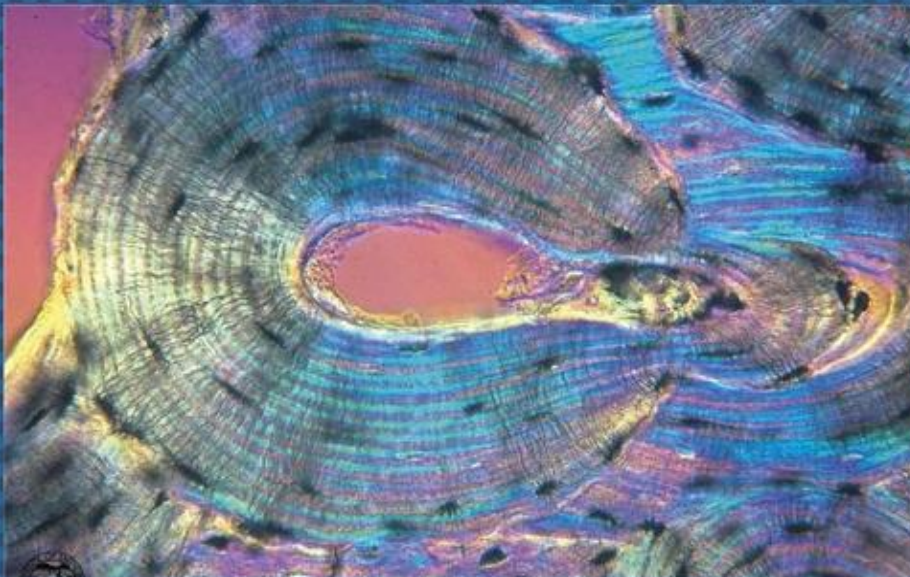




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Possible Protective Role of L-carnitine against Cisplatin-induced Testicular Changes in Adult Male Albino Rats: A Histological and Morphometric Study

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ABSTRACT

Background: Cisplatin (CIS) is a famous chemotherapeutic drug. Although its potency, it has serious side effects on organs, including testis. L-carnitine is an amino acid with an encouraging impact. **Aim of the work:** To assess the CIS effect on the testis of the adult male rat and the protective role of L-carnitine. **Material and Methods:** 30 adult male albino rats were divided into three equal groups. Control group; received 1 ml saline (0.9% NaCl) daily for 30 days via a gastric tube and were injected i.p. with 6 ml saline on the 27th day. CIS-treated group; received 1 ml saline daily for 30 days via a gastric tube and on the 27th day, they were injected with a single dose of CIS (7.5 mg/kg body weight; i.p.). CIS+L-carnitine-treated group; was treated with L-carnitine (100 mg/kg body weight /day) via a gastric tube for 30 days and on the 27th day, they received a single dose of CIS (7.5 mg/kg body weight; i.p.). At the end of the experiment (on the 30th day), the rats were sacrificed. The left testes were extracted, fixed and processed for histologic and morphometric studies. **Results:** The seminiferous tubules were deformed with marked depleted Sertoli and germ cells, the interstitial tissue was shrunken, and the seminiferous tubular diameters and epithelial heights were morphometrically decreased in the CIS group. Administration of L-carnitine markedly attenuated these CIS-induced testicular injury. **Conclusion:** L-carnitine has a protective role versus CIS-provoked damaging effects on the testis.

INTRODUCTION

The testis is the male primary reproductive organ. It profits two main functions: secretion of testosterone which is the male sex hormone, and the production of sperms. These functions are key for both maintenances of the male characteristics and preservation of species (Lara *et al.*, 2018).

Cisplatin (CIS) is a widely used chemotherapeutic agent. It is used to treat cancer like mesothelioma and osteosarcoma. It has been recommended by the United States Food and Drug Administration since 1978 (Abdel-Moneim, 2014).

CIS is an alkylating agent that exerts its effect by persuading DNA cross-links and making breaks in the DNA double strand causing what is called the programmed cell death (PCD) and apoptosis (Reddy *et al.*, 2016). PCD leads to oxidative stress by generating Reactive Oxygen Species and lipid peroxidation, causing cellular necrosis (Fallahzadeh *et al.*, 2017).

Even though cisplatin is a potent chemotherapeutic drug, it produces marked side impacts on the body (Ku *et al.*, 2015). It was observed that CIS had induced hepatic, renal and testicular degeneration (Dos Santos *et al.*, 2012; Waseem *et al.*, 2015; Purena *et al.*, 2018).

L-carnitine is a natural amino acid with a chief role in energy creation inside the cells. It was also found that L-carnitine possesses powerful antioxidant, anti-inflammatory and anti-apoptotic characteristics (Sayed-Ahmed, 2010; Radwan *et al.*, 2012).

Moreover, L-carnitine is highly concentrated in the optimized function of the testis. Nutritional supplementation with L-carnitine was found to improve both rat and mice sperm quantity and quality (Kanter *et al.*, 2010; Ahmed *et al.*, 2014) exposed to X-ray irradiation. Moreover, it was found that L-carnitine had protective effects on the testes of atherosclerotic rat models (Salama *et al.*, 2015). Hence, the present study aimed to assess the protecting role of L-carnitine on the testis of the CIS-treated adult male albino rat.

MATERIAL AND METHODS

A total number of 30 adult male albino rats (220-250 gm) were gotten from the Animal House of Assiut University. They were independently housed in ventilated cages, acclimated for 5 days before starting the experiment, at the room temperature and the ordinary daylight/dark cycle with free standard nourishment and water access.

