Effect of pyridine on the histological structure of adult male rabbit's testes *Orectolagus cuniculus*

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Abstract

The current study aimed to identify the effect of pyridine on the histological structure of the testis in rabbits. The study included 15 rabbits, with different ages ranging between 6-9 months and with different weights of 1.100 -1.653 kg. The animals were divided into three groups, and each group contained 5 rabbits. The animals of each group were placed independently and were as follows: the first group was the control group, and the second and third group were the experimental groups and included 5 rabbits for each group. The animals of the three groups were injected with pyridine subcutaneously at a concentration of (100,200) µl of pyridine / kg of body weight for a period of time 30 days with one concentration for each group. After the completion of the experiment, the animals were sacrificed, dissected and neutered, and their tissue sections were prepared . The results of the current study showed clear changes in the weights of rabbits treated with pyridine, as the weights of all experimental group animals decreased, and this decrease was affected by the increase in concentration, reaching between (0.850-1.400) kg. The treatment with pyridine also led to clear pathological changes in the histological structure of the testis, as it led to a change in the thickness of the wall of the seminiferous tubes and their shrinkage, as their shape became wavy and irregular, and atrophy in some seminiferous tubules, and the germ cells had shedding and depletion and their collection in the lumen of the seminiferous tubules and dissolution in some Sertoli cells. It was also found that the sperm cells had separated, the epithelial layer had separated from the basement membrane, and hemorrhage had occurred in some areas. In addition, Leydig cell slackening, degeneration, and increased interstitial space occur. In the study, it was also found that the seminiferous tubules ruptured, the Sertoli cells degeneration, and the cells of the basement membrane were deformed and aggregation in the lumen of the seminiferous tubules. Also, there was an expansion in the diameter of the seminal tubules, which reached (50.2 and 54.6) micrometers, respectively, compared to the control group, which amounted to (45.8) micrometers .

Keywords: histological structure; adult male rabbit's; *Orectolagus cuniculus*

1. Introduction

Pyridine is one of the natural compounds on which the formation of other chemical compounds is based in a number of applied industrial fields such as agricultural chemistry and the pharmaceutical industry. The increasing demand for it has led to the development of synthetic reactions of simple compounds such as acetaldehyde and ammonia. Annually, more than 20 thousand tons of the compound are produced all over the world, and these new prepared compounds were identified through several technologies (FT.IR, H.NMR, MASS) (Mahmood and Aljamali, 2020). The heterocyclic aromatic has the molecular formula C5H5N (Altaf ,et al, 2015). It is used in industry as a chemical solvent and reagent, as well as in the synthesis of DNA in the laboratory, and it is prepared by cyclization reaction and condensation reaction (Mahmood and Aljamali, 2020). Pyridine was first made in 1876 from acetylene and hydrogen cyanide (Shimizu ,et al, 1993). The most abundant source is coal tar and coke ovens, and it has been commercially produced from coal tar sources since the 1920s. During the fifties of the last century, synthetic processes developed to provide alternatives to sequestration from coal tar sources (Scriven ,et al, 1996);(Santodonato, et al, 1985). The emission of waste containing pyridine causes great harm to human health and environmental quality (Gupta ,et al, 2019).The male reproductive system consists of 1- pairs of testis, the primary gonads, located in the scrotum. Spermatozoa and male reproductive hormones produce testosterone. 2- Reproductive ducts, such as the epididymis, vas deferens, and ejaculatory duct. 3- Accessory sex glands, which include a pair of seminal vesicles, a single prostate, and a pair of bulbourethral glands. 4- Penis. (Gunasegaran, 2010) It is also known that chemicals in the environment have a significant impact on the male reproductive system and that contribute in one way or another to infertility events among males (Oliva ,et al, 2001). The testicles have two main functions : 1- Production of male reproductive cells (sperms)2- Production of male sex hormones androgens, testosterone (Te) hormoneThe testis contains a network of twisted tubules called seminiferous tubules and is usually surrounded by interstitial tissue and Leydig cells that secrete (Te), which targets the seminiferous tubules and affects the process of spermatogenesis pituitary gland from the hypothalamus, and there are factors that have an impact on the functions of the testicles are age and environmental conditions such as temperature and duration of lighting as well as food (Guyton and Hall, 2006). The seminiferous tubules develop during the period of 43-50 days after conception and from the ninth to the tenth week, Leydig cells begin to secrete (Te). During fetal development, when this hormone is secreted, it transforms undifferentiated structures into external male reproductive organs such as the penis and scrotum, which is the sac that contains the testicles. In its absence, these external structures grow into female genital organs (Raven, 2002).

doi.org/10.31838/hiv22.02.663 Received: 30.06.22, Revised:12.07.22, Accepted: 16.08.22

2. Materials and working methods

Dosage preparation

The dose of pyridine was prepared based on the LD50, which is valuable in rabbits (µl of pyridine/kg of body weight). Two doses of pyridine were selected to test its toxicity, which are (100 and 200) µl/kg. The weights of rabbits used in the experiment ranged between (1.100 - 1.653) kg, and the rabbits were injected with the required amount of pyridine, according to the required dose, and once a day for 30 days for each concentration. It was possible to calculate the amount of pyridine injected into rabbits used in this study based on the following equation: Whereas:

$\boldsymbol{x}$: represents the substance to be injected into the rabbits in the experiment, measured in microliters

