Effect of Vanadium Sulfate on The Histological Structure of The Testes in The White Rabbit (Oryctolagus Cuniculus)

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Keywords: New Zealand white rabbits (Oryctolagus cuniculus), vanadium sulfate, testes, seminal ducts, and germ cells.

Abstract: Vanadium is considered a hazardous heavy metal because it has a highly harmful effect on living organisms and because scientific studies in the literature on its effects are insufficient. Therefore, the current study aimed to investigate the effect of one of the widely used compounds of this metal, namely vanadium sulfate, on the fertility by determining its effect on the histological structure of the testes of New Zealand white rabbits (Oryctolagus cuniculus). To achieve the objective of this study, 12 rabbits were used, equally divided into three groups. The groups were classified into a control group, whose animals were injected only with distilled water, and two experimental groups, whose animals were continuously injected with concentrations of 10 and 15 mg/kg, respectively, of vanadium sulfate solution for a specific period of 30 days. The following day, the rabbits were sacrificed, the testes were removed, and the histological sections were prepared according to the known reference method. Microscopic examination of the prepared tissue sections revealed that the overall appearance of the seminiferous tubules had become wavy, and that the walls of some of these tubules had suffered marked shrinkage, while the wall thickness had increased in others. Treatment with vanadium sulfate led to the shedding of some germ layer cells and their accumulation in the seminiferous tubules, and the appearance of nuclear condensation and inter-sperm vacuoles in some of these tubules. Follow-up examination of the tissue sections indicated that some germ cells suffered from nuclear lysis, as well as hydro-degeneration, appearing swollen with dark-colored nuclei in their center due to fluid accumulation within them.

Introduction: The environment contains a variety of chemical compounds, including heavy metals, which are powerful modifiers of many physiological functions. It is known that some of these substances alter certain stages of spermatogenesis, leading to reproductive dysfunction [1]. One of the most notable heavy metals with subtle effects is vanadium and its compounds. According to the periodic table of chemical elements, vanadium is classified as a transition element. It is widely distributed in the Earth's crust and is used in many industries [2]. At the same time, it is a trace element found in almost all living organisms, including humans. Environmental levels of vanadium are increasing due to vanadium releases into the atmosphere from multiple human-generated sources [3]. Vanadium is one of the main metals in fossil fuels, and the burning of petroleum, coal, and heavy oil provides one of the most significant sources of environmental pollution with this element. Vanadium accounts for the remainder of the particulate matter emissions from human industrial activities [4]. For humans, the main sources of vanadium pollution are airborne dust, food, and smoking. Vanadium salts interfere with a core set of enzyme systems such as various ATPases, protein kinases, ribonucleases, and phosphates. However, vanadium has recently been considered an "atypical biologically important element" due to its uses and the potential applications of its pharmaceutical compounds [5]. The therapeutic activity of vanadium compounds has been identified in several areas, including the treatment of type 1 diabetes, as vaginal contraceptives, as nutritional supplements, for the prevention of animal carcinogens, and for combating HIV [6]. Vanadium pentoxide caused a marked increase in the number of hypoploid cells, an increase in DNA migration, and increased micronucleus formation in mice; eye and respiratory irritation and bronchospasm; neutrophil influx; splenomegaly in rats and mice; leukocytosis and phagocytosis in these animals; skeletal abnormalities; delayed ossification; and decreased fetal body weight and length [7]. Vanadium compounds have the ability to regulate various physiological processes such as cell growth, differentiation, and

glucose and lipid metabolism [8]. Despite their benefits, the toxic effects of some vanadium compounds on humans and animals are now well-known. However, until recently, little information was available regarding the male reproductive toxicity of vanadium compounds [9]. Furthermore, the involvement of oxidative mechanisms in the toxic manifestations of vanadium compounds in the male reproductive system is not yet fully understood. Numerous pieces of evidence indicate that an increase in vanadium leads to toxic effects in industrial workers, possibly due to oxidative stress [10]. Approximately 25% of soluble vanadium compounds are absorbed via inhalation. Vanadium is slowly absorbed in the gastrointestinal tract; therefore, ingested vanadium compounds are primarily eliminated in the feces. Shortly after absorption, a fairly uniform distribution of vanadium is observed in soft tissues; however, the long-term storage sites of vanadium are bones, muscles, kidneys, liver, and testes. Urinary excretion is the predominant route of elimination of absorbed vanadium [11]. Once absorbed, vanadate is converted to vanadyl(IV) by glutathione in red blood cells or by ascorbic acid and other reducing agents in plasma and transported by albumin and transferrin. Vanadium is stored particularly in certain organs, especially bones, kidneys, and liver. It plays a crucial role in the reproductive system because it is involved in the formation of gametes [12]. Excessive levels of vanadium in the body, exceeding beneficial levels, can alter its behavior and potentially have adverse effects on the reproductive system. However, there is little research describing the potential in vivo reproductive toxicity of vanadium in males [13]. Therefore, the current study attempts to fill the research gap regarding the effect of vanadium on the male reproductive system by adding novelty through investigating the potential effects of vanadium sulfate on the testes of white rabbits (Oryctolagus cuniculus) by studying histological sections and identifying structural changes in these organs as a result of treatment with concentrations of 10 and 15 mg/kg of vanadium sulfate for 30 days.