CIS vials (10mg/10ml) were obtained from Mylan, Saint-Priest, France, and dissolved in 0.9% NaCl saline. L-carnitine was gotten from the Company of MEPACO, Egypt.

The rats have haphazardly separated into 3 groups; 10 rats each:

- **Group A** (Control group): The rats have gotten 1 ml saline (0.9% NaCl) daily for 30 progressive days through a gastric tube. On the 27th day, they were infused intra-peritoneal with 6 ml saline.
- **Group B** (CIS-treated group): The rats have gotten 1 ml saline daily for 30 days

via a gastric tube and on the 27th day, they were injected with a single dose of CIS (7.5 mg/kg body weight; intra-peritoneal). This dose of CIS is corresponding to the human therapeutic dose with the least side effects (Kindler, 2008; Reagan-Shaw *et al.*, 2008; Adeyemi *et al.*, 2017).

- **Group C** (CIS+L-carnitine-treated group): The rats have received 100 mg/kg body weight/day L-carnitine via a gastric tube for 30 days (Avsar *et al.*, 2014) and a single intra-peritoneal dose of 7.5 mg/kg body weight CIS on the 27th day.

At the end of the experiment (on the 30th day), all rats were anaesthetized by inhalation of ether and then they were sacrificed. The left testes were removed, cut into small (~1 mm³) cubes and settled in glutaraldehyde. Semithin sections were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate (Tizro *et al.*, 2019). The slides were inspected and captured at Assiut University Electron Microscopic Unit.

Statistical Analysis:

The semithin sections were also examined morphometrically to measure the means of the diameters and the epithelial heights of the seminiferous tubules of the different studied groups utilizing the picture analyzer computer system (Leica Qwin 500). The measured parameters were analyzed statistically using a one-way analysis of variance test (ANOVA) of the SPSS program version 21.0.

RESULTS

A) Histological results:

1- Control Group (Group A):

The control rat testis was comprised of rounded or oval seminiferous tubules lined with spermatogenic cells at distinctive stages of development (spermatogonia types A and B, pachytene primary spermatocytes, rounded spermatids and mature sperms) with Sertoli cells in-between. Groups of Leydig cells with blood vessels were seen within the interstitial tissue among the seminiferous tubules (Fig. 1).

Ultrastructural examination of the control group testis showed that the seminiferous tubules were lined with different germ cells. The type A spermatogonia were characterized by wide contact with the basement membrane. The nucleus was oval, exhibited a basal position and contained a prominent nucleolus. The type B spermatogonia appeared smaller with less contact with the basement membrane than type A. The nucleus was spherical and contained more than one nucleolus with multiple chromatin masses attached to the inner aspect of the nuclear membrane. The pachytene primary spermatocytes had rounded nuclei with even chromatins. Mitochondria, endoplasmic reticulum and free ribosomes were present in the cytoplasm. The rounded spermatids revealed spherical nuclei. The acrosomal cap extended over one pole of the nucleus. The cytoplasm showed an acrosomal granule in association with the nucleus, multiple mitochondria and endoplasmic reticulum. The Sertoli cells were resting on a regular basement membrane and had euchromatic nuclei and nucleoli. The cytoplasm contained multiple mitochondria, endoplasmic reticulum and lipid droplets. The Leydig cells appeared as small clumps with spherical or polyhedral shape and pale vacuolated cytoplasm in the interstitial tissue. Their nuclei were characterized by rounded or oval vesicular appearance with a thin peripheral rim of chromatin material and fine indentations. The cytoplasm displayed abundant organelles (Fig. 4).

2- Cisplatin-Treated Group (Group B):

In the Cisplatin-treated group, the seminiferous tubules appeared shrunken and deformed with an irregular basement membrane. Marked reduction and degeneration of the spermatogenic cells with the appearance of empty intercellular spaces in between. Spermatogonia lost contact with the basement membrane. Pachytene primary spermatocytes had vacuolated cytoplasm. The germ cells separated from each other and revealed vacuolated cytoplasm. Sertoli cells were separated from the basement membrane.

Shrunken interstitial tissue was also observed (Fig. 2).

Electron microscopic examination of the CIS-treated testicular sections showed marked degenerative changes affecting most of the spermatogenic, Sertoli and Leydig cells. Type A spermatogonia were shrunken. Type B spermatogonia had severely vacuolated cytoplasm containing swollen mitochondria. Pachytene primary spermatocytes showed an interruption of the nuclear envelope. The cytoplasm was severely rarified. Loss of junctional integrity between the primary spermatocytes and the adjacent spermatids was observed. Rounded spermatids were separated from each other by wide intercellular spaces containing cellular debris exhibiting damaged mitochondria. Their cytoplasm revealed many vacuoles, damaged mitochondria and a huge perinuclear vesicle. Sertoli cells appeared loosely separated from the basement membrane and contained vacuolated cytoplasm. Leydig cells revealed shrunken nuclei. The cytoplasm was rarified and contained damaged mitochondria (Fig. 5).

3- Cisplatin+L-Carnitine-Treated Group (Group C):

The testicular specimens of the rats received L-carnitine in addition to cisplatin revealed improvement of the morphology of the seminiferous tubules. Seminiferous tubules had patent lumen and revealed spermatozoa. The spermatogenic cells were well-organized. Spermatogonia, pachytene primary spermatocytes and round spermatids were similar to the control ones. Residual empty spaces and irregular basement membranes were noticed. The interstitial tissue appeared less