated that sloughing of the seminiferous epithelium which was accumulated in the centre of the seminiferous tubules form of atrophy and degenerated tissue. El-Beltagi et al., 2017 was explained that by the disruption of tight junctions of blood–testis barrier, upon exposure to the reactive oxygen species (ROS), leading to entry of excess water and toxic agents between the spermatogenic cells, with consequent widening of the intercellular spaces[22].

The previously mentioned structural alterations in the testis could be explained by the accumulation of gemcitabine in different organs, and hence the testis where it caused testicular damage. These pathological changes could be contributed to the drug toxic effect on the normal cells.

Gemcitabine is targeting cells in their multiplication phase or any other phase in the cell cycle and damaging their DNA. The toxic effect of gemcitabine is related to its ability to bind with DNA molecule, thus it is efficient in inhibiting DNA synthesis during multiplication phase. It is well known that spermatozoa formation is a series of direct and indirect cellular divisions resulted in cellular multiplication where each germinal cell divided into four spermatids with DNA multiplication. DNA represents the basic genetic material of cells and carries the genetic code that transferred by spermatozoa. These explained the distortion and reduction in numbers of spermatocytes and spermatozoa[73].

According to this results on male reproductive tract of domestic rabbits can be considered the testis as a target for gemcitabine in the treated animals which expose to this compound can cause histological damage on testicular tissue.

Sections from the testes of animals received therapeutic dose of gemcitabine and treated by grape seed extract, group (C) were showing partial preservation of the normal structure of the seminiferous tubules. Most of them more or less resembled the normal structure.

Grape seed extract, now available as a dietary supplement, contains a number of polyphenols including procyanidins and proanthocyanidins, which are powerful free radical scavengers[74].

The exact chemical characteristics and the mechanism of action of grape seed extract have not yet been completely understood, and the experimental findings are inconsistent. Some studies have shown that grape seed extract has beneficial effects in prevention of colorectal cancer[75] and it also prevents low-density lipoprotein oxidation, predominantly through its antioxidant properties[76].

Presence of antioxidant property in grape seed extract (GSE) has shown to exert a much stronger oxygen free radical-scavenging effect. Compounds those are having antioxidant properties able to protect the cells against oxidative stress[77]. Many papers published in recent decades have documented that, the putative beneficial effects of polyphenols are frequently related to their antioxidant activity. There in all these implying beneficial role of grape seed extract[9].

Another effect of grape seed extract is its DNA protection. It inhibits oxidative damage to DNA in the ischemia-reperfusion injury model. Grape seed extract also blocks cell death signaling.

In addition, grape seed extract (GSE) has been reported to exert anticarcinogenic effects[78].

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The beneficial effects of grape seeds extract are well documented in earlier studies.
Pari and Arumugam 2008[79]; Safa et al., 2010[80]; Zhao et al., 2014 [16]; Wang et al., 2019 [81].
This partial preservation could be attributed to the ability of grape seed extract to overcome the oxidative stress and upregulate the endogenous antioxidant defense system. In addition, grape seed extract was found to be beneficial in ameliorating the degree of apoptotic cell death[82].
On the basis of the histological findings obtained from this study, it could be concluded that gemcitabine caused marked alterations in the structure of the seminiferous tubules of the rabbit testis that may be minimized by administration of grape seed extract. These findings are in accordance with the results that reported by El-Beltagi et al., 2017 in the effect of gibberellic acid on the seminiferous tubules of testis of adult albino rat and the possible protective role of grape seeds proanthocyanidin extract [22].
The results of the present study confirmed that grape seeds exerts potential effects against oxidative stress, and may inhibit the production of free radicals, as described in a previous study in effects of alpha tocopherol on cadmium induced toxicity in rat testis and spermatogenesis [83].
The results of our study in (group C) demonstrated that there was an improvement in the seminiferous tubule structure, with mild cytoplasmic vacuolation of few spermatogenic cells. The interstitial space was wide and cells of Leydig were seen seemingly normal. Mild congestion was present in the interstitial blood vessels. These findings are consistent with those reported in other studies [23, 38,84, 85].
The protective effect of grape seeds extract might be attributed to not only its potential effects against oxidative stress induced by gemcitabine, but also the inhibition of the free radical production with subsequent maintaining of the affected antioxidant protection system.
Oxidative stress is one of the major pathogenic mechanisms, and can cause morphological and functional damage to the testis. Oxidative stress in the testes has already been considered as potent inducers of cell apoptosis. Our findings simply revealed that grape seeds extract treatment significantly inhibits testicular cell apoptosis, which suggests that apoptosis may be one of the underlying mechanisms by which grape seeds extract protects against gemcitabine induced defective spermatogenesis.

