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Alpha-Lipoic Acid Mitigates Copper Sulfate-Induced Male Reproductive Toxicity in Albino Rats

Khalil Gazar Chelab Al-Nailey

Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq.
khalil.Chelab@qu.edu.iq

Abstract

A total of 40 albino rats, all about a month old and weighing between 200 and 220 grams, were randomly split into four groups for the experiment. In the first group, the rats received a daily dose of 40 mg of CuSO4/kg BW via stomach tube for two months. The second group was administered 40 mg/kg BW CuSO4 through a stomach tube and 100 mg/kg BW intraperitoneal once-daily injections of alpha-lipoic acid (ALA) for two months. The third group received intraperitoneal injections of alpha-lipoic acid at a dose of 100 mg/kg BW once daily for two months, whereas the fourth group, which served as the control group, received intraperitoneal injections of 0.2 ml of normal saline once daily for two months. After two months, all of the animals were sacrificed, and the testes and epididymis were removed for histological analysis. Based on the results of the first group, the amount of spermatogonia, spermatocytes, spermatozoa, and Leydig cells in the testicles of male albino rats exposed to CuSo4 was statistically lower ($p \leq 0.05$). Rats exposed to CuSO4 with lipoic acid (2nd group) had significantly higher levels of spermatogonia, spermatocytes, spermatozoa, and Leydig cells compared to the 1st group, in which CuSO4 alone significantly ($p \leq 0.05$) decreased these parameters compared with the control group. In rats treated with just lipoic acid, however, the increase in spermatogonial cells, spermatocytes, spermatozoa, and Leydig cells was not statistically significant compared to the control group (4th group). Whereas these microscopic pathological changes showed severe toxicological lesion in both testes and epididymis of rats in the 1st group (CuSo4 group) if compared to a control group and other experimental groups. These lesions due to the toxic effects of CuSo4 on the male reproductive toxicity. ALA's preventive and ameliorative impact against the toxicity of CuSo4 resulted in the second group (CuSo4 plus ALA) exhibiting much more favorable and healing changes than the first group.

Keywords: ALA. Copper Sulfate. Reproductive. Rats.

Introduction

Copper sulphate, an inorganic compound consisting of copper and sulphate, is available in liquid or powder form and goes by a variety of other names, including basic copper sulphate, and BSC copper fungicide (1). Some natural plant derivatives were also used in the process of chemical pesticide application (2). Common chemical pesticides included the Bordeaux mixture (copper sulfate, quicklime, and water) and preparations containing mercury, lead, and sulfur. These pesticides can accumulate in the soil thanks to their inert precipitates, which are then washed away by rain and carried by water to the water table and rivers to be toxic sources for fish and other aquatic creatures (3). Copper sulfate is a chemical compound with the formula CuSO4, and it is in the form of blue crystals powder. It

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has many uses in the veterinary and agricultural fields in combating harmful fungi and disinfecting animals (4).It is widely used for the preparation of other copper compounds. In chemical laboratories, it is also used in pest control and sterilization. It is also used in the oil and metal processing industries and in glazing (5). Considering its toxicity, copper sulfate is typically applied to plants as a fungicide or herbicide (6). To boot, it helps in curing fruits like melons and berries by warding off parasites and fungi (7). When free reduced copper binds to sulfhydryl groups inside cells, it stops enzymes like glucose-6-phosphate dehydrogenase and glutathione reductase (GR) from working. This can damage and kill cells (8). Alpha-lipoic acid is an essential catalyst in enzymatic reactions related to glucose metabolism (9). The cofactor of alpha-lipoic acid is an essential, consisting of four mitochondrial enzymes (10). It is also available in nutritional supplements that may be used without a prescription. One of the most important functions of alpha lipoic acid is to stop the action of free radicals damaging the mitochondria, thus preserving the only source of energy for the cell (11). The goal of this study was to determine if supplementation with -lipoic acid can protect rats against CuSo4-induced pathophysiological and histopathological alterations.

Materials and Methods:

Animals:

Forty Wistar male rats weighing 200–220 g were received from the Animal House of the Veterinary Medicine College/University of Al-Qadisiyah, Iraq. All of the rats were housed in a temperature-controlled room 25 ±2 °C) in standard plastic cages (10 rats per cage) before becoming acclimated to the experimental environment for two weeks. The animals in the experiment were given a regular diet ad libitum and access to water at all times.