$\boldsymbol{D}$: represents the specific dose of pyridine (C5H5N) which is (100,200) in µl/kg $\boldsymbol{Wrabbit}$: the weight of the rabbit used in the experiment in kgIn the current study, 15 male rabbits were used, obtained from the animal house in the Department of Life Sciences / College of Education for Pure Sciences / University of Diyala, at different ages ranging between 6-9 months and with different weights of 1.100-1.653 kg. It was divided into three groups and it was as follows: The first group represented the control group, the second and third group represented the experimental groups with a concentration of (100-200) µl/kg and 5 rabbits for each group. The dosing process for the second group with a concentration of 100 continued for 30 days, while the third group with a concentration of 200 lasted only for a period of 19 days. After completing the experiment, the animals were dissected and the testis were removed. Then the samples were fixed with formalin solution for 24 hours, then washed with tap water and transferred to 70% alcohol for preservation. The tissue sections were prepared according to the method used in (Suvarna ,et al, 2013).The tissue sections were stained using(Haemotoxylin and Eosin (H&E) Stain).After completing all the steps, the samples were examined and photographed using a light microscope equipped with a digital camera .

3. Results

It was observed in the transverse sections of the testis of male rabbits of the experimental group treated with pyridine at a concentration (100 µl/kg) for a period of (30 days/dose) that cellular changes occurred in the testis tissue represented by the occurrence of dissociation in the cells of the spermatic line and the separation of the epithelial layer from the basement membrane. The study showed that some of the walls of the seminiferous tubes appeared irregularly and wavy, as they contracted, and some of the walls thickened, in addition to that, vacuolation in the seminiferous tubes, degeneration in Sertoli cells and desquamation occurred. It was also seen that the cells separated from the basement membrane and aggregation in the lumen of the seminiferous tubules as in Figure (1) (2)

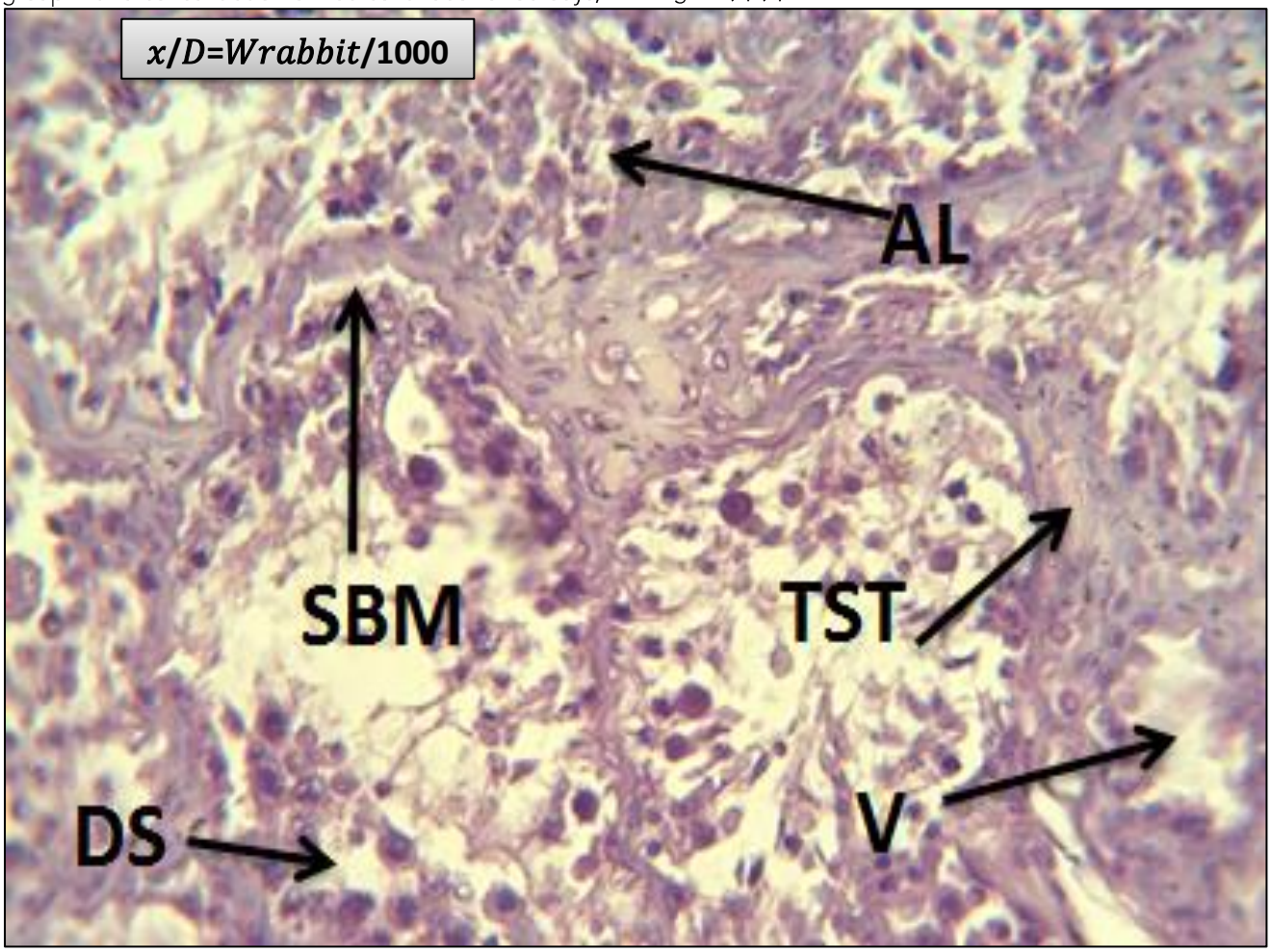

Figure (1) A cross section of the testis of male rabbits of the experimental group treated with pyridine at a concentration of 100 µl/kg for 30 days. DS: Sertoli cell degeneration, V vaculation in the seminiferous tubules, TST thickness in the seminiferous tubule wall, BM detachment of the basement membrane from the epithelial layer, AL Cells aggregation within the central lumen stain (H&E 40 x)