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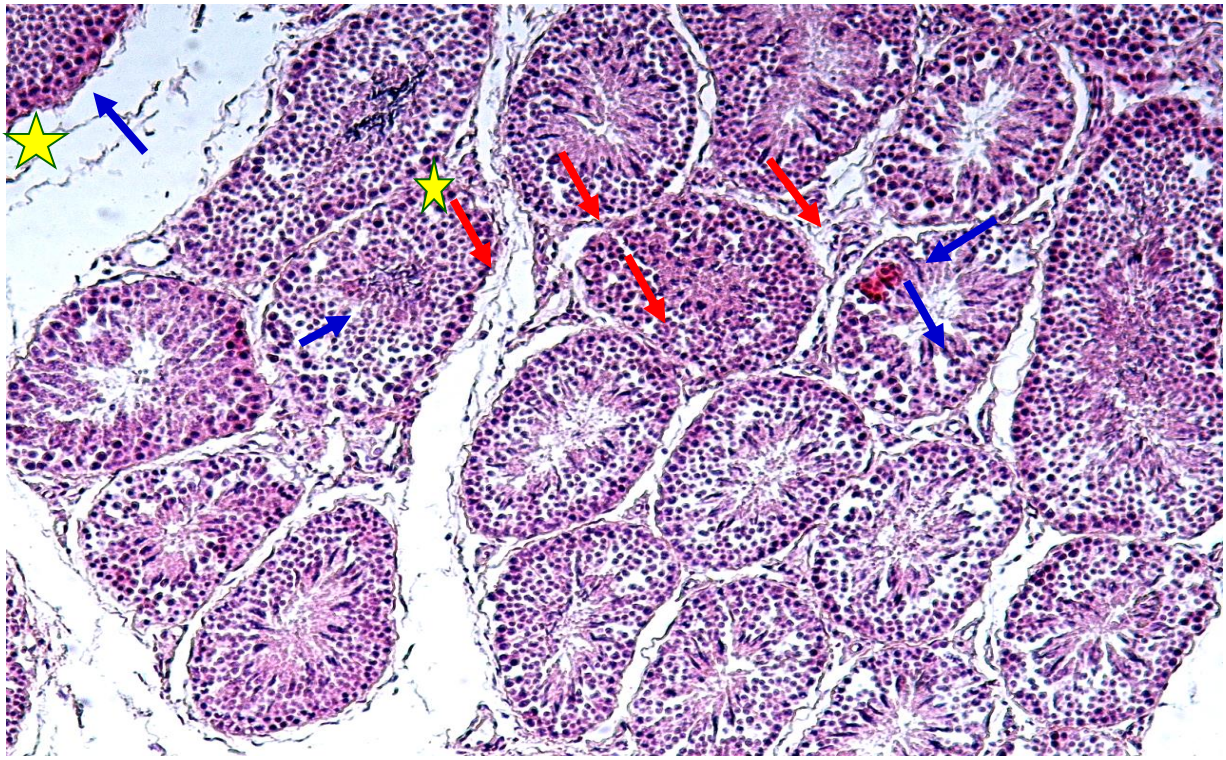

Figure 1a: Photomicrograph illustrates the general structure of the normal rabbit testis (control group).
Note, numerous septae divided the testicular parenchyma into numerous lobules (blue arrow). Each lobule contained the seminiferous tubules (red arrow). Blood vessels (star). H&E stain. (X10).

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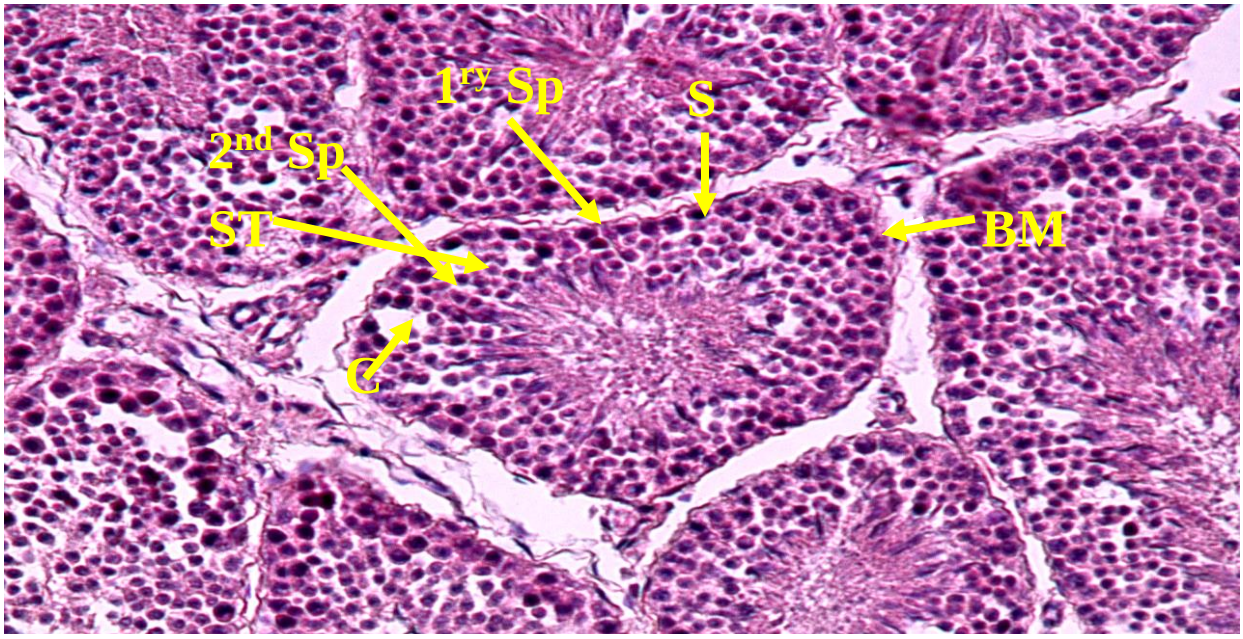