Experimental Design:

Following an acclimation period, 40 rats were randomly assigned to one of four groups, each with 10 animals.

***Group 1*:** Copper sulfate (CuSo4) was administered orally to experimental animals at a dosage of 40 mg/kg BW for a period of two months

***Group 2*:** The animals were administered ALA (100 mg/kg BW intraperitoneally once daily) and CuSO4 (in the same dose as in group 1) orally after 3 hours. This treatment lasted for two months.

***Group 3*:** The animals were given the same dosage of alpha-lipoic acid as group 2.

***Group 4*:** The control group received intraperitoneal injections of 0.2 ml of normal saline for two months.

Counting of spermatogonia, spermatocytes, spermatozoa and Leydig cells:

Calculating the numbers of spermatogonia, spermatocytes, and spermatozoa present inside the seminiferous tubules were counted at the rate of ten seminiferous tubules for each animal by means of 40X objective lens and averaged them. Also the Calculation of Leydig cells numbers in the interstitial tissue performed between every three seminiferous tubules and ten readings were repeated for each animal using the objective lens with a power of 40X according to (12) to calculate the average numbers of those cells.

Histopathological Examination:

Tissues from each group's testicles, epididymis, and vas deference were preserved in 10% buffered formalin and embedded in paraffin for light microscopic analysis. To examine the tissues, paraffin slices were processed as usual, then cut to a thickness of 5 μm and stained with haematoxylin and eosin (13).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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Statistical analysis:

SPSS, version 17, was used for the statistical analysis of all the data sets. Methods of testing comprise a one-way analysis of variance (ANOVA) for group comparisons and a least significant difference (LSD) test for pairwise comparisons. Statistics were recorded as significant when the p-value was less than or equal to 0.05. All data are presented in the form of means ± standard error (SE) (14).

Results

Counting of spermatogonia, spermatocytes, spermatozoa and Leydig cells of testicular tissue of male albino rats:

Table (1) shows the values of spermatogonia, spermatocytes, spermatozoa and Leydig cells counting in all experimental groups. In comparison with control values, the levels of spermatogonia, spermatocytes, spermatozoa and Leydig cells were statistically decreased in rats exposed to CuSo4 (1st group). Significant elevations in the counts of spermatogonia, spermatocytes, spermatozoa and Leydig cells were observed in rats exposed to CuSo4 plus α-lipoic acid (2nd group) compared to the 1st group in which it's obviously the α- lipoic acid notably increased the levels of these parameters in CuSo4-treated rats, while there is significant decreasing of these levels compared to the control group (4th group). But there is non significant increasing in the counts of spermatogonia, spermatocytes, spermatozoa and Leydig cells were noted in rats treated with only α-lipoic acid (3rd group) compared to the control group.

Table (1): Effect of α-lipoic acid treatment on the counts of spermatogonia, spermatocytes, spermatozoa and Leydig cells of male albino rats treated with CuSo4:

groups	spermatogonia	spermatocytes	spermatozoa	Leydig cells
1st Group	48.29 ±1.7 B	51.08±1.9 B	54.84±0.92 G	15.8±0.05 B
2nd Group	63.06 ±0.59 C	77.08±0.53 C	80.59 ±1.3 F	20.5±0.32 C
3rd Group	85.05 ±0.6 A	121.03±1.96 A	123.12 ±2.23 A	24.8 ±0.74 A
4th Group (control group)	84.13±0.83 A	119.04 ±1.4 A	121.91±1.66 A	23.5±0.13 A

The various superscript letters indicated that there were significant differences p≤0.05

Histopathology:

The first group (copper sulfate-exposed rats group):

Examination of testicular section of copper sulfate-exposed rats showed that the testis had lost its characteristic architecture. There is vacuolation of seminiferous tubules and suppression of spermatogenesis and pronounced reduction in sperm numbers in the lumen of seminiferous tubules and many

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tubules are totally devoid of sperms. Also increased diameter of seminiferous tubule's lumen as in Fig. (1). Desquamation of germ cells resulting in several small spaces showed inside the seminiferous tubule. There is vacuolation of spermatogonia and its detached from basal lamina of seminiferous tubules, with presence of spermatid multinucleate giant cells which indicate severe degeneration within the seminiferous tubules as in Fig. (2).

