Evaluation of Histological Changes Resulting from Effect of Antimony on Structure of the Testes in the White Rabbit Orectolagus Cuniculus

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Abstract

The current study was conducted to investigate the effect of antimony in the testes of male adult white rabbits, Orectolagus cuniculus, treated with two concentrations 20 and 30 mg/kg/day for a period of 30 days. 18 rabbits were used, divided into three groups, each group included 6 rabbits. The first group (the control group) was dosed with distilled water. The second group (the first experiment group) was dosed with antimony at a concentration 20 mg/kg/day, and the third group (the second experiment group) was dosed with antimony at a concentration 30 mg/kg/day, and the tissue sections were prepared using the paraffin method. The results of histopathological changes of the testis tissue showed the occurrence of dissociation in the germ cells. And the separation of the epithelial layer from the basement membrane and a contraction in the walls of some semiferous tubules, where their general appearance became wavy and irregular Irregularity of the epithelial layer of some seminiferous tubules, The results of the study showed an increase in the thickness of the wall of some seminiferous tubules. In addition, it was observed and the appearance of vacuolation between germ cells in some seminiferous tubes. Degeneration is degeneration in Sertoli cells. It was also observed that some germ layer cells sloughed off and gathered in the lumen of the seminiferous tubules, Pyknosis of some germ cells and Karyolysis of some germ cells, where the nuclei completely disappeared, and Hydropic Degeneration was also seen in some germ cells.

Keywords: Hydropic Degeneration, antimony, testes, Seminiferous tubules, Pyknosis

1. Introduction

Antimony is a pentavalent element, and it contains two compounds, meglumine antimoniate and sodium stibogluconate. For more than half a century, antimony has been used in the treatment of parasitic leishmaniasis, although antimonials are still first-line drugs, they show many limitations, Including its severe side effects, the need for daily injections, administrative follow-up, and drug resistance (1). Moreover, the molecular structure of antimony, its metabolism and its mechanism of action are still under study, and some recent studies indicate that pentavalent antimony acts as a prodrug that is converted into the most active and toxic triple antimony, and this importance was documented in early medicine, because of the controversy that It was about its use in that period (2). The most important clinical use of organic antimony was during the last century in the treatment of leishmaniasis, which is an infectious parasitic disease that is endemic in 88 countries, including 22 developed countries and 66 developing countries, which mainly affected the population, including the poor and low-income populations. During clinical manifestations of the disease that can involve the skin, such as the appearance of skin lesions and mucous secretions and may lead to death if the patient is not treated (3). At the beginning of the last century, Gaspar Vianna, the leading researcher in the treatment of leishmaniasis, reported the efficacy of antimony (III) potassium tartrate (tartar emetic) for the treatment of mucocutaneous leishmaniasis (Vianna,1912). This efficacy was documented in one study in India, but the clinical use of this compound was discontinued due to its severe side effects. The study by Guerin et al. (4) about the use of less toxic antimonials, treatment with them is often accompanied by local pain during intramuscular injection and systemic side effects, which require very close medical supervision. Typical side effects include nausea, vomiting, weakness, muscle pain, abdominal cramps, diarrhea, rash, and hepatotoxicity, with possible drug resistance in the treatment of this disease. In fact, until recently, little was known about the chemical composition of pentavalent antimony drugs and the methods used in industry to prepare them. However, improved

doi.org/10.31838/hiv22.02.518 Received: 07.06.22, Revised: 21.07.22, Accepted: 19.08.22

bioavailability of drugs, new insights into chemistry and the opening of new mechanisms of action have led to the development of new strategies that include the development of compounds and products to improve their treatment (5). And the detection of serious side effects produced by some commercial forms of pentavalent organic antimony (6). The aim of the current study is to determine the effect of antimony on the histological structure of the testes in the rabbit.