Figure 1.b: Photomicrograph illustrates the normal rabbit seminiferous tubules (control group). Note, the seminiferous stratified epithelium was resting on basement membrane (BM) and contained spermatogonia (S), primary spermatocytes (1ry Sp), secondary spermatocytes (2nd Sp) and spermatids (ST). Intertubular connective tissue (C). H&E stain. (X20).

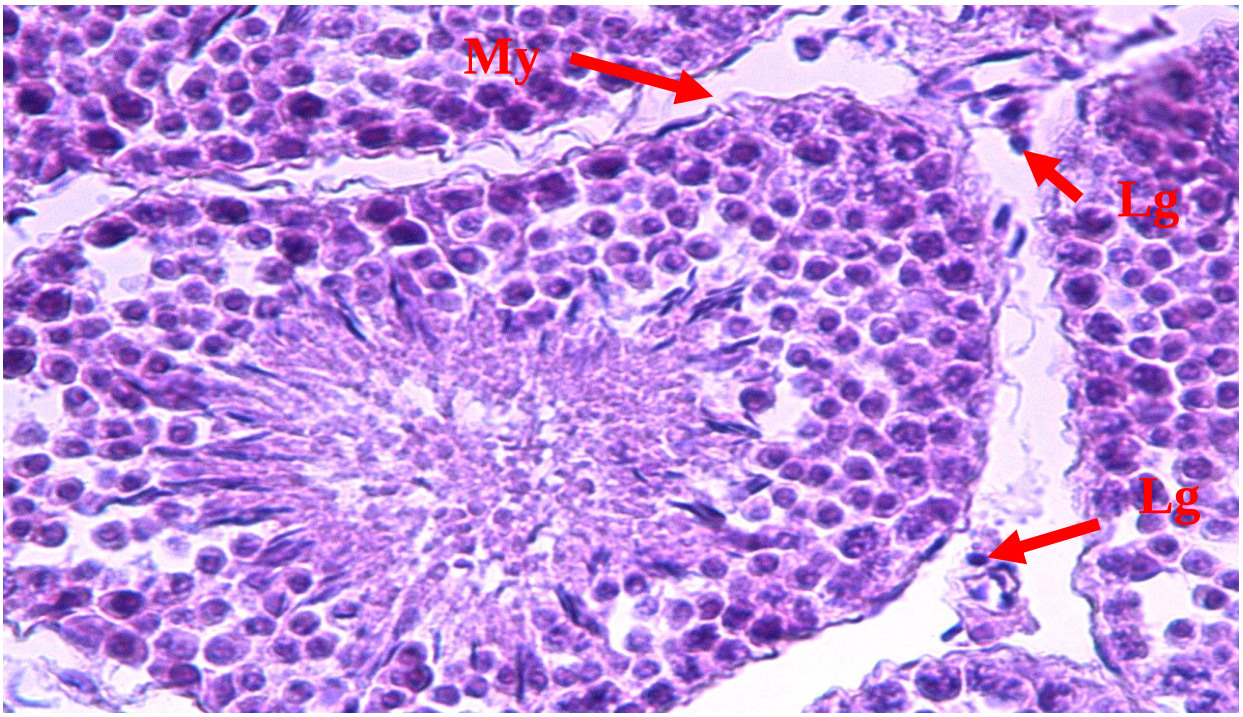

Figure 1.c: Photomicrograph illustrates the normal rabbit seminiferous tubules (control group). Note, in between the seminiferous tubules there were pyramidal shaped Leydig cells (Lg) and continuous sheet of single layered flattened polygonal myoid cells (My) outside the basal membrane.
H&E stain. (X40).