The epididymal tubules showed severe changes which characterized by dilated lumen and empty of sperms as in Fig (3) & (4).

The second group (CuSo4 plus *α*-lipoic acid group):

The histopathological changes of testicular sections of copper sulfate-exposed rats which supplemented with alpha lipoic acid showed a significant amelioration and reduction in the lesion severity. The histological changes characterized by complete spermatogenesis without vacuolation or spaces within the seminiferous tubules. Presence of mild to moderate numbers of sperms in the lumen of these tubules as in Fig. (5). Presence of characteristic architecture of seminiferous tubules which appeared circled, and compact with decreased diameter of seminiferous tubule's lumen and presence of spermatozoa in the lumen of these tubules as in Fig. (6). The epididymal tubules showed less affected some these tubules filled with sperms but others were empty as in Fig. (7) & (8).

The third group (only alpha lipoic acid supplementation):

The testicular sections of this group (which supplemented with alpha lipoic acid) showed marked positive changes which characterized by normally arranged seminiferous tubules which showed circled and compact. Full spermatogenesis, including spermatogonia, spermatocytes, and spermatids all in their proper places alongside spermatozoa in the seminiferous tubule lumen, much like in Fig. (9). Swarms of spermatogonia, spermatocytes, and spermatids containing spermatozoa populate the seminiferous tubule lumen. Leydig cells are hyperplastic in the interstitial tissue as well (see Fig. (10). The epididymal tubules showed normally dilated and fully filled with sperms as in Fig. (11), the epididymal tubules showed lined by normal pseudostratified columnar epithelium with long stereocilia and filled with sperms as in Fig. (12).

The forth group (Control group):

The testicular sections of control group did not demonstrate any changes which characterized by normally arranged seminiferous tubules which showed dilated and compact as in Fig. (13). Also the epididymis of this group showed normal histological tissue as in Fig. (14).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
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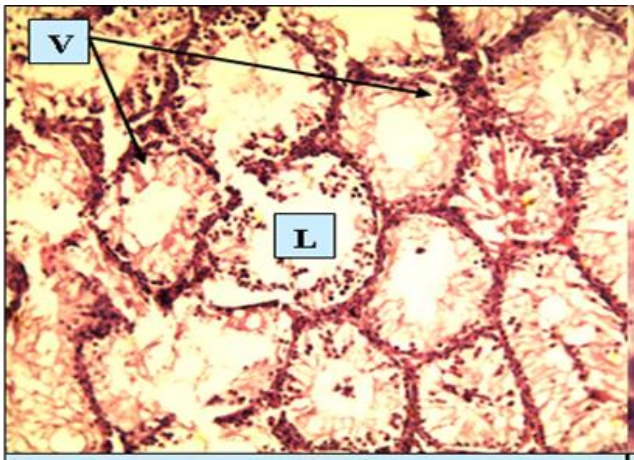

Fig (1): Testes of rat (1st group). There is vacuolation of seminiferous tubules (V) and suppression of spermatogenesis and pronounced reduction in sperm numbers in the lumen of seminiferous tubules and many tubules are totally devoid of sperms. Also the diameter of seminiferous tubule's lumen was increased (L). 50X H&E.

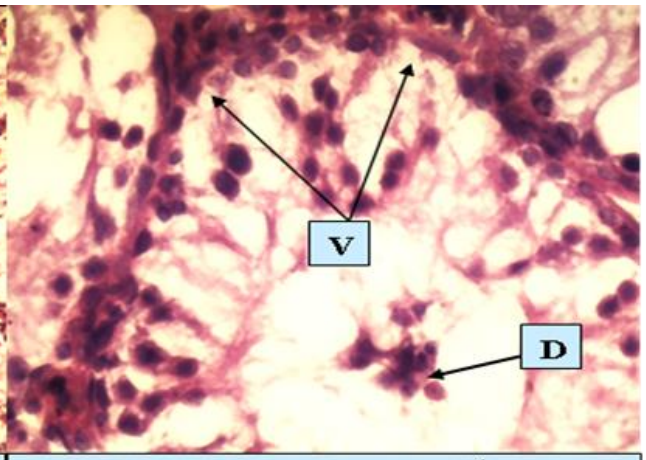

Fig (2): Testes of rat (1st group). Desquamation (D) of germ cells resulting in several small spaces showed inside the seminiferous tubule (V). There is vacuolation of spermatogonia and it's detached from basal lamina of seminiferous tubules. 200X H&E.