2. Materials and Methods

The specific dose of antimony was prepared based on the lethal half dose (LD50), which is valued in the rabbit 370 mg of antimony per kg of body weight. Two concentrations (two doses) of antimony were chosen to test its toxic effect, namely 20 and 30 mg/kg, while the weights of rabbits used in the experiments ranged between (500-1000) g, and the rabbits were injected with the required amount of antimony according to the concentration (dose). One day for one month 30 days per concentration. It was possible to calculate the amount of antimony injected into rabbits used in this study, based on the following equation:

$$\boxed{\frac{x}{D} = \frac{W_{\text{rabbit}}}{1000}}$$

Whereas: x: the amount of antimony to be dosed to rabbits (gm) in the experiment, D: The specified dose of antimony is either 20 or 30 (mg of antimony/kg of body weight), W_{rabbit} : the weight of the rabbits used in the experiment, which ranged between 20-30 g

Animals used in experiments and histological studies

In this study, 18 white male rabbits (Orectolagus cuniculus) were used, obtained from the animal house of the Department of Life Sciences - College of Education for Pure Sciences / University of Diyala, and their average weight ranged between 500-1000 g. These rabbits were randomly divided into three groups, the details of which were as follows: the first group was the control group with 6 rabbits, and the second group was the test group, which numbered 12 rabbits, and this group in turn was divided equally into two secondary groups (6 rabbits for each group). The rabbits of the two secondary groups were injected with antimony at a concentration (20 and 30 mg of antimony/kg of body weight) daily for 30 days. On the last day, the rabbits were anesthetized with chloroform and the testicles were removed from their site. Then the samples were fixed with formalin solution for 24 hours and then washed with tap water and transferred to 70% alcohol for preservation. The tissue sections were prepared according to method (7), where the samples were passed for decantation with an ascending series of ethyl alcohol, then placed in xylene solution for leaching, then embedded with paraffin wax. The prepared wax molds were cut using a rotary microtome with a thickness of 7 microns. The obtained glass sections were stained using the Haemotoxylin and Eosin (H&E) stain according to the method used in (8). The glass sections tinted with Canada substance carried a balsam, after which the samples were examined and photographed using a light microscope equipped with a digital camera.

3. Results

The results of the histological study of the testicles of white rabbits treated with a concentration of 20 mg/kg of antimony and during a period of 30 days showed changes in its histological structure, represented by the occurrence of dissociation in the cells of the spermatogonia and the separation of the epithelial layer from the basement membrane as in Figure 1. The study also showed a shrinkage in the walls of some semiferous tubules, where their general appearance became wavy and irregular, and the epithelial layer irregularity of some seminiferous tubules became (Figure 2). The results of the study showed an increase in the thickness of the wall of some seminiferous tubules. In addition, it was noted that the vacuolation between germ cells in some seminiferous tubes and degeneration in Sertoli cells appeared (Figures 2 and 3).

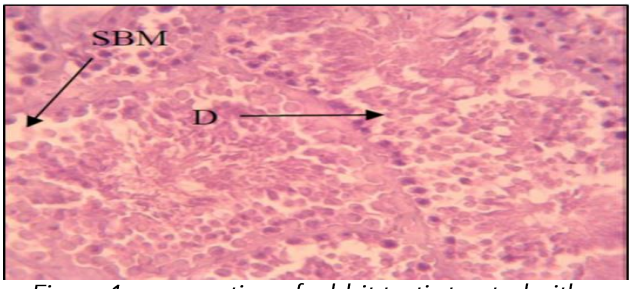

Figure 1 cross section of rabbit testis treated with a concentration of 20 mg/kg of antimony for 30 days. Note: Dissociation in germ cells (D) Detachment of the basement membrane from the epithelial layer (SBM). Hematoxylin-eosin stained (×40).

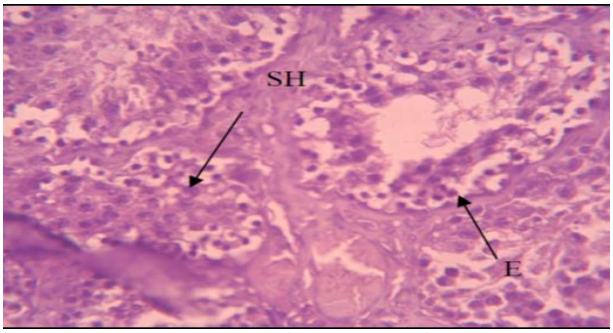

Figure 2 cross section of the testis of rabbits treated with a concentration of 20 mg/kg of antimony for 30 days. Note: irregular epithelial layer (E) shrinkage of seminiferous tubules (SH). Hematoxylin-eosin stain (×40)

The histological sections showed that some germ layer cells had sloughed off and collected in the lumen of the seminiferous tubules as in Figure 4. It was also seen that the nuclei of some germ cells have thickened, as they appeared in a dark color and a small size in the middle of the cells, and Karyolysis nuclei were also observed in some germ cells, where the nuclei completely disappeared, and the cell was seen in a uniform color, and hydropic degeneration

was seen in some germ cells as it was seen With a swollen appearance with dark-colored nuclei centrally located as a result of fluid accumulation inside them, as in Figure 5.