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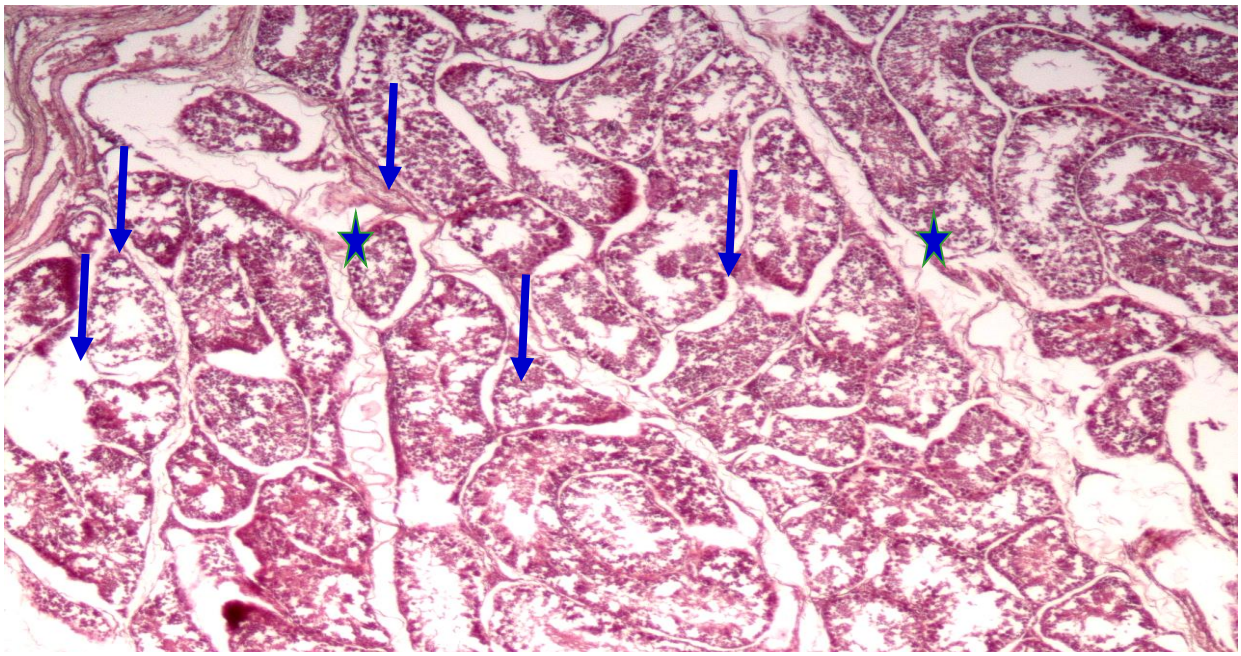

Figure 2: Photomicrograph illustrates cross section of testis treated with gemcitabine (B group).
Note, disorganization and distortion of the seminiferous tubules. Numerous seminiferous tubules were sloughed and atrophic (arrow). Slightly congestion of interlobular septae (star). H&E stain. (X10).