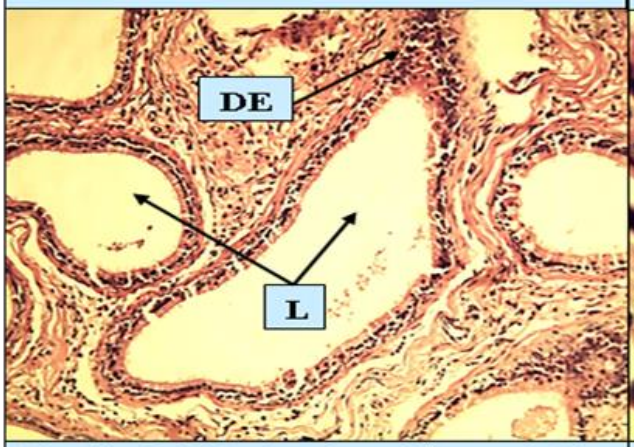

Fig (3): Epididymis of rat (1st group). The epididymal tubules showed severe changes which characterized by dilated lumen (L) and empty of sperms with degeneration (DE) in the pseudostratified columnar epithelium. 50X H&E.

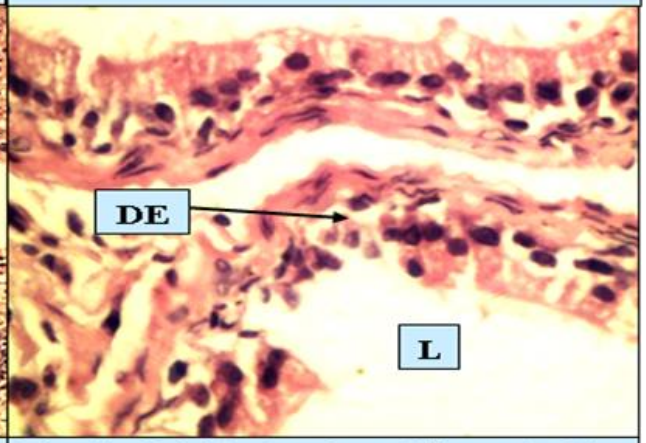

Fig (4): Epididymis of rat (1st group). The epididymal tubules showed severe changes which characterized by marked degeneration (DE) in the lining of theses tubules with dilated lumen (L) and empty of sperms. 200X H&E.

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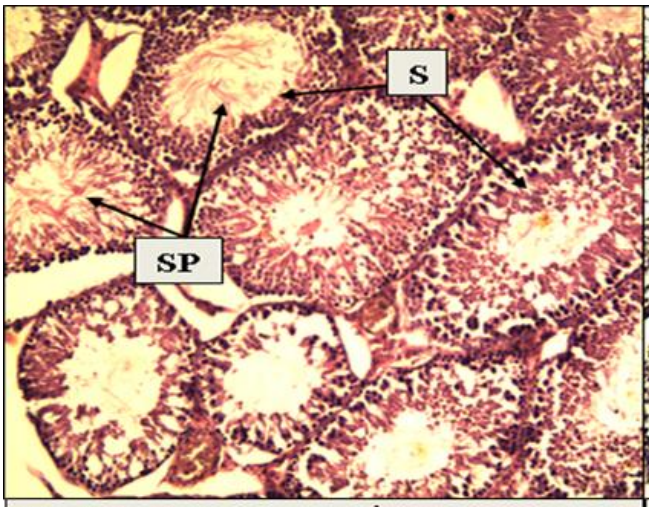

Fig (5): Testes of rat (2nd group). Complete spermatogenesis without vacuolation or spaces within the seminiferous tubules (S). Presence of mild to moderate numbers of sperms in the lumen of these tubules (SP). 50X H&E.