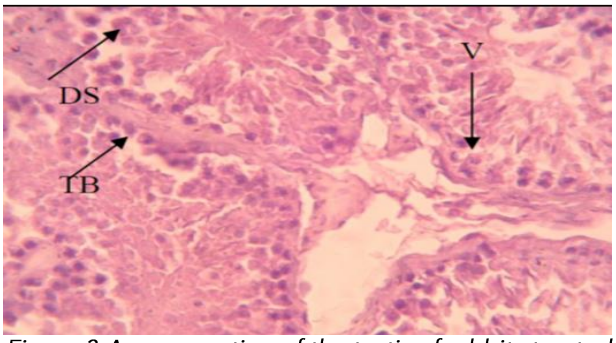

Figure 3 A cross section of the testis of rabbits treated with a concentration of 20 mg/kg of antimony for 30 days. Note: The thickness of the wall of some seminiferous tubules (TB) Sertoli cell degeneration (DS) Vaculation in the germ layer (V) stained with hematoxylin-eosin (×40)

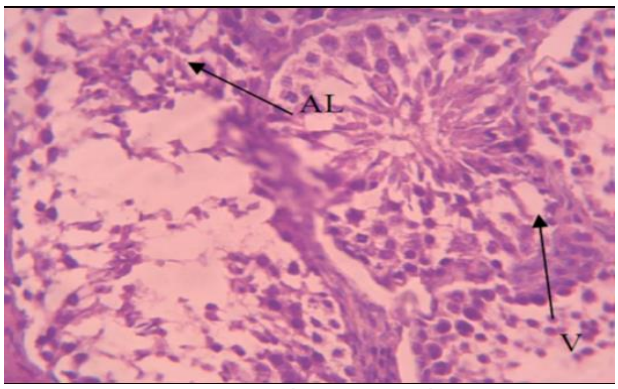

Figure 4 cross section of the testis of rabbits treated with a concentration of 20 mg/kg of antimony for 30 days. Note: The accumulation of cells within the central lumen (AL) vacuolation in the germ layer (V), hematoxylin-eosin stain (×40)

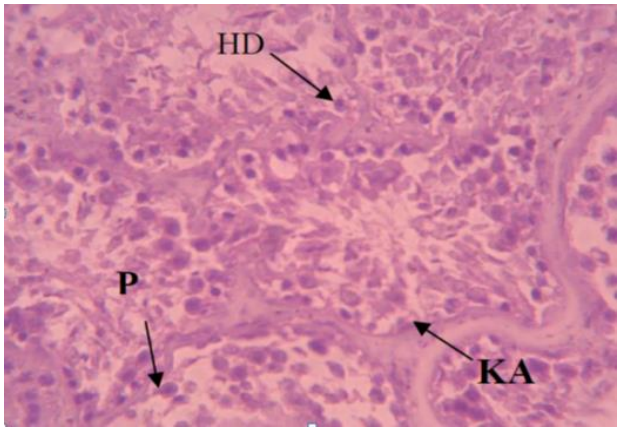

Figure 5 cross section of rabbit testis treated with a concentration of 20 mg/kg of antimony for 30 days. Note the occurrence of Hydropic Degeneration (HD), Pyknosis (P), Karyolysis (KA). Hematoxylin-eosin stained (×40)

As for the group of rabbits treated with antimony at a concentration of 30 mg/kg for a period of 30 days, the effects increased significantly with the increase in the dose given to the experimental animals. The changes in the testes tissue can be observed, which can be summarized by the appearance of small spaces between germ cells and the occurrence of degeneration in Sertoli cells and the increase in the space between Sertoli cells Neighboring, Vaculation was also observed among the germ line, as well as degeneration of primary germ cells and spermatids and necrosis of the germ layer as shown in Figure 6 It was also observed that some cells of the germ layer sloughed and collected in the lumen of the seminiferous tubules and the separation of the seminal epithelium from the epithelial separation as in Figure 7 Increasing shrinkage of the seminiferous tubules and depletion of depletion in some germ layers of the seminiferous tubules, and at the same time led to the occurrence of degeneration, rupture, and apoptosis in the spermatids, the primary sperm cells, the spermatids and the mature sperm, and the return of the spermatids and the mature sperms into the seminiferous tubules. On the other hand, the results showed the absence of spermatozoa in some cavities of the seminiferous tubules. Figure 8 It was also seen that the nuclei of some germ cells have thickened, as they appeared in a dark color and a small size in the center of the cells. It was seen with a swollen appearance with dark-colored nuclei centrally located as a result of fluid accumulation inside them, Figure 9.