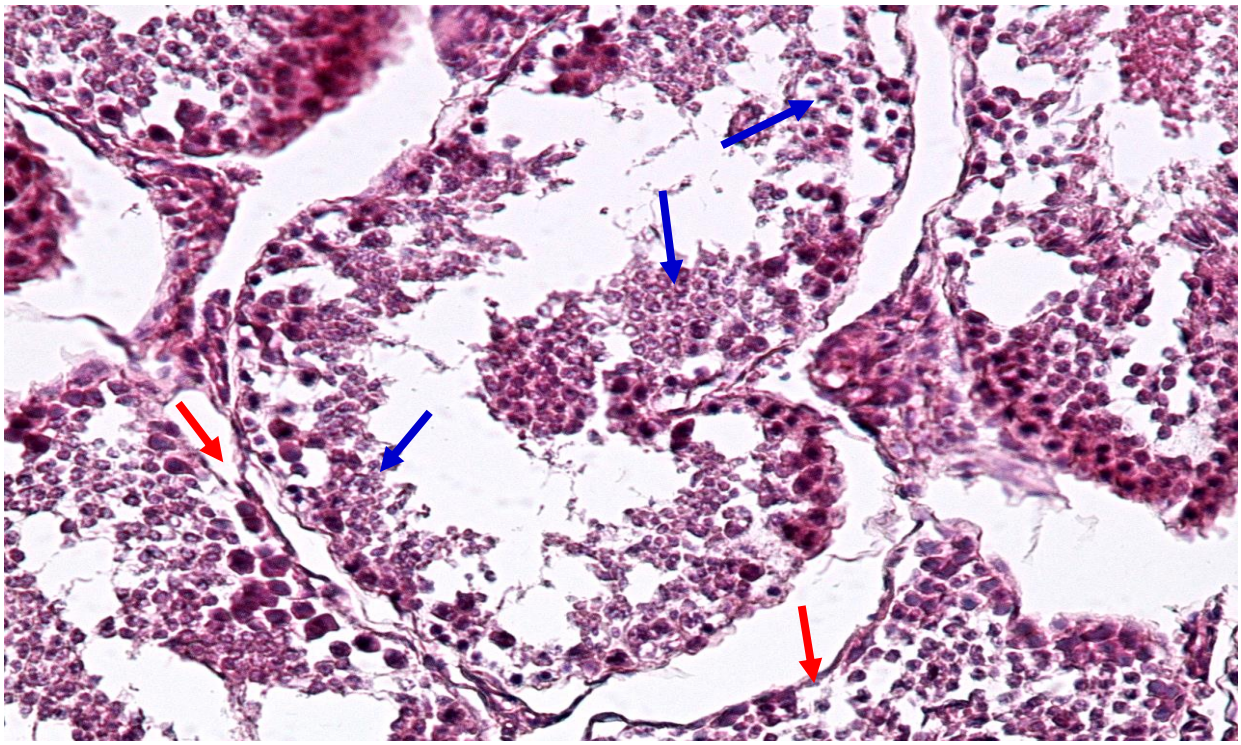

Figure 3: Photomicrograph illustrates cross section of the seminiferous tubules treated with gemcitabine (B group). Note, distorted and necrotic spermatocytes (blue arrow), reduction in the numbers of spermatogenic cells, separation of tubular basement membrane (red arrow). H&E stain. (X20).

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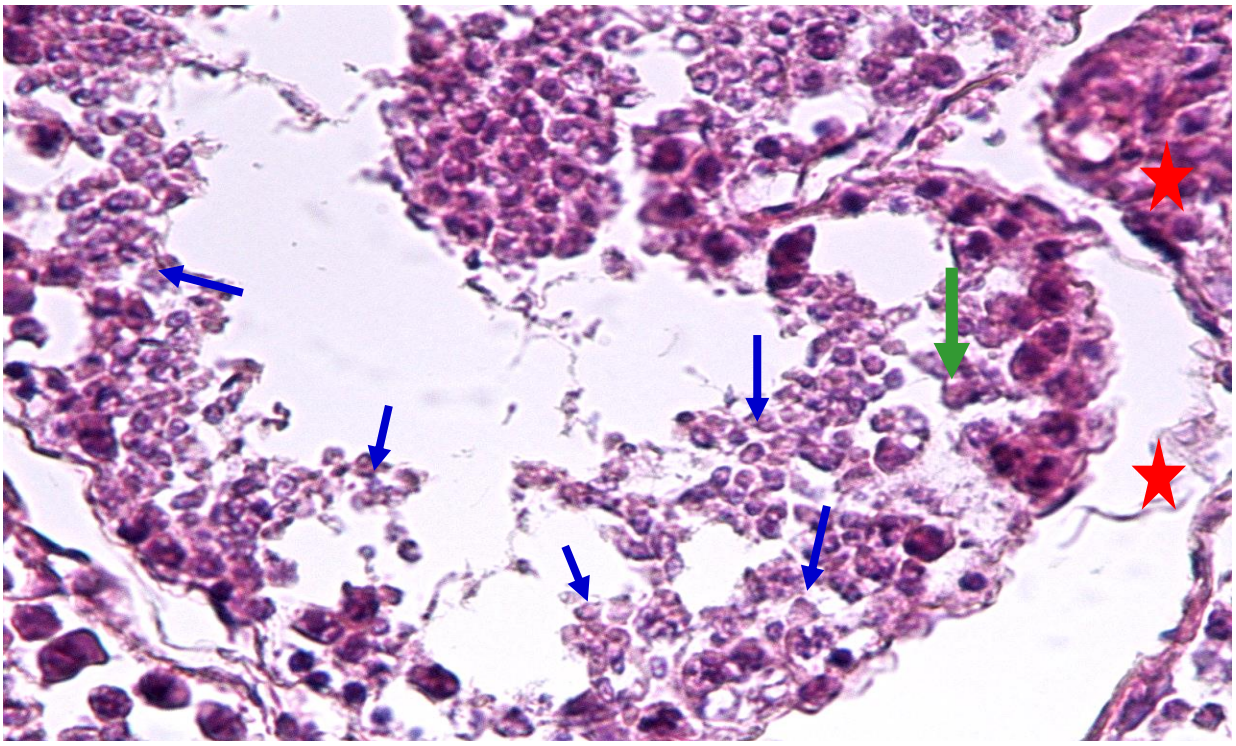

Figure 4: High magnification photomicrograph illustrates cross section of the seminiferous tubules treated with gemcitabine (B group). Note, degenerated spermatogenic epithelium and cellular detachment. Pyknotic nuclei of the spermatogenic cell (blue arrow) Necrotic Sertoli cells (green arrow) and flat myoid cells (star). H&E stain. (X40).