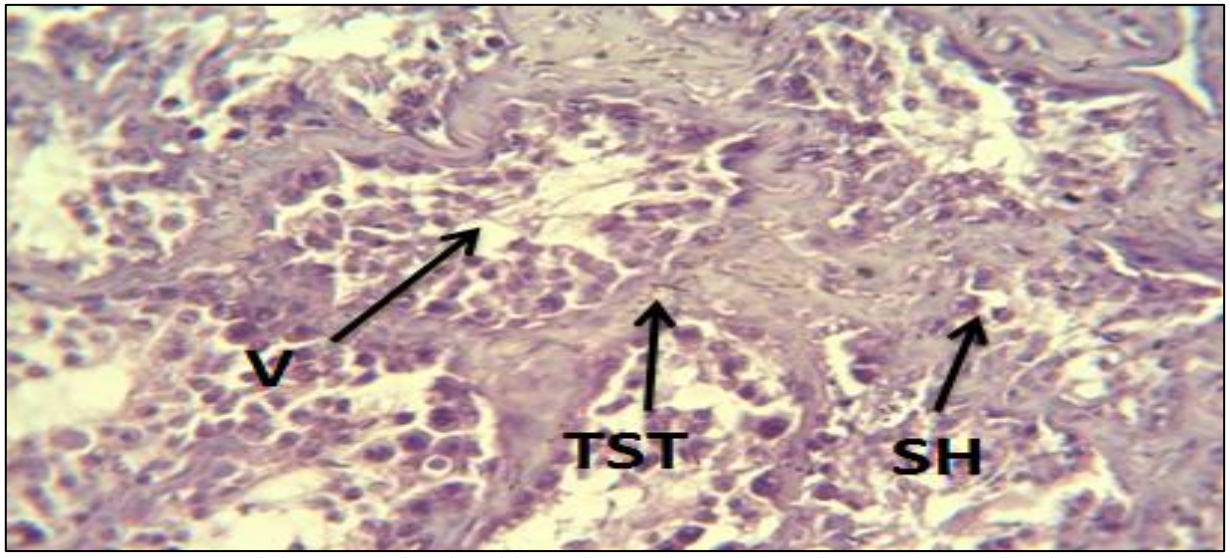

Figure (2) Cross-section of the testis of male rabbits of the experimental group treated with pyridine at a concentration of 100 µl/kg for 30 days. SH contraction of seminiferous tubules, V vaculation of seminiferous tubules, TST thickness of seminiferous tubule wall .stain (H&E 40x)The results also showed a thickening of the nuclei of some germ cells and their appearance in a dark color and a small size in the middle of the cells, as well as the expansion of the seminiferous tubule and its diameters changed. . It was observed in Figure (4) that the nuclei of some germ cells were decomposed by Karyolysis, whereby the nuclei had completely disappeared, and the cell was seen in a uniform color .

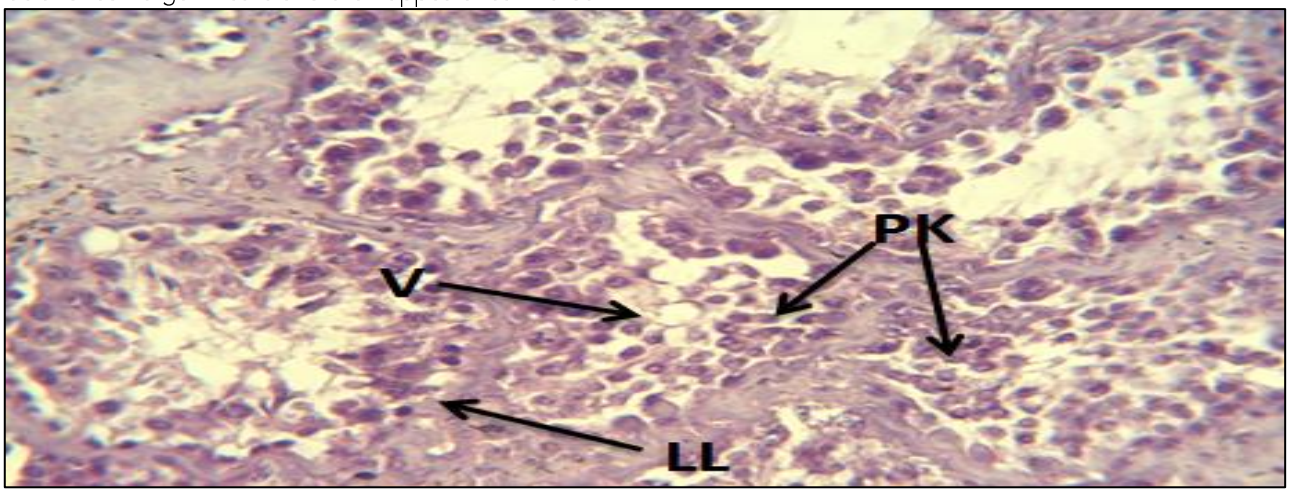

Figure 3. A cross section of the testis of male rabbits of the group treated with pyridine at a concentration of 100 µl/kg for 30 days. PK Pyknosis was observed, LL expansion of the median lumen of the seminiferous tubule, V vaculation. stain (H&E 100x)