Methodology:

Experimental animals: The rabbits were sourced from the national center for drug control and research (NCDCR). Twelve male New Zealand White rabbits (Oryctolagus cuniculus) were carefully selected and confirmed to be disease-free and physically healthy. The animals used in this study ranged in age from 7 to 8 weeks and in weight from 1 to 1.7 kg.

Animal care: The animals were cared according to the method described in [(14)], by placing them in a plastic cage with a mesh lid containing sawdust and fed standard rabbit feed (Rabbit Chow), a pellet-based feed containing all the necessary nutrients for rabbit health without the need for additional supplements, thus ensuring a consistent diet throughout the experiment. The animals were also provided with tap water for the same duration, while lighting was provided by a 50-watt electric lamp for 12 h/day. All laboratory conditions were designed to suit the rabbits and facilitate their adaptation, with the cages being regularly washed and disinfected using 70% ethanol.

Experimental Design: The selected animals were randomly and equally divided into three groups of four rabbits each: two experimental groups and a control group. The rabbits in the control group were fed their usual feed and given tap water, and were injected with distilled water only. This was the same procedure applied to the experimental groups. However, the rabbits in the first and second groups were injected daily for 30 consecutive days with concentrations of 10 mg/kg and 15 mg/kg of vanadium sulfate, respectively.

Preparation of Specific Doses of Vanadium Sulfate: The specific dose of vanadium sulfate was selected based on the LD_{50}, which is 50 mg of vanadium sulfate/kg of body weight in rabbits [15]. The required concentrations were prepared by weighing a precisely measured quantity of vanadium salt using an electronic balance, according to Equation 1 [16], and dissolving it in an appropriate amount of distilled water.

$$\frac{x}{D} = \frac{w}{1000} \qquad \dots (1)$$

Where: x: mass of vanadium sulphate must be injected to rabbit male, (g); D: required dosage of vanadium sulphate, which is 10 or 15 $\frac{mg_{VOSO_4.5H_2O}}{kg_{B.W}}$; w: weight of rabbit male used in each experiment, (1000-1700 g).

Removal of the targeted organs: After the end of the specified period of time for the experiments, which is 30 days, the animals were sacrificed by anesthetizing them with chloroform, and then they were dissected by fixing

them on the dissection board, by making a longitudinal incision below the middle of the abdomen up to the sternum using dissection scissors and separating the testes from the tissues and organs associated with them, and then they were transferred to a neutral saline solution, before being transferred to a formalin fixative with a concentration of 10% for the purpose of preparing the microscopic slides [17].

Preparation of microscopic slides: The microscopic slides were prepared based on the method described in [18], which involved fixing the removed testicular specimens in a 10% formalin solution for 72 hours, followed by washing the fixed specimens with water for half an hour, before passing the specimens through an ascending series of concentrations of ethyl alcohol (100%, 90%, 80%, 70%) for one hour for each concentration, for the purpose of removing water from the tissues of the specimens by the dehydration process. After that, the samples are cleared using a 1:1 solution of 100% absolute ethyl alcohol to xylene for half an hour, and then transferred to xylene for a quarter of an hour to give them the required transparency. The cleaned samples are immersed for no more than half an hour in a 1:1 mixture of xylene and molten paraffin wax at 58°C in an electric oven, before being filtered by placing the samples in pure, molten paraffin wax in three stages, for one hour each. The samples are embedded in special molds filled with molten paraffin wax. Air bubbles that form around the sample during the casting process are removed using a hot needle, and then the molds are left to cool and harden. The wax molds containing the samples are trimmed with a sharp scalpel to remove excess wax, then fixed to the base of the rotary microtome and cut into 5 micrometer-thick transverse sections, before being transferred to a water bath at a temperature of 45°C. Clean, labeled glass slides coated with a layer of Mayer's albumen are prepared for spreading the textile sections on them, before being heated to 37°C using a hot plate in order to fix the textile sections, complete the spreading process, and remove excess water. The hematoxylin-eosin stain was used to stain the samples, and they were then mounted using the DPX Di-butylphthalide Polystyrene Xylene loading medium, then covered with a glass cover slide, before being dried using a hot plate at 37°C [19].

Microscopic examination and photography: Histological sections of testicular tissue from male New Zealand White rabbits (Oryctolagus cuniculus) were studied using a compound light microscope (Meiji Techno, Japan) at a magnification of 40×. Selected histological sections were photographed using a digital camera (Sony, Japan) attached to the microscope. The study was conducted in the Animal Laboratory, Department of Biology, College of Education for Pure Sciences, University of Diyala.