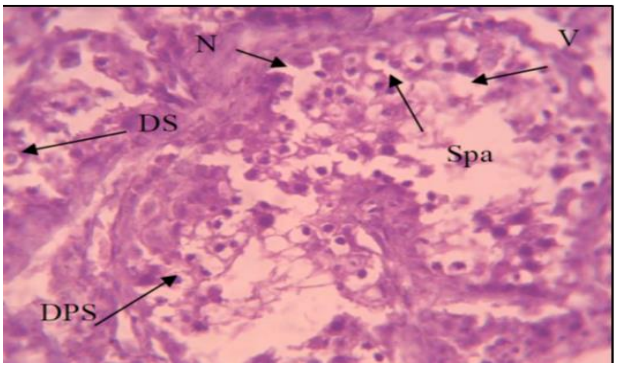

Figure 6 cross section of rabbit testis treated with a concentration of 30 mg/kg of antimony for 30 days The appearance of narrow spaces between the cells of the spermatozoa (Spa) Degeneration of Sertoli cells (DS) Vaculation between cells of the spermatic line (V) Degeneration of the cells Primary germ and spermatids (DPS) necrosis in the germ layer (N). Hematoxylin-eosin stain (×40)

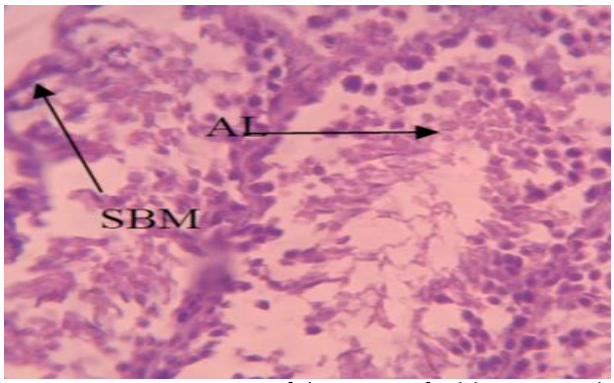

Figure 7 cross section of the testis of rabbits treated with a concentration of 30 mg/kg of antimony for 30 days. Cells collect within the central lumen (AL) Detachment of the basement membrane from the epithelial layer (SBM). Hematoxylin-eosin stain (×40)

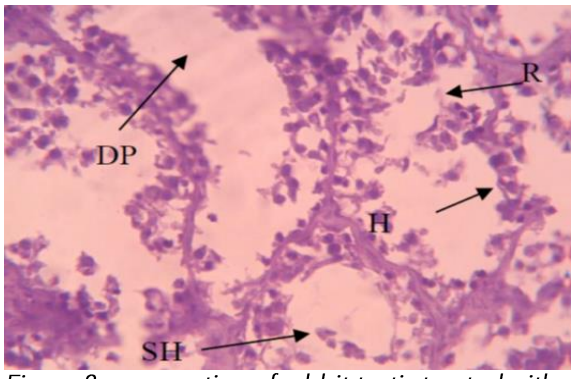

Figure 8 cross section of rabbit testis treated with a concentration of 30 mg/kg of antimony for 30 days. Note: Hypoplasia (H) Depletion of germ cells in some seminiferous tubules (DP) Shrinkage of seminiferous tubules (SH) Reverse spermatogenesis (R). Hematoxylin-eosin stain (×40).