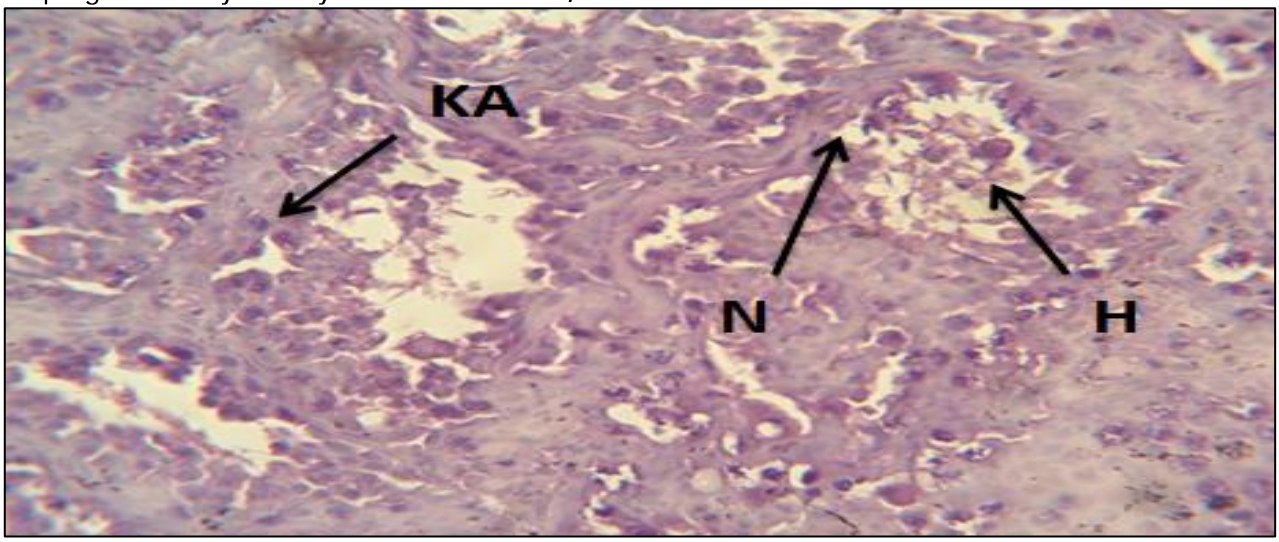

Figure (4) A cross section of the testis of male rabbits of the experimental group treated with pyridine at a concentration of 100 µl/kg for 30 days. KA Karyolysis observed nucleolysis, H germ cells, N necrosis in the testicular tubules. Stain (H&E 100x)The results also showed hydropic degeneration in some germ cells, as it was seen with a swollen appearance with dark-colored nuclei centrally located as a result of fluid aggregation inside them as in Figure (5)

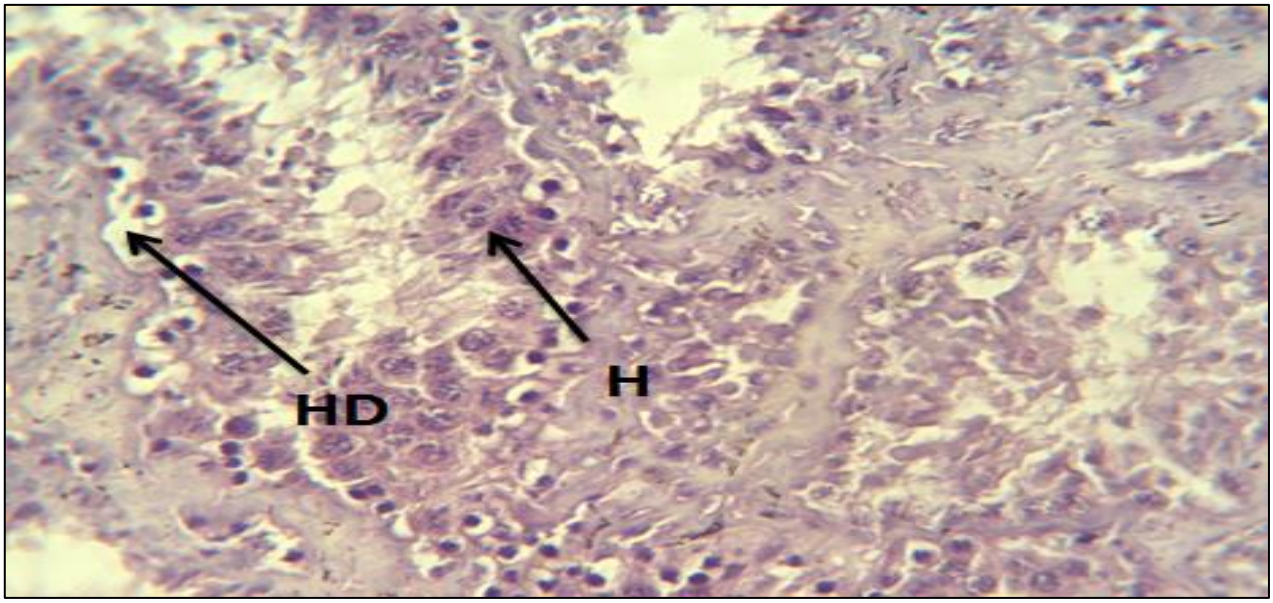

Figure (5) A cross section of the testis of male rabbits of the experimental group treated with pyridine at a concentration of 100 µl/kg for 30 days. HD Hydropic degeneration is observed, H germ cells. stain (H& E 40x) The results of the current study of testicular tissue treated with pyridine at a concentration of (200 µl/kg) of body weight for a period of 19 days/dose showed clear histological changes similar to the previous concentration, but they are more severe and negatively controlled, and represent damage to the occurrence of Distortion and Rupture in the capsule. It was also noted that there were clear histological changes represented by an expansion in the median lumen of the seminiferous tubules, where the average diameter of the tubule reached (54.6) µm compared to the control group, which amounted to (45.8) µm, as well as the appearance of small spaces between the cells of the spermatic line and an increase in the space between Sertoli cells, as well as degeneration was seen. Hydropic degeneration in some germ cells, as it was seen with a swollen appearance with dark-colored nuclei centrally located as a result of the aggregation of fluids inside them as shown in Figure (6)