Results:

Animals of the first experimental group treated with a concentration of 10 mg/kg of vanadium sulfate: After analyzing the results obtained from examining the histological sections of the testicles of New Zealand white rabbits (Oryctolagus cuniculus) in the first experimental group treated with 10 mg/kg concentration of vanadium sulfate for 30 consecutive days at a rate of one dose/day, and comparing them with the testicles of same rabbits in the control group, clear changes in the histological structure were recorded, reflecting the negative effect of the tested substance. The changes, indicated in Figure 1, include disorganization of the spermatogenic series, where the normal hierarchical order in which stem cells (spermatogonia) begin and develop through primary and secondary spermatocytes to spermatids and mature sperm is lost. This results in gaps and spaces where sperm cells have fallen into the seminiferous tubules. Figure 1 also indicates the occurrence of the phenomenon of basal detachment, which is the separation of the germinal epithelium from the basement membrane. Upon microscopic examination of the histological sections of the treated testes, as shown in Figure 2, a clear shrinkage of the walls of some seminiferous tubules was observed, where these walls lost their normal roundness and elongation, becoming generally wavy and irregularly shaped.

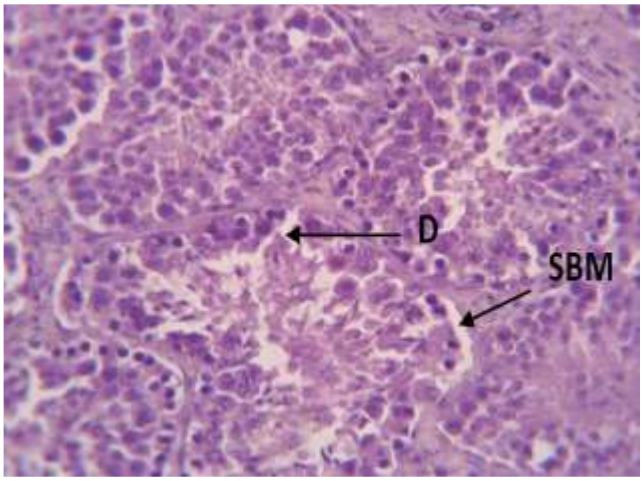

Figure 1 A cross section of rabbit testis treated with a concentration of 10 mg/kg of vanadium sulfate for 30 days. Note: Disintegration in the spermatogenic line (D) detachment of the basement membrane from the epithelial layer (SBM). Hematoxylin-eosin stained ×40

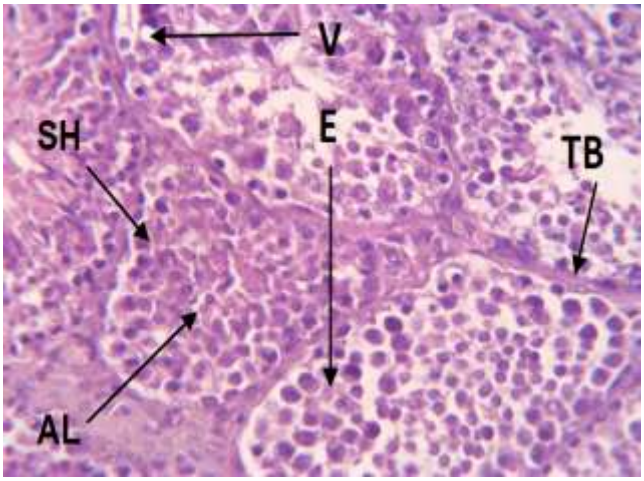

Figure 2 A cross-section of the testis of mice treated with a concentration of 10 mg/kg of vanadium sulfate treatment for 30 days. Note: Irregularity of the epithelial layer (E) shrinkage of seminal tubes (SH), thickness of the wall (TB) vacuolation (V) Cells collect inside Central cavity (AL) Hematoxylin-eosin stain ×40

This shrinkage was accompanied by a marked irregularity in the epithelial layer lining the seminiferous tubules (the spermatogenic layer). This layer was no longer uniform in thickness or evenly distributed as in the healthy tubules of the control group. Instead, in some sections, it appeared wavy or of varying thickness from one area to another, with areas where the cells appeared compressed or abnormally close together. Furthermore, Figure 2 also revealed additional structural disturbances in the form of gaps between germ cells in some seminiferous tubules, where these gaps appeared as pale, wide areas separating cells that were normally adjacent and tightly packed. The same figure also clearly showed the shedding and separation of germ layer cells from their original positions along the basement membrane of the seminiferous tubules, followed by the aggregation of these shed cells – which appear in different forms, including germ cells at multiple developmental stages. In addition to dead or degenerating cells, the seminiferous tubules become filled with these scattered and accumulated cells instead of containing only mature sperm, as compared to the normal tissue of the control group. Histological examination of different animal samples indicated a variation in the severity of these changes among the different seminiferous tubules, as some tubules were greatly affected with almost complete shedding of germ cells and the appearance of large cavities filled with shedding cells, while other tubules showed less severe changes. The changes recorded in the testes of rabbits treated with a concentration of 10 mg/kg were not limited to the above. Figure 3 indicates the occurrence of nuclear condensation in the nuclei of some germ cells in the seminiferous tubules, as evidenced by the appearance of these nuclei in a very dark color, compared to the normal, lighter-colored nuclei found in the testes of rabbits in the control group. Furthermore, their size was noticeably reduced, transforming them into a small, dense mass concentrated in the center of the cell or slightly displaced towards the periphery. This phenomenon was observed in a number of primary and secondary spermatocytes and in some seminiferous spermatids that were in advanced stages of differentiation, while the spermatogonia that remained close to the basement membrane were less affected by this change than others. This nuclear condensation, accompanied by hyperchromatic and pyknosis, is completely different from the normal appearance, where normal nuclei are large and regularly distributed with pigment. The cells affected by this condensation are distributed irregularly within the lumen of the tubule, where other normal cells with intact nuclei are adjacent to each other in the same tissue space. In addition to the above, Figure 3 shows that nuclear lysis occurred in some germ cells. These cells appeared to have completely lost their nuclear identity, as their nuclei disappeared entirely. Instead of the nucleus appearing as a granular or localized structure, the affected cell exhibited uniform, regular cytoplasmic staining devoid of any clumps or condensations of genetic material. This resulted in the appearance of an empty space or a region stained