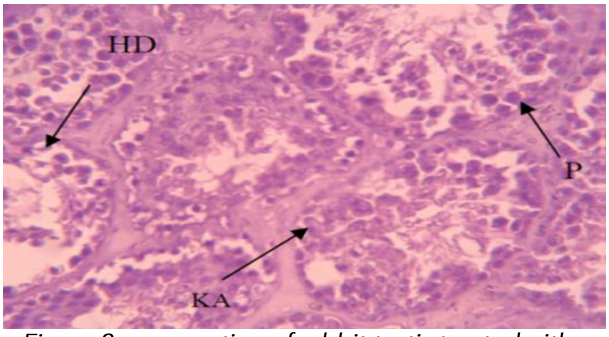

Figure 9 cross section of rabbit testis treated with a concentration of 30 mg/kg of antimony for 30 days. Note the occurrence of Hydropic Degeneration (HD), Pyknosis (P), Karyolysis (KA). Hematoxylin-eosin stained (×40)

4. Discussion

There are many different factors that affect spermatogenesis, and among these are chemical factors such as drugs, compounds, and toxic chemical elements that pollute the environment (9). The current study showed clear histological changes represented by the noticeable increase in the thickness and shrinkage of the walls of the seminierous tubules. The result of this study agrees with the findings of (10) in their immunological and morphological study about proteins and vitamins outside the testicles that the thickness of the seminiferous tubules wall weakens the relationship between it and the interstitial tissue and with the increase in wall thickness many pathological disorders appear inside the testis, especially in the function of Sertoli cells. It affects the differentiation of germ cells and inhibits spermatogenesis. While (11) showed that Sertoli cell secretes collagen fibers type IV, which causes thickening of the walls of seminiferous tubules, and thus leads to poor spermatogenesis. Treatment with antimony may lead to the oxidation of polyunsaturated fatty acids, and this causes the dissolution of phospholipids, which eventually leads to damage to the cellular structure of the testes. Or there may be a strong relationship between antimony-induced toxicity and lipid peroxidation LPO (12). In addition, the results showed the absence of spermatozoa in some cavities of the seminiferous tubules. The results agreed with what was observed by (13), who indicated that the intensity of the oxidative stress caused by antimony may affect the composition of the cytoplasm, mitochondria and the membranes of the peripheral bodies in the testes and nerve centers. The terminal bodies, but at the same time the membranes become vulnerable to damage, oxidative stress increases the oxidative stress affecting the number of sperm cells. Interactions between testicular antioxidant and steroidal enzyme systems may be physiologically relevant, and testicular antioxidants in antimony-treated animals support the observed changes in the activities of steroidal enzymes that indicate a defect in the redox and antioxidant system, leading to oxidative stress in mice. Exposed to vanadium and reactive oxygen species can produce both endogenous and exogenous substances, and lead to tissue damage by initiating a self-diffusing lipid peroxidation reaction (14). The results also indicated the emergence of Vaculation in some areas of the testicle, and it was clear that the space between the germ cells widened, their sloughing from the epithelial tissue and their assembly in the lumen of the seminiferous tubules. This result is consistent with the findings of the researchers (13) who indicated that the percentage of sperm degeneration was high after treatment with antimony. The study also showed the presence of degeneration in germ cells and Sertoli cells. In the study of (15) that dealt with reproductive risks and their relationship to Sertoli cells indicated that a disorder in Sertoli cells will inevitably affect the germ cells and eventually lead to a defect in the testicular tissues. Whereas (16) mentioned in their study on the advantages and disadvantages of the Sertoli cell as a model in male reproductive toxicology, that the Sertoli cell has a necessary role in the development of germ cells through the formation of the blood-testis barrier that protects the germ cells and the transfer of nutrients and hormones to the germ cells. It is believed that all these pathological signs are due to a defect in the structure and function of Sertoli cells. The results of the current study showed that dosing rabbits with a concentration of 30 mg/kg of antimony led to an increase in the shrinkage of the seminiferous tubules and depletion of depletion in some germ layers of the seminiferous tubules, and at the same time it led to degeneration, apoptosis, and apoptosis in spermatids and spermatogonia. This study agrees with the study of (17) that the programmed death in the number of germ cells (apoptosis) increased in different stages of spermatogenesis, and that the presence of abnormal sperms might affect the process of spermatogenesis when treated with antimony. The decrease in antimony-induced spermatogenesis could have affected cell division and cellular structure-dependent processes during the conversion of spermatids to sperm or the antimony might cause DNA breakage via free radical interactions (18). DNA damage may also be responsible for the increase in

the percentage of abnormal sperm shapes. Recently it has been shown that calcium K and sodium Na (ATPase) [a group of enzymes that catalyze the hydrogenation of the phosphate bond in ATP to Adenosine diphosphate (ADP), located in the central part of the flagellum of mature sperm cells, is essential for normal sperm function, and antimony is known to inhibit the activity of Na,K ATPase by binding to the ATP hydrolysis site due to its close similarity with phosphates (Nechay et al, 1984).

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