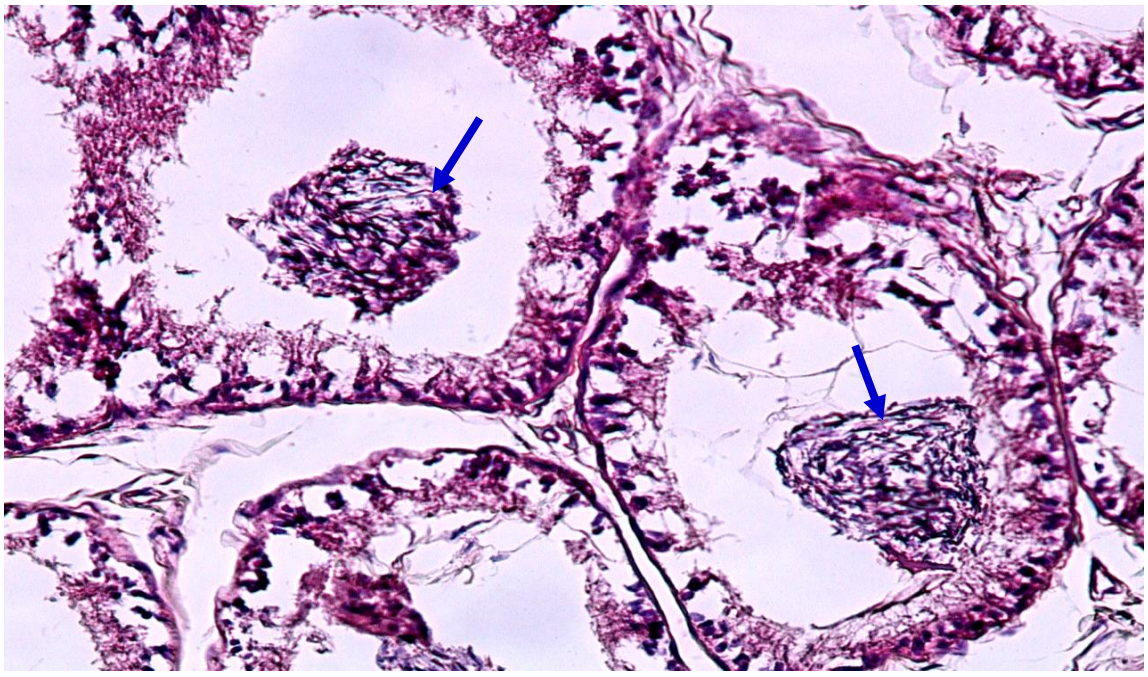

Figure 5: Photomicrograph illustrates cross section of the seminiferous tubules treated with gemcitabine (B group). Note, accumulation of fibrosis within the lumen of the seminiferous tubules (arrow). H&E stain. (X20).

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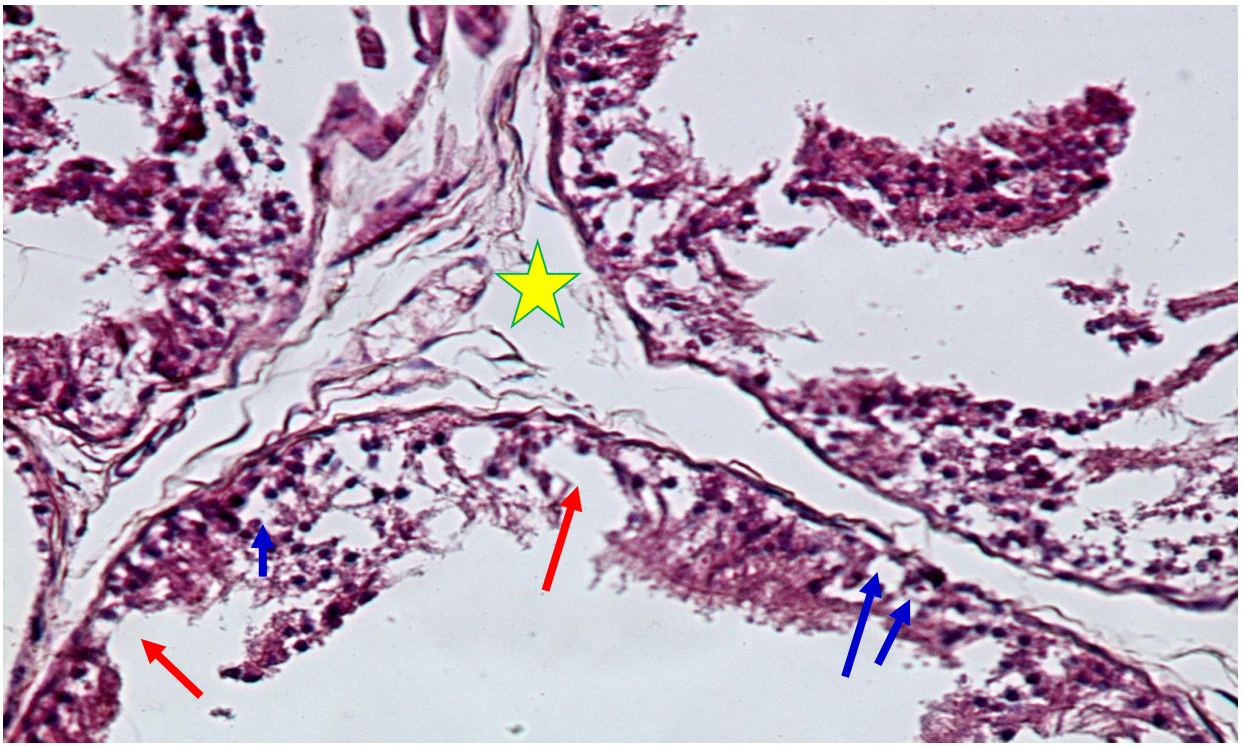