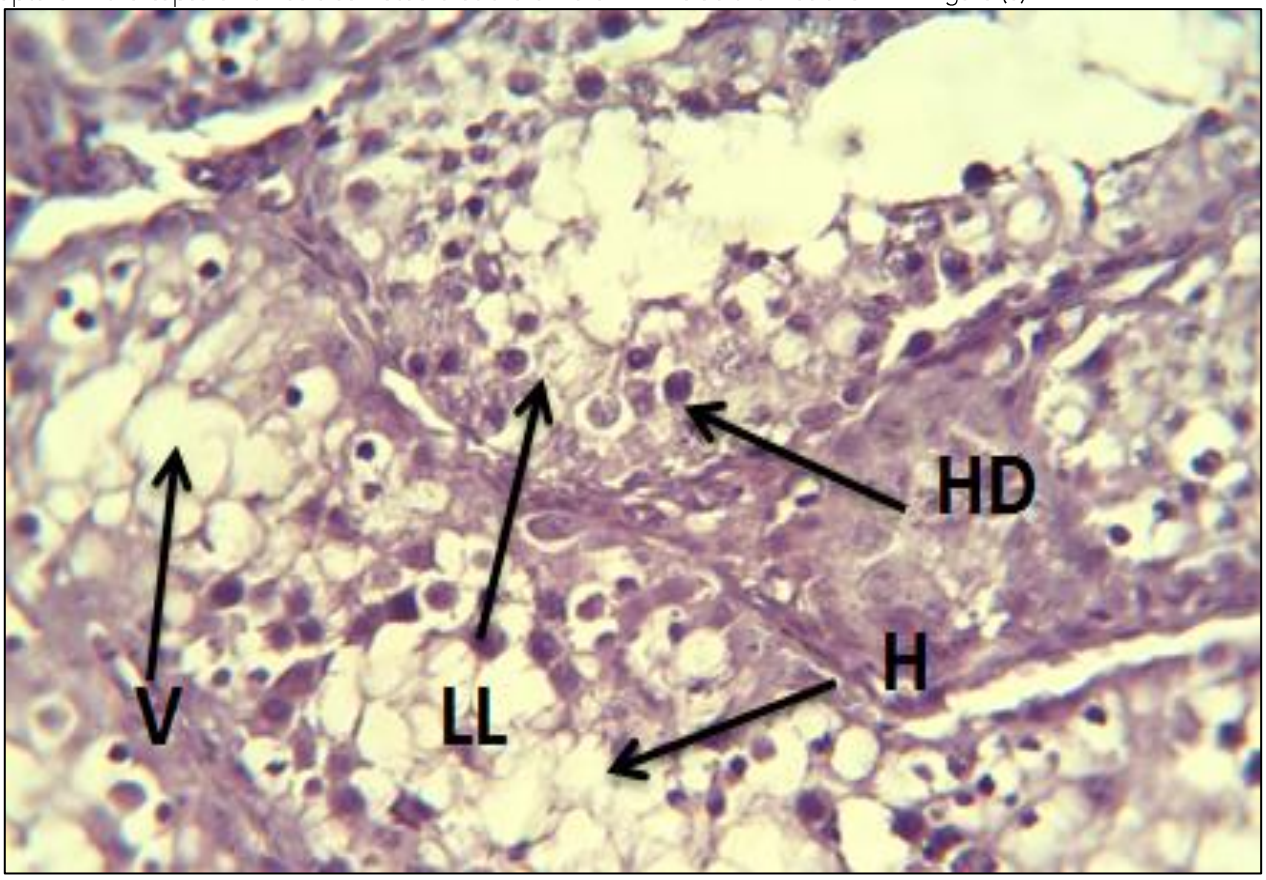

Figure (6) A cross section of the testis of male rabbits of the group treated with pyridine at a concentration of 200 µl/kg for 19 days. LL was observed, V vaculation between Sertoli cells, HD Hydropic degeneration, H germ cells . stain(H&E 40 x). The study showed that some of the walls of the seminiferous tubes appeared irregularly and wavy, as they contracted, and some of the walls thickened. In addition, it was shown that the vaculation of the seminiferous tubes, degeneration in Sertoli cells and desquamation, as shown in Figure (7). It was also seen that the cells separated from the basement membrane and aggregation in the lumen of the seminiferous tubules, as in Figure(8).