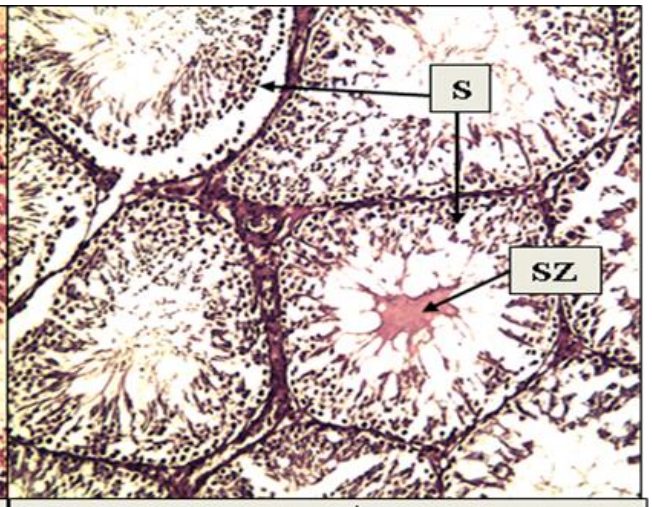

Fig (6): Testes of rat (2nd group). Presence of characteristic architecture of seminiferous tubules which appeared circled, and compact (S) with decreased diameter of seminiferous tubule's lumen and presence of spermatozoa in the lumen of these tubules (SZ). 50X H&E.

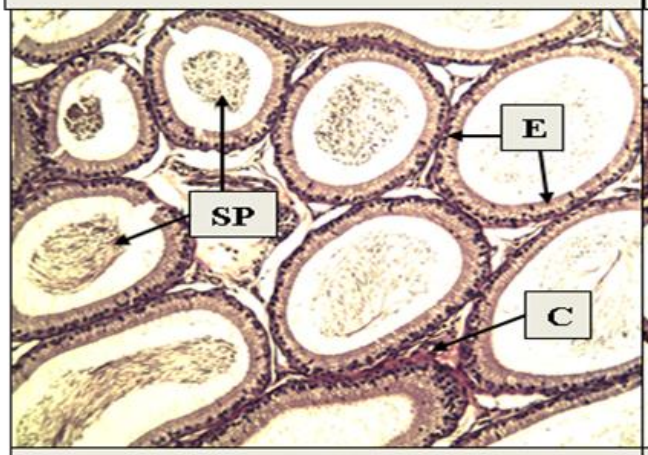

Fig (7): Epididymis of rat (2nd group). The epididymal tubules showed lined by normal pseudostratified columnar epithelium (E), less affected some these tubules filled with sperms (SP) but others were empty. Slightly congestion in the interstitial tissue (C). 50X H&E

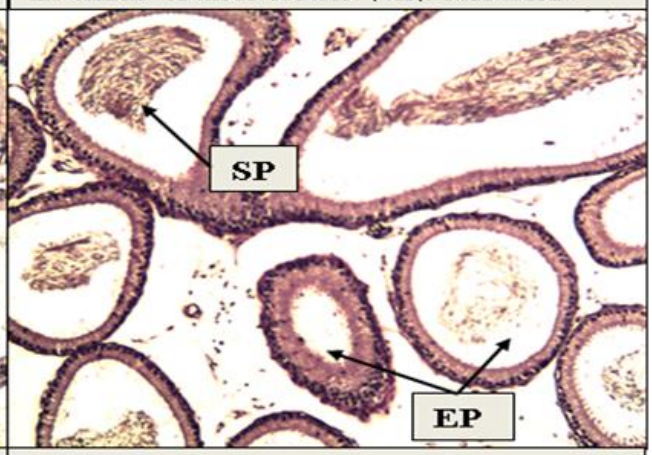

Fig (8): Epididymis of rat (2nd group). The epididymal tubules showed less affected some these tubules filled with sperms (SP) but others were empty (EP). 50X H&E.