Figure 6: Photomicrograph illustrates cross section of the seminiferous tubules treated with gemcitabine (B group). Note, vacuolization (blue arrows) and distorted in testicular tissues. Disappearance of interstitial cells of Leydig (star), necrotic Sertoli cells (red arrow) and pyknotic lesions in spermatogonia. H&E stain. (X20).

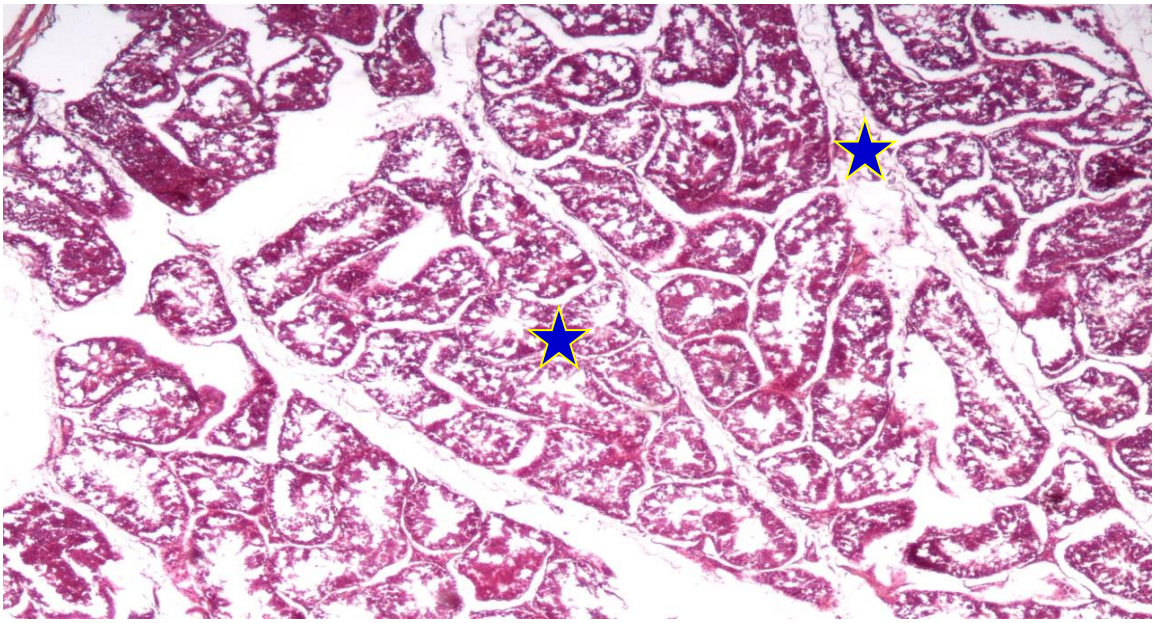

Figure 7: Photomicrograph illustrates cross section of the seminiferous tubules of group (C). Note, reorganization of tubules. Most of them more or less resembled the normal structure. Mild congestion in interlobular sptae (stars). H&E stain. (X10).