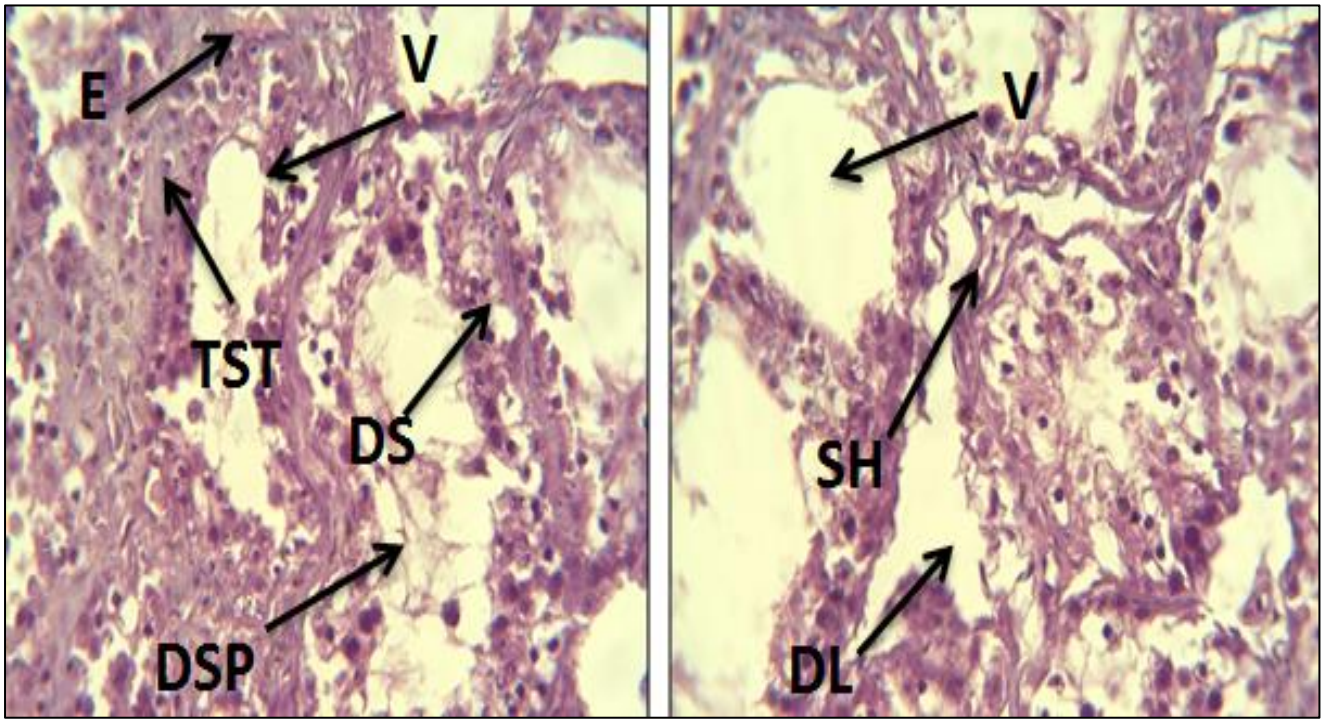

Figure (7) A cross section of the testis of male rabbits of the experimental group treated with pyridine at a concentration of 200 µl/kg for 19 days. SH contraction of the seminiferous tubules, TST thickness in the walls of the seminiferous tubules, V vaculation in the seminiferous tubules, DS degeneration of Sertoli cells, DL degeneration and increased interstitial space, DSP spermatids, E edema. Stain (H&E 40 x)