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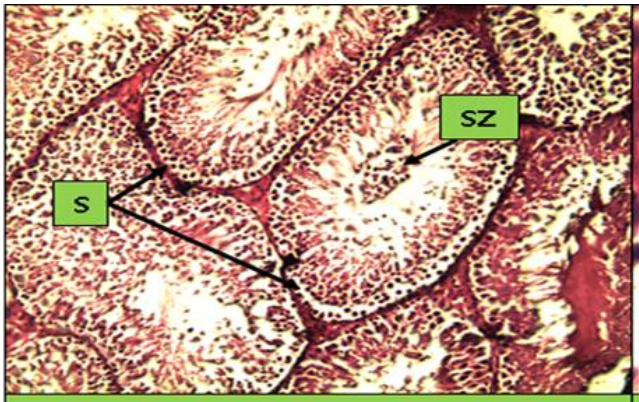

Fig (9): Testes of rat (3rd group). Normally arranged seminiferous tubules which showed circled and compact (S). Complete spermatogenesis in which spermatogonia, spermatocytes and spermatids were regularly arranged with spermatozoa (SZ) in the lumen of seminiferous tubules. 50X H&E.

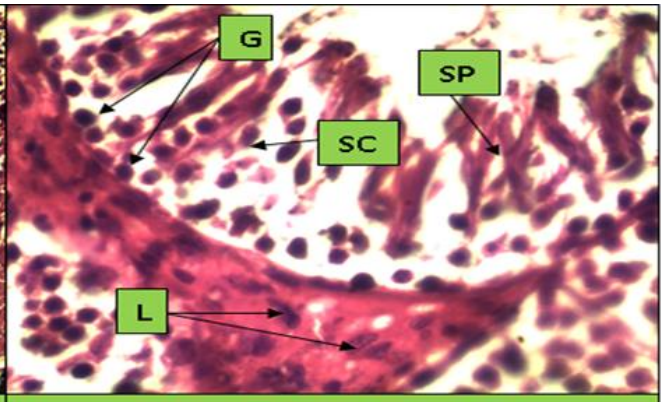

Fig (10): Testes of rat (3rd group). Normally arranged seminiferous tubule in which showed there is high numbers of spermatogonia (G), spermatocytes (SC) and spermatids (SP) in the lumen of seminiferous tubules. Also there is hyperplasia of Leydig cells (L) in the interstitial tissue. 200X H&E.

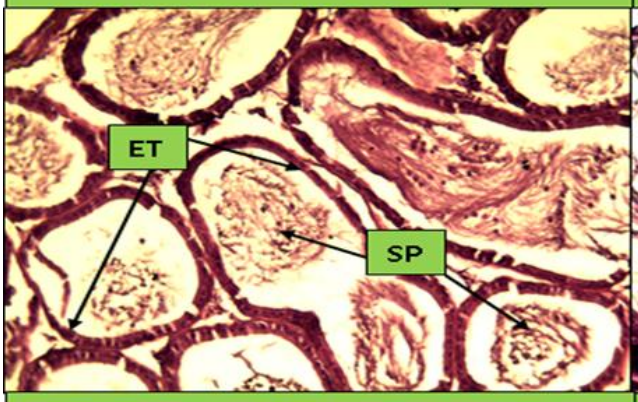

Fig (11): Epididymis of rat (3rd group). The epididymal tubules showed normally dilated (ET) and fully filled with sperms (SP). 50X H&E.

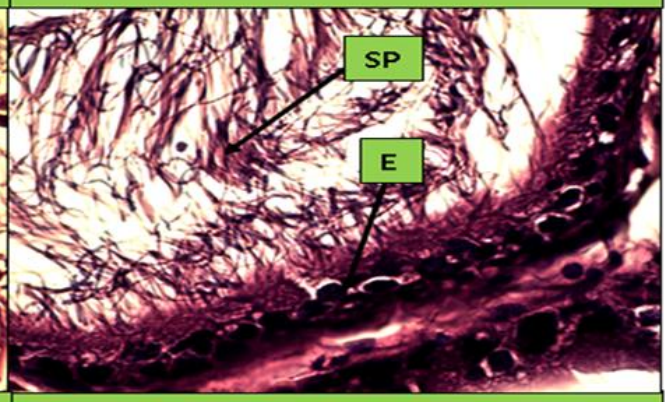

Fig (12): Epididymis of rat (3rd group). The epididymal tubules showed lined by normal pseudostratified columnar epithelium (E) with long stereocilia and filled with sperms (SP). 200X H&E.

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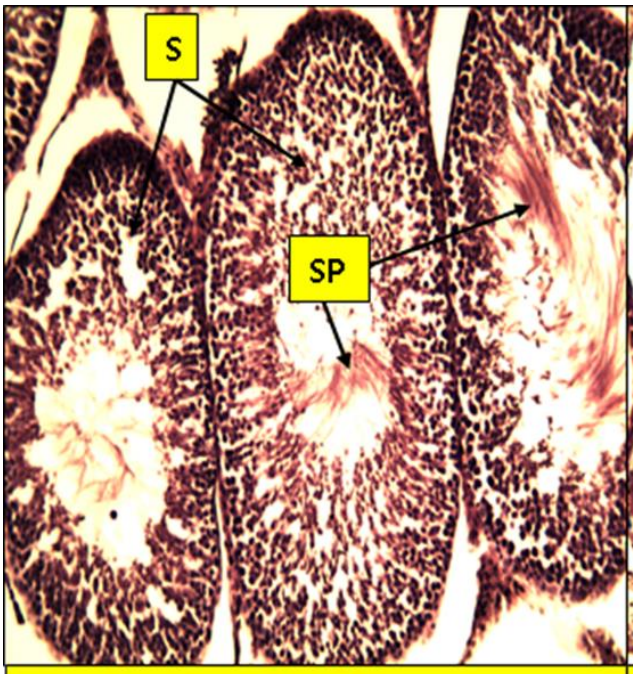

Fig (13): Testes of rat (4th group as control group). Note presence of characteristic architecture of seminiferous tubules (S) which appeared circled, compact with decreased diameter of their lumen and sperms in these lumens (SP). 50X H&E.

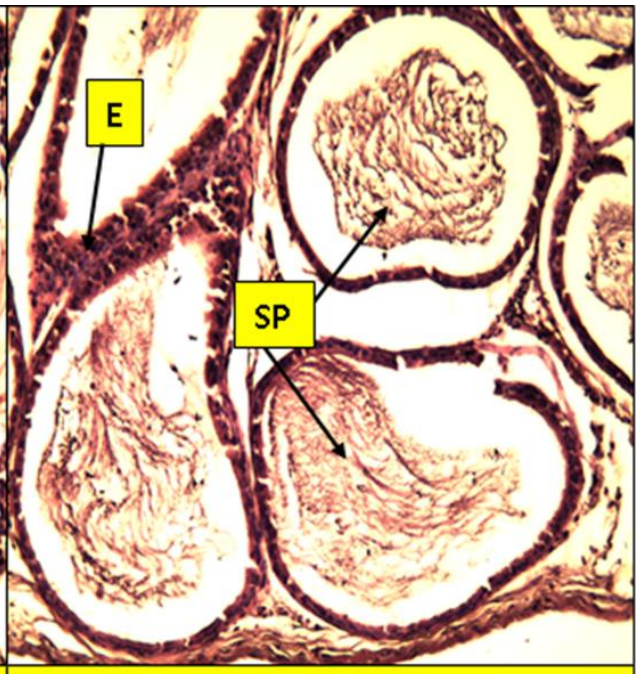

Fig (14): Epididymis of rat (4th group as control group). The epididymal tubules normally dilated with filled with sperms (SP) in their lumen and lined by pseudostratified columnar epithelium (E). 50X H&E.

Discussion

Counting of spermatogonia, spermatocytes, spermatozoa and Leydig cells:

In the Counting of spermatogonia, spermatocytes, spermatozoa and Leydig cells of testicular tissue of male albino rats, the results of 1st group showed the levels of spermatogonia, spermatocytes, spermatozoa and Leydig cells were statistically decreased in rats exposed to CuSo4. The reason may be due to a direct effect of CuSo4 in reducing the serum testosterone hormone, or an indirect effect through the toxic effect of CuSo4 on Leydig cells, which are the cells responsible for secreting the testosterone. Ultimately, this will be reflected in a clear inhibition of the process of spermatogenesis and a reduction in the number of spermatocytes within the seminiferous tubules. This is consistent with what (15).mentioned, they provided that using copper chloride results in reduction in plasma testosterone level in albino rats. Furthermore, it was reported (16) that Copper Sulphate causes

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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reproductive dysfunction that affects sperm quantity, morphology, and motility by destroying Sertoli, Leydig, and germ cells, thereby affecting the organs (testis, epididymis, and seminal vesicle). Other reason for reduction of the number of spermatocytes is production of oxidative stress by CuSo4 resulting in the destruction of spermatocytes particularly spermatogonia within the seminiferous tubules. Copper may play a crucial role in spermatogenesis and male infertility, as suggested by (17), who demonstrated that Copper may act as a mediator of the effect of oxidative damage.Rats exposed to CuSo4 plus -lipoic acid (2nd group) had significantly higher levels of spermatogonia, spermatocytes, spermatozoa, and Leydig cells compared to rats exposed to CuSo4 alone (1st group) because -lipoic acid obviously significantly increased levels of these parameters in CuSo4-treated rats. The reason for this improvement is the positive and ameliorative effect of ALA on the male reproductive system through its antioxidant role as well as its direct effect in activating Leydig cells and thus increasing the testosterone hormone and then increasing the number of spermatocytes and completion of spermatogenesis. This corresponds with (18) they indicate a cytoprotective role of lipoic acid against testicular toxicity in cyclophosphamide-exposed rats through its antioxidant action. Also the current study coincided with (19) they showed that the administration of ALA leads to the improvement of hormonal estimation, declines the testicular and body oxidative damage in lead acetate-exposed rats.

Histopathology:

The testes also showed severe changes like distortion of seminiferous tubules with complete suppression of spermatogenesis and disintegration of connective tissue between them. The reason of all these changes indicate the major effect of free radicals and oxidative stress which resulting from the exposure to CuSo4. These findings corroborate those of (20), who show that CuSo4-induced production of (ROS) and consequent oxidative stress impaired testicular function. Researchers (17) found that CuSo4 negatively affected male reproductive organs via oxidative stress. The presence of small spaces within the seminiferous tubules indicate the apoptotic or toxic effect of CuSo4 on the germ cells within the seminiferous tubule through production of ROS (21). Also the reason of these spaces within the seminiferous tubules may be attributed to accumulation of lipids in these tubules due to inhibition of spermatogenesis (22). Our results in the testes tissues demonstrated that there was reduction in the sperm amounts within the lumen of seminiferous tubules and within the lumen of epididymis. These changes may be due to the effect of CuSo4 on the sex hormones like (testosterone, luteinizing hormone (LH) and follicular stimulating hormone (FSH)) and this resulting in a direct effect on the spermatogenesis. This evidence was provided by (23) who provided that CuSo4 can be considered as an endocrine disruptor. Also (24) they showed that CuSo4 may affect sexual hormone levels and spermatogenesis. Therefore, this alteration in the secretion of sex hormones like testosterone is also responsible for the reduction of sperm numbers in CuSo4 exposed rat and this construe devoid the epididymal tubules from the sperms.On the contrary, using or supplementation with alpha lipoic acid could ameliorate all these testicular changes which occur due to exposure for the CuSo4. The alpha lipoic acid plays an important protective role against the reproductive toxicity of CuSo4 through reduced the production of ROS via reducing malondialdehyde (MDA) level and improving the antioxidant defense system activity in the testicular cells. These results coincided with (25) who explained that ALA supplementation

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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significantly decreased Malondialdehyde (MDA) levels and might lead to an amelioration in lipid peroxidation. It is an action completely opposite to the action of copper sulfate, as it was found to increase MDA and thus cause an increase in the generation of free radicals, as mentioned by (26), that CuSo4 caused elevation in rainbow trout gill and liver MDA resulting in increased the free radicals formation. Also alpha lipoic acid can ameliorate the reproductive toxicity of CuSo4 through increase synthesis and secretion of sex hormones and resulting in high production of sperms and this opposite effect of alpha lipoic acid against the reproductive toxicity of CuSo4.This evidence is supported with other studies, (27) provided that alpha lipoic acid able to maintain normal steroidogenesis and spermatogenesis, and it can be modulate adriamyin-induced testicular toxicity.In conclusion, Alpha-Lipoic Acid protects albino rats from the copper sulfate-induced male reproductive toxicity.

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