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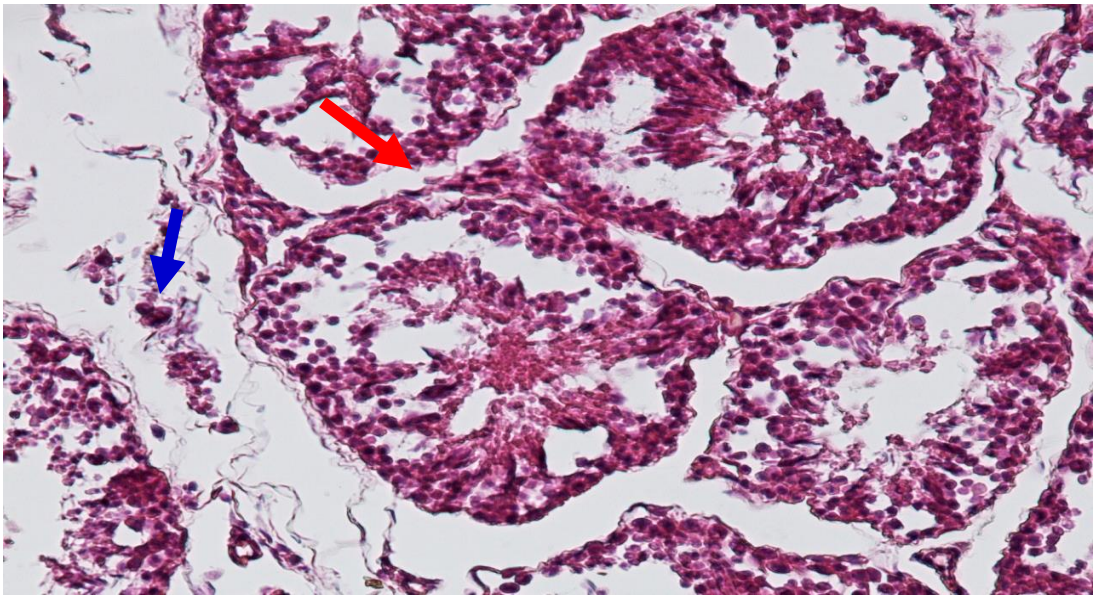

Figure 8: Photomicrograph illustrates cross section of the seminiferous tubules of group (C). Note, there was an improvement in the seminiferous tubule structure, with mild cytoplasmic vacuolation of few spermatogenic cells. Leydig cells (blue arrow). The basement membrane (red arrow).
H&E stain. (X20).

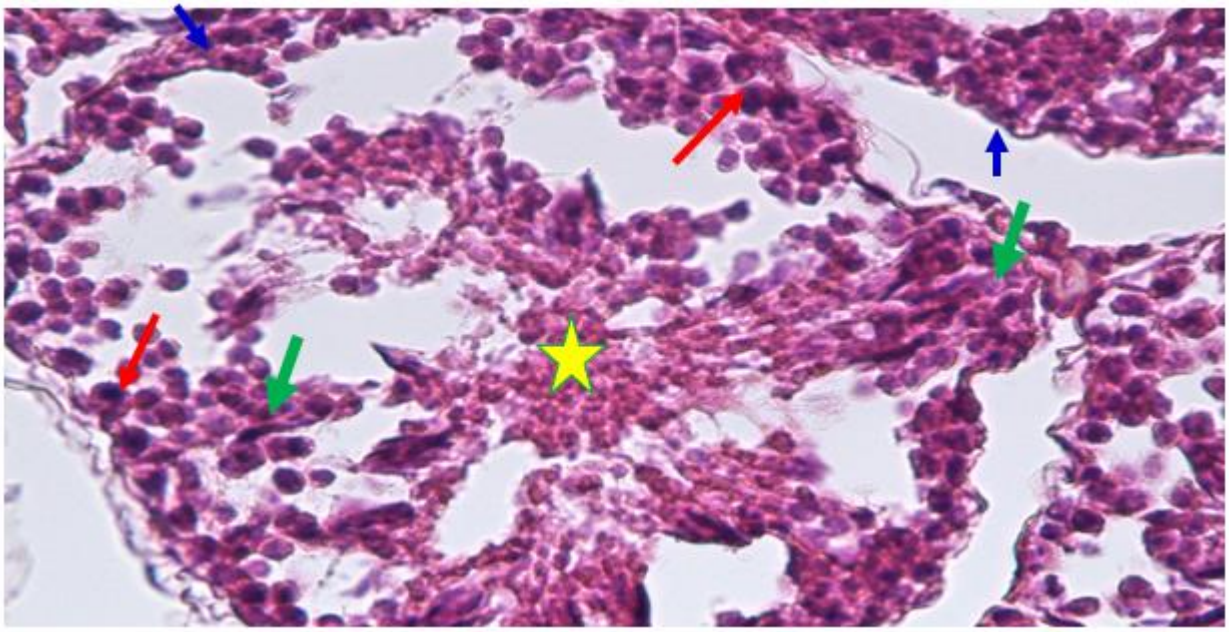

Figure 9: High magnification photomicrograph illustrates cross section of the seminiferous tubules of group (C). Note, limited histological changes as slightly blood congested in the center of their tubules (star). Spermatogonia with round nucleus and condensed chromatin (red arrow). Myoid cells (blue arrow) Sertoli cell (green arrow). H&E stain. (X40).

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