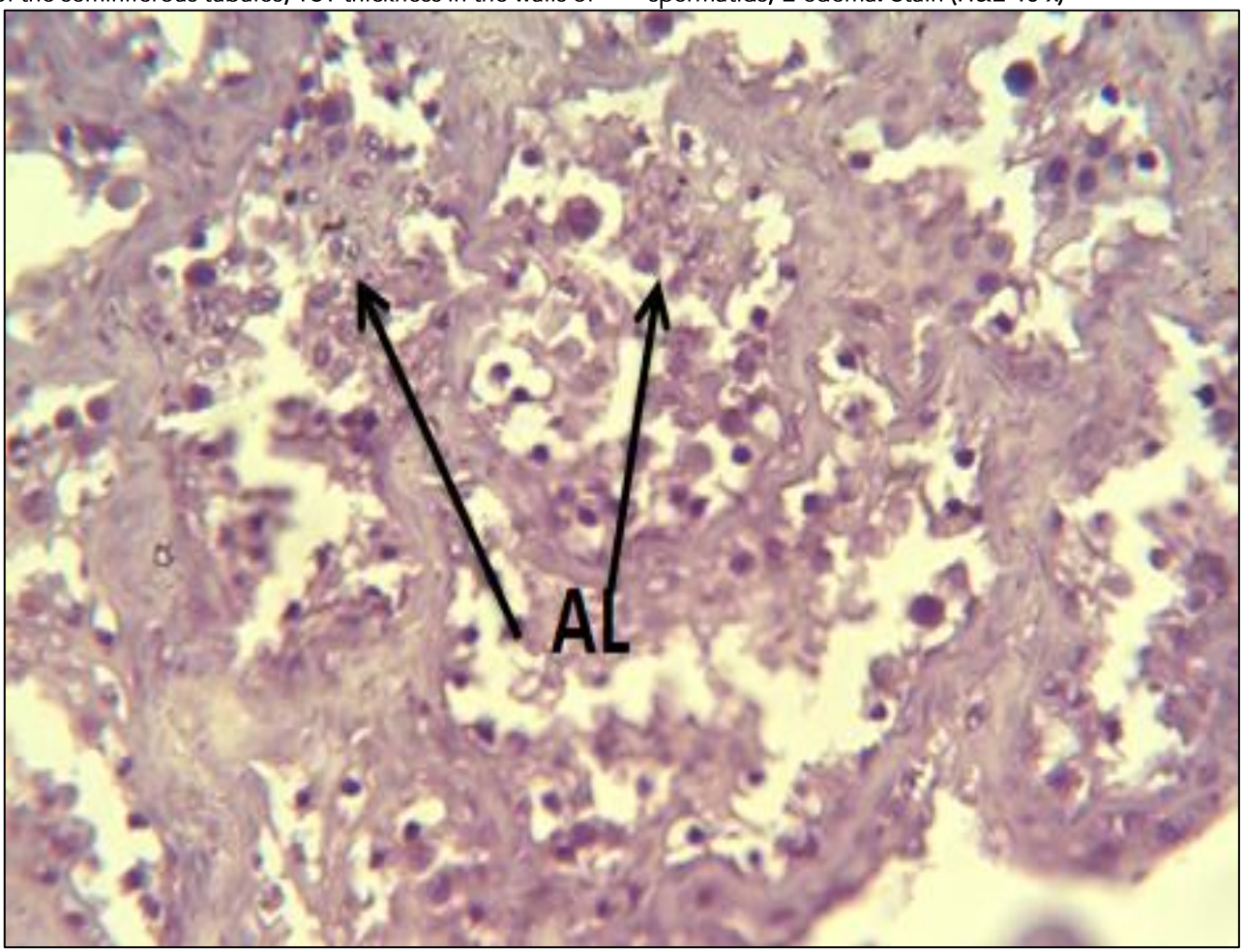

Figure (8) Cross section of the testis of male rabbits of the group treated with pyridine at a concentration of 200 µl/kg for 19 days. AL cell aggregation within the central lumen is observed. stain (H&E 40 x)It was also seen that the nuclei of some germ cells thickened, as they appeared in a dark color and a small size in the middle of the cells, and for others, Karyolysis occurred in some germ cells, where the nuclei completely disappeared, and the cell was seen in a uniform color as shown in Figure (9).

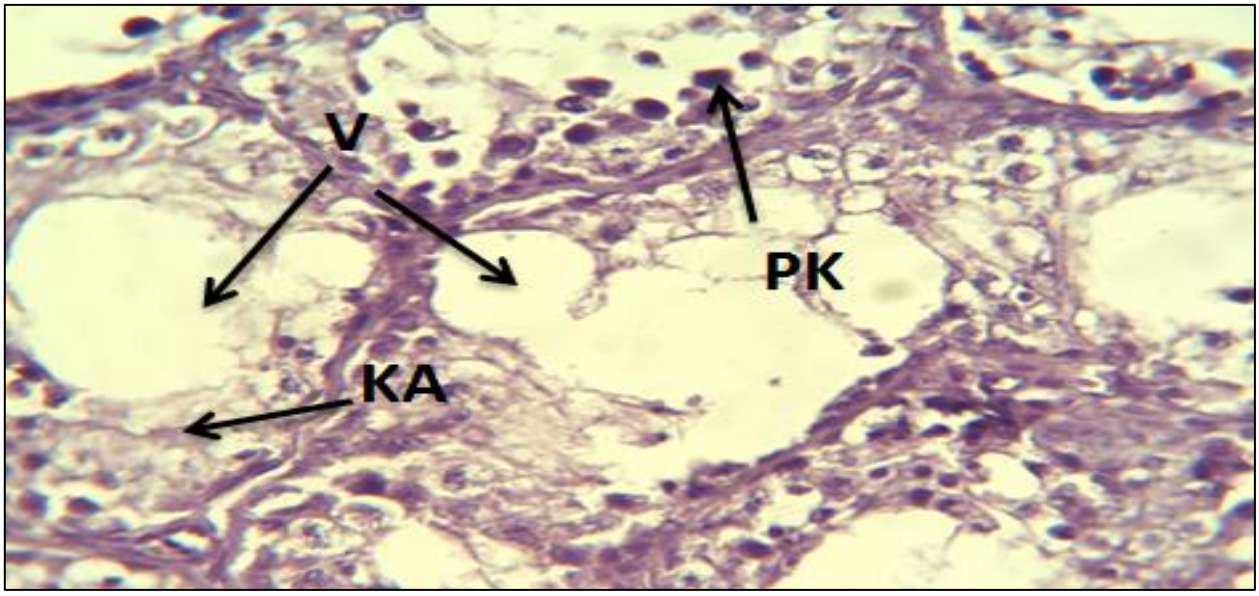

Figure (9) A cross section of the testis of male rabbits of the group treated with pyridine at a concentration of 200 µl/kg for 19 days. PK was observed, the occurrence of Pyknosis, V vaculation , KA of the occurrence of Karyolysis. Stain (H & E 40 x) It was also found to have an effect on Leydig cells and the interstitial tissue, where necrosis, lysis and the appearance of the ductus arteriosus occurred, as well as the appearance of phagocytic cells inside the lumen of the seminiferous tubules and a depletion of the germinal epithelium as shown in Figure (10).