with a single, homogeneous color, in which the nucleus could not be distinguished from the rest of the cell's components. Clear changes were recorded in the connective tissue surrounding the seminiferous tubules (interstitial tissue), as the histological section in Figure 4 of the group treated with a concentration of 10 kg/kg of vanadium sulfate showed marked congestion and dilation of the blood vessels (veins, small arteries, and capillaries) running within the interstitial space of the testis. The diameter of the lumen of these vessels was markedly larger than that observed in the testes of the control group animals. They also appeared densely filled with normal-shaped red blood cells. This vascular dilation and congestion reflect a state of hyperemia, and this congestion extended to all the micro-vessels associated with the walls of the seminiferous tubules and vesicles.

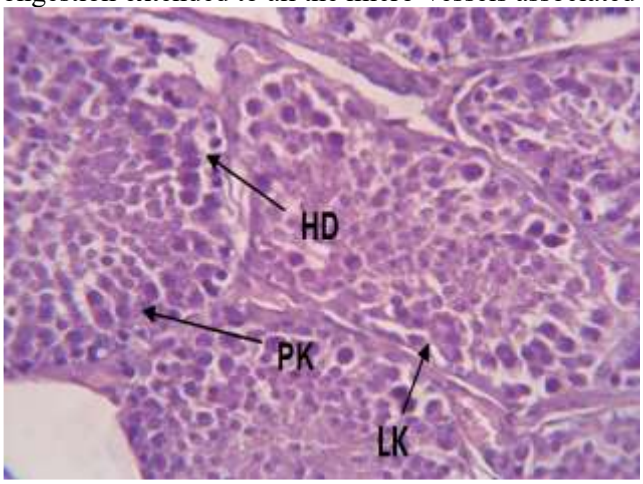

Figure 3 A cross section of rabbit testis treated with a concentration of 10 mg/kg of vanadium sulfate treatment for a period of 30 day. Note: Pyknosis (PK), Karyolysis nuclei (HD) Hydropic Degeneration (LK). Hematoxylin-eosin stained ×40

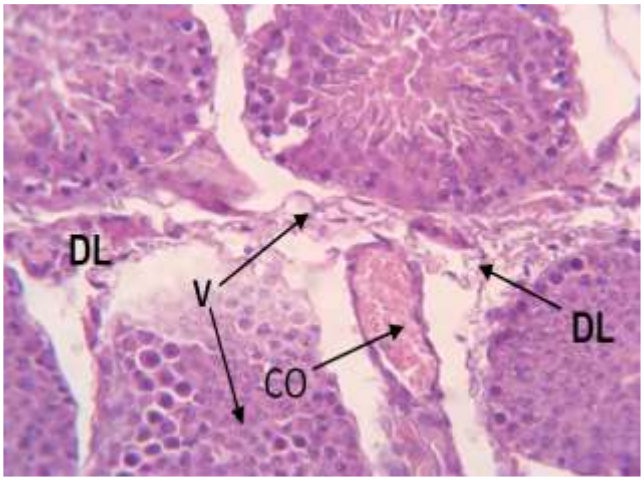

Figure 4 A cross-section of the testis of mice treated with a concentration of 10 mg/kg of vanadium sulfate for 30 days, congestion (CO), vacuolation (V), degeneration of Leydig cells and interstitial tissue (DL). Hematoxylin-eosin dye ×40.

On the other hand, the examination of the tissue treated with vanadium sulfate in Figure 4 indicated a clear pathological histological response that particularly affected Leydig cells and the interstitial tissue surrounding the seminiferous tubules, as the histological sections with different stains showed the presence of distinctive cellular signs of degeneration, represented by a change in cell shape, loss of their clear membrane boundaries, pyknosis, and karyorrhexis. In addition to the appearance of cells with degenerated cytoplasm containing vacuoles of varying sizes, areas of necrosis were clearly observed in the interstitial tissue. These areas were characterized by the loss of normal tissue structure and the accumulation of dead cell debris amidst inflammatory cellular infiltration around small blood vessels. Alongside these degenerative and necrotic changes, another pathological formation, detailed in Figure 4, was also recorded: the presence of abnormal accumulations or the formation of unusual structures within the interstitial space.