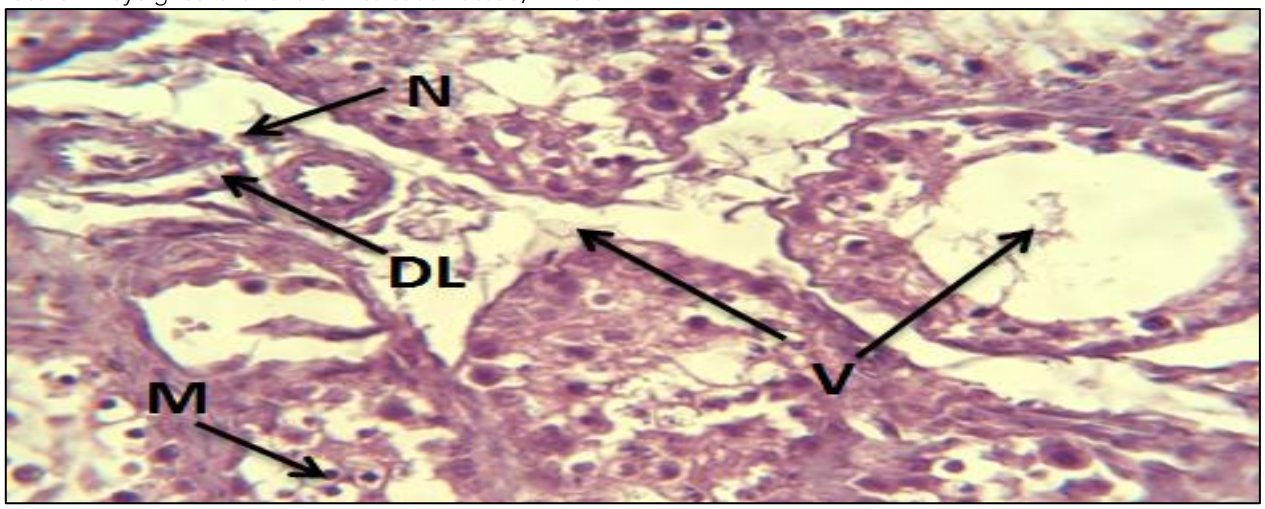

Figure (10) A cross section of the testis of male rabbits of the experimental group treated with pyridine at a concentration of 200 µl/kg for 19 days. M Macrophages, DL cells, Leydig cell degeneration and interstitial tissue, V vaculation, N necrosis, were observed. stain (H&E 40x).

4. Discussion

Another study (Mathur and D'cruz, 2011) showed the effect of environmental toxicants on the testis, and it was found that they have an effect on male reproductive health, as they affect natural estrogen, target sperm formation, steroid formation, and the function of both Sertoli cells and Leydig cells, as they stimulate the types of Reactive oxygen, thus causing oxidative stress state in different parts of the testicles.

The study of researchers (Ahmed ,et al , 2019) indicated as a result of the use of zinc oxide on the histological structure of the testis in rats, which led to disorders in the seminiferous tubules, an increase in thickness, separation of the basal membranes, dilation of interstitial tissues, an increase in the thickness of blood vessels within the interstitial tissue, and degeneration of Sertoli cells. This is in agreement with the results of the current study.

And (Zheng ,et al , 2002) noted in their study that the thickness of the seminiferous tubules wall weakens the relationship between it and the interstitial tissue and many pathological disorders appear within the testicle as a result of the increase in wall thickness, especially in the function of Sertoli cells, which affects the differentiation of germ cells and poor spermatogenesis. Sertoli cells play an important and necessary role in spermatogenesis by providing support and nutrients and controlling germ cell proliferation and programmed cell death, which is essential for spermatogenesis (Tvrda , et al , 2015). The researchers also showed the importance of Sertoli cells for differentiation and maintenance of spermatogenesis through the secretion of specific factors such as glial cell line-derived neurotrophic factor (GDNF) and endocrine gland interactions (Ortman and Siegel, 2020).The researcher (Winters, 2004) indicated that the poor formation of sperm is caused by the thickening of the walls of the seminiferous tubules due to the secretion of collagen fibers of the fourth type, Collagen fibers IV, by Sertoli cell.It was found that pyridine and its derivatives contribute to a decrease in testosterone, and this is what I agree with the researcher's result (Li , et al , 2019). And the researchers (Yao , et al , 2022) indicated that the chemicals cause endocrine disorders that cause male infertility and an imbalance in sperm formation, movement, activity and

composition, as well as a decrease in the level of testosterone through their study of the effect of cadmium on mice. The results of the current study showed the absence of sperm in some cavities of the seminiferous tubules, the appearance of vaculation in some areas of the testicle, lesion in the epithelial tissue of the seminiferous tubules, the expansion of the distance between germ cells and their sloughing and their collection in the lumen of the seminiferous tubules, degeneration of Sertoli cells and an increase in the space between neighboring cells. It agreed with (Yang , et al , 2021) as a result of their study of nickel chloride testis in mice In addition, the results of the current study showed the appearance of edema in the interstitial fluid. And that edema is evidence of increased permeability of blood vessels and thus leads to fluid accumulation in the interstitial spaces, which results in degeneration of most of the epithelial cells that make up the walls of the seminiferous tubules (Kumar , et al , 2002). The appearance of edema and degeneration of the seminiferous tubules, lysis and necrosis, a change in the level of testosterone and a decrease in the number of sperms, all these results agreed with the results of researchers (Bouabdallah , et al , 2021) for their study of types of chemical pesticides on male mice .It was found that the phenomenon of sloughing of the germ cells is caused by damage to Sertoli cells due to the incorrectness of the seminiferous tubules (Kurmar , et al , 2006) and the decrease in testicular lipids, which is important in the adhesion of germ cells to the seminiferous tubules (Lanco-Rodrignez and Martinez-Garcia, 1998).

5. Conclusions

Treatment with pyridine at different doses for one month caused severe histopathological damage to the testis, which was represented by congestion, hemorrhage, rupture, detachment of part of the epithelial layer from the basement membrane and follicular degeneration. And pyridine has an effect on the average diameter of the seminiferous tubules in the testis of adult rabbits.

6. Recommendations

It is important to be fully aware of the effect of chemicals and care must be taken when dealing with them. Using Immunohistochemistry to know the changes in the histological structure of the testis when using pyridine. Conducting more studies to know the effect of pyridine on other organs such as the kidneys, liver and spleen.

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