Animals of the second experimental group treated with a concentration of 15 mg/kg of vanadium sulfate: After analyzing the results obtained from examining the histological sections of the testicles of rabbits in the second experimental group treated with a concentration of 15 mg/kg of vanadium sulfate for 30 consecutive days at a rate of one dose per day, and comparing them with the testicles of rabbits in the control group, more pronounced changes in the histological structure were recorded compared to the effects recorded in the animals of the previous group, thus reinforcing the negative effect of the tested substance with increasing dose. One of the important effects recorded on the testes of rabbits treated with vanadium salt at this concentration, according to Figure 5, is the appearance of small spaces between primary spermatocytes, where these cells became less compact than they are in normal tissue, in addition to the occurrence of a clear degeneration in Sertoli cells, represented by a reduction in their size, the appearance of cytoplasmic vacuoles within their cell bodies, and a change in the shape of their nuclei. This degeneration led to a significant increase in the distance between adjacent Sertoli cells, making the connection between them less regular. It was observed that these widened spaces between the supporting cells

were always accompanied by small spaces between the primary spermatocytes. Histological examination of the rabbits further revealed changes compared to animals treated with the previous concentration, showing more pronounced structural abnormalities, including spaces and gaps of varying sizes located between the primary spermatocytes within the seminiferous tubules. Clear degeneration and damage were also observed in primary germ cells, manifested in morphological changes such as the breakdown of their nuclei and the disappearance of their cellular boundaries. In addition to the degeneration of spermatids, which took on an abnormal shape with the formation of fragmented and condensed nuclei, the most prominent feature was extensive necrosis of the entire germ cell layer. This region was characterized by the presence of degenerated cells with torn membranes and a higher degenerated nucleus than recorded in the effect of a concentration of 10 mg/kg, which led to a significant loss of the cells that make up the germinal epithelium and the formation of large spaces in their place, as shown in Figure 5. As indicated by the histological examination of the testes of rabbits treated with a concentration of 10 mg/kg, the same changes were recorded, but more significantly and clearly. The histological examination shown in Figure 6 indicated the shedding of a number of germ layer (spermatogonia) cells of various types, from stem cells to mature spermatocytes. These cells detached from their normal positions between Sertoli cells and were released within the seminiferous tubules, causing irregular cellular accumulations that impede the normal flow of seminal fluid. This coincided with a marked separation of the entire seminiferous epithelium, including germ cells and supporting Sertoli cells, from its connecting basement membrane, resulting in distinct gaps between the cellular base of the epithelium and the surrounding interstitial tissue. Figure 7 shows advanced pathological changes affecting all stages of cell differentiation in seminal tissue, including cell degeneration, membrane rupture, and programmed cell death (apoptosis) in spermatogonia, primary spermatocytes, spermatids, and mature spermatozoa within the seminiferous tubules. The manifestations of this degeneration included a reduction in cell size, condensation of the nucleus, and transformation of the cytoplasm into eosinophilic granules, along with the appearance of programmed cell death bodies within the tubule lumen.

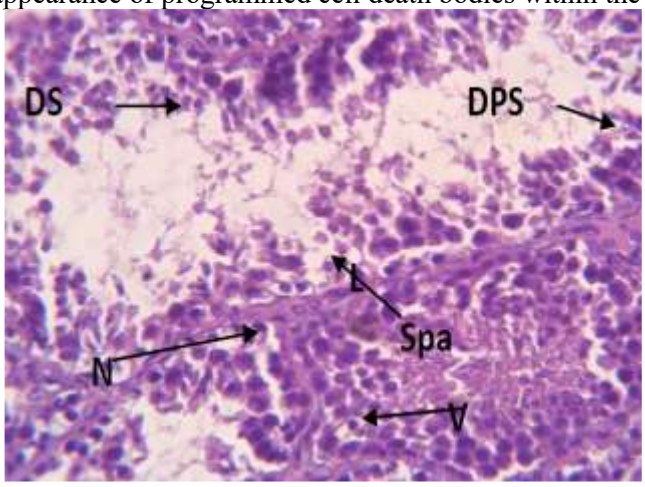

Figure 5 A cross section of the testis of mice treated with a concentration of 15 mg/kg of vanadium sulfate for period of 30 days. The appearance of narrow spaces between the cells of the spermatid line (Spa) and the degeneration of Sertoli cells (DS) vacuolation among the cells of the spermatic line (V) Degeneration in Primary germ cells and spermatids (DPS) necrosis in the germ layer (N). Hematoxylin-eosin stain ×40

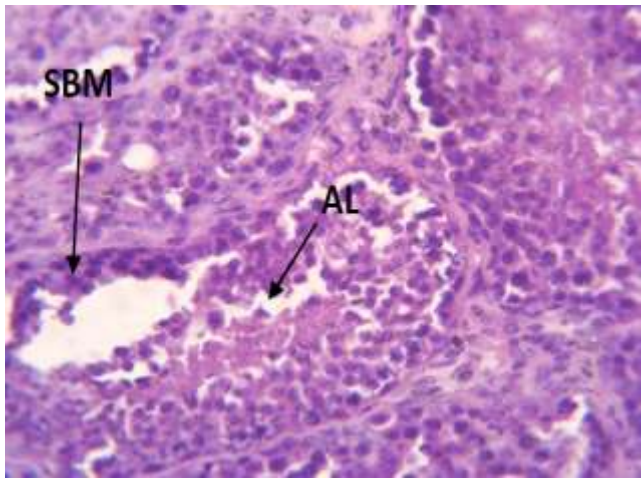

Figure 6 a cross-section of the testis of mice treated with a concentration of 15 mg/kg of vanadium sulfate for period of 30 days. Cells collect within the central cavity (AL) and the basement membrane is separated from the epithelial layer (SBM). Hematoxylin-eosin stain ×40.

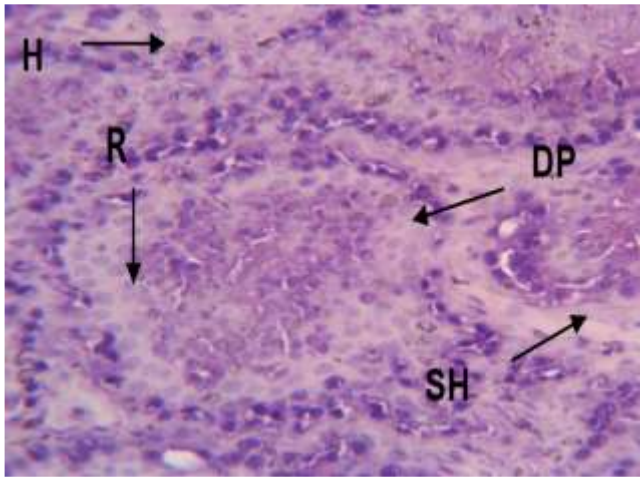

Figure 7 a cross section of the testis of mice treated with a concentration of 15 mg/kg of vanadium sulfate for period of 30 days. Note: Hypoplasia (H) Depletion of germ cells in some seminiferous tubules (DP) Shrinkage of seminiferous tubules (SH) Reverse spermatogenesis (R). Hematoxylin-eosin stain ×40.

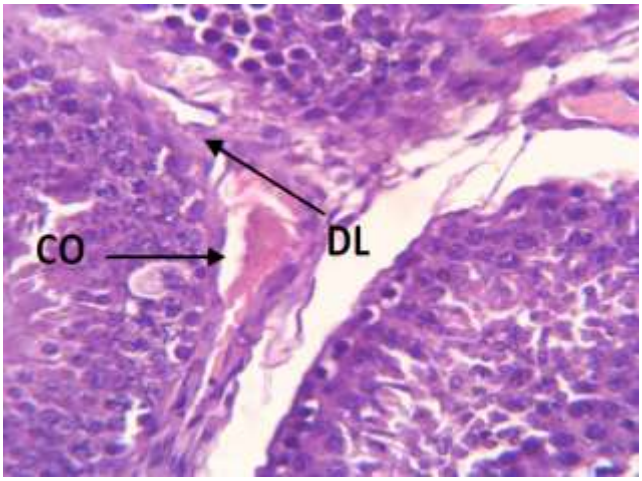

Figure 8 A cross section of the testis of mice treated with a concentration of 15 mg/kg of vanadium sulfate for period of 30 days. Lydic cell degeneration (DL) vascular congestion (CO). Hematoxylin-eosin stain ×40.

Most significant is the unilateral return of mature sperm into the seminiferous tubules rather than their progression towards the epididymis, where some sperm with condensed heads and long flagella were observed positioned in the layers between sperm cells or attached to the membrane of the periwinkle cells, accompanied by the separation of the lining cells and the rupture of the tight intercellular junctions between Sertoli cells. Figure 7 also indicated increased contraction of the seminiferous tubules and a decrease in some of their germ layers, allowing mature cells to reflux towards the basement membrane, along with leakage of tubular fluid into the interstitial spaces and compression of the surrounding blood vessels, without any accompanying changes in the thickness of the basement membrane or the normal distribution of collagen in the interstitial tissue. When comparing the results of the pathological histological changes recorded from the histological examination of the testes of animals treated with 15 mg/kg vanadium sulfate with those of animals treated with 10 mg/kg or the control group, Figure 8 indicates a marked dilation of blood vessels within the interstation. The vascular lumen became filled with large quantities of red blood cells, leading to severe congestion and an increase in vessel diameter. Additionally, there was marked degeneration of the Leydig cells responsible for testosterone secretion. In addition, these cells undergo a change in their normal shape from a polygonal to an irregular shape, with the appearance of cytoplasmic vacuoles and sometimes the disappearance of the nucleus. Thickening was also noted in the walls of these cells and in the surrounding interstitial tissue, which may indicate a disturbance in their secretory function. In addition, extensive infiltration of inflammatory cells with polymorphic nuclei, such as lymphocytes, neutrophils, and macrophages, into the interstitial spaces surrounding the seminiferous tubules was observed, reflecting a clear local inflammatory response. In a related matter, Figure 8 also indicates the appearance of signs of cytopathic abnormalities, represented by the presence of clear edema in the interstitial space between the seminiferous tubules. Varying amounts of light-colored serous fluid accumulated in the interstitial spaces, leading to the dilation of these spaces and an increase in the distances between adjacent seminiferous tubules. Some tubules were observed to be compressed as a result of this edematous dilation. In addition, the same figure indicated the occurrence of pyknosis in the nuclei of some germ cells, characterized by the condensation of chromatin into a homogeneous, dark-colored, highly dense mass. Furthermore, the size of these nuclei was significantly reduced compared to the normal nuclei of healthy germ cells in the control group. These thickened, dense, dark-colored nuclei were concentrated in the center of the infected germ cell, giving a distinctive appearance to spermatogonia, primary and secondary spermatocytes, and spermatids at different stages of damage. The irregular distribution of these thickened nuclei within the different layers of the germinal epithelium, without a specific location, has resulted in some cells appearing as if their nuclei have been transformed into multiple small dark fragments instead of a single condensed

nucleus. Histological examinations of testes treated with 15 mg/kg vanadium sulfate, as shown in Figure 9, revealed more pronounced nuclear degradation than in animals treated with 10 mg/kg or in the control group. In some germ cells, this degradation manifested as complete disappearance of the nuclei and loss of nucleic acid staining, resulting in a uniformly colored cell with no distinction between the cytoplasm and the nucleus. This led to the transformation of cells into homogeneous masses of color without any internal details. This nuclear decomposition is attributed to the cessation of vital processes within the nucleus and the disintegration of genetic material, resulting in the disappearance of normal nuclear features seen in healthy cells, such as nuclear and nucleolar membranes and chromatin aggregates. Figure 9 also recorded a distinct pattern of hydropic degeneration affecting a specific class of germ cells in the seminiferous tubule epithelium. This phenomenon was characterized by abnormal swelling of the cell bodies, making them appear larger and more rounded compared to the surrounding healthy cells. The accumulation of clear, slightly mottled fluid within these cells led to cytoplasmic expansion and the displacement of cellular contents towards the periphery. Simultaneously, the nucleus of each cell appeared distinctly swollen, located almost in the center of the cell. These nuclei were abnormally dark and pyknotic, indicating increased condensation of their genetic material as a direct result of the pressure exerted upon them by the surrounding fluid. It was also observed that in some areas, cellular swelling reached a point where cell membranes ruptured, releasing the contents of the damaged cells into the seminiferous tubule lumen. Other cells remained in the initial swelling stage with a clear, dark nucleus despite changes in their cytoplasm.

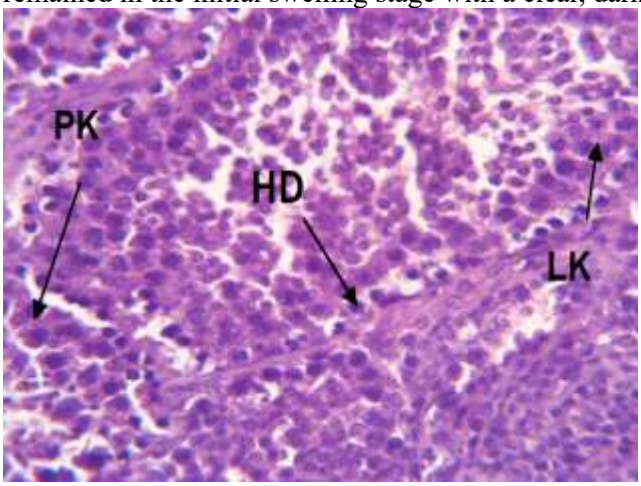

Figure 9 A cross section of rabbit testis treated with a concentration of 15 mg/kg of vanadium sulfate treatment for a period of (30) days. Note: Pyknosis (PK)), Karyolysis nuclei (HD) Hydropic Degeneration (LK). Hematoxylin-eosin stained ×40

Figure 9 further illustrates that the severity of this hydrolysis varied between individual seminiferous tubules and between different sections. Some tubules appeared completely free of this phenomenon, while others showed a clear accumulation of swollen, hydrolyzed cells in multiple layers of the spermatogenic line.

Discussion: The results of the current study showed that animals treated with doses of 10 and 15 mg/kg of vanadium sulfate suffered from marked histological changes, with the severity of these changes increasing with the dose. The testes of rabbits treated with 10 mg/kg vanadium sulfate showed less damage and fewer histological changes than those treated with 15 mg/kg, indicating that this substance has a clear negative effect on animal fertility. The most significant histological changes observed in the testes of New Zealand White rabbits (Oryctolagus cuniculus) as a result of treatment with different concentrations of vanadium sulfate can be described as the disintegration of germ cells, detachment of the epithelial layer, hydrolytic degeneration of cells, and programmed cell death (apoptosis) in germ cells. It was also observed that gaps and spaces were formed within the seminiferous tubules, and that there was a marked increase in the proportion of germ cells with non-standard shapes or sizes, as well as an indication of a defect in the adhesion of Sertoli cells, which support the germ cells, to the basement membrane.

The pathological changes observed in this study are largely consistent with the findings of (Chandra et al. 2007) [20] regarding vanadium-induced oxidative toxicity in the testes of rats treated with a vanadium compound. Histopathological examination revealed that a 400-µg dose of sodium metavanadate administered for 26 days (less than 3 weeks) was sufficient to induce oxidative stress, characterized by a marked increase in lipid peroxidation products in the testes and inactivation of enzymes responsible for steroid and antioxidant synthesis. Histologically, the confirmed effects included the selective loss of mature and elongated sperm cells, as well as a significant decrease in spermatogenesis.

(Aragon et al. 2005) [21] studied the reproductive toxic effects of vanadium in male laboratory rats during spermatogenesis by tracking germ cell apoptosis patterns. Detailed histological examination of testicular tissue from animals treated with two different doses (9.4 and 18.8 mg/kg body weight) of vanadium tetraoxide for 60 days revealed that germ cells were targeted by dose-proportional apoptosis at specific stages of spermatogenesis, including stages I-III and 10-12, at doses of 9.4 and 18.8 mg/kg, respectively.

The reproductive toxicity of vanadium and its effects on the fertility and sperm of male Sprague-Dawley rats were evaluated by measuring several biomarkers and histological markers, as well as monitoring DNA damage (Bharathi Vijaya et al., 2015) [22]. Histological examination of the testes of vanadium-treated rats showed that exposure to a 1 mg/kg daily dose for 90 days resulted in a significant decrease in serum testosterone levels, a marked reduction in both sperm count and motility, and an increased percentage of morphological abnormalities compared to the control group. Furthermore, histological examinations revealed pathological changes in testicular structure, and molecular analysis confirmed that vanadium induces sperm DNA fragmentation, reduces androgen bioavailability in tissues, and increases free radical generation, collectively leading to integrated structural and functional disturbances in the male reproductive system.

Chandra et al. (2010) [23] investigated the complex toxic effects of vanadium, classified as one of the most potent and damaging pollutants to the male reproductive system (reprotoxic), by examining its ability to induce testicular dysfunction in male rats. The study was conducted by examining the histological changes in animals treated with 400 µg/kg sodium metavanadate for 26 days. This treatment resulted in a near-complete cessation of spermatogenesis, clearly demonstrated by the inhibition of spermatization, accompanied by the appearance of numerous gaps and vacuoles within the seminiferous tubules due to germ cell loss. Functionally, the number of sperm stored in the epididymis decreased significantly, with a marked increase in the proportion of abnormal and malformed sperm. At the hormonal level, the analysis revealed a sharp decrease in blood levels of testosterone and other reproductive hormones, leading to inhibition of the activity of steroidogenesis and antioxidant enzymes within testicular tissue. These changes were accompanied by histological evidence of severe degeneration of the reproductive organs, as well as advanced biochemical indicators of oxidative stress, such as elevated cellular lipid degradation products. Taken together, these findings provide a comprehensive model for understanding the reproductive toxicity mechanism of vanadium, classifying it as a highly toxic substance to reproductive function.

The study by (Dehghani et al., 2010) [24] aimed to investigate the effect of vanadium sulfate (VS) on spermatogenesis in male rats. The study was conducted by administering vanadium sulfate orally to male rats for one month at a dose of 32 mg/kg/day. Blood testosterone levels were measured, and seminal fluid was analyzed to determine the effect of vanadium sulfate. Vanadium sulfate had detrimental effects on spermatogenesis. Examination of the epididymis and testes of the treated rats revealed a 51% decrease in blood testosterone levels and a significant 80% decrease in sperm count compared to the control group. However, sperm motility, morphology, and the histological structure of the testes and epididymis were unaffected and did not differ from those of the control group.

Conclusions: Although numerous studies have investigated its effects on the reproductive systems of mice and rats, the effects of vanadium sulfate on the male reproductive system of New Zealand White rabbits (Oryctolagus cuniculus) have not been previously explored. The results obtained confirmed that vanadium sulfate had a clear detrimental effect on the histological structure of the testes, and that this effect increased with increasing concentration. The current study found that treating rabbits with concentrations of 10 and 15 mg/kg for 30 consecutive days resulted in varying behavioral and clinical changes in all animals in the experimental groups. At the histological level, the treatment was the primary cause of spermatogenesis and detachment of the epithelial layer from the basement membrane. Shrinkage of the walls of some seminiferous tubules and inters perm cleavage in some seminiferous tubules were also observed. Vanadium sulfate affected Sertoli cells, with histological

examination indicating degeneration and damage to blood vessels. The shedding of some germ cells and their accumulation in the seminiferous tubules, along with the thickening of the nuclei of some of these cells and the degeneration of others, were caused by exposure to this chemical. Finally, the study recorded diffuse thrombotic necrosis in testicular tissue, as well as the accumulation of glycogen granules in the basement membrane of the tubules, the interstitial tissue, sperm cells, spermatoblasts, and blood vessels, as a result of treatment with vanadium sulfate at the specified concentrations. Therefore, it is essential to address any type of environmental pollution with vanadium, whether in soil, air, or water, and to exercise caution and implement occupational safety standards in all industrial sites where this substance, or any other substance containing vanadium, is used or produced (especially crude oil production fields or oil refineries), given its clear toxicity to the male reproductive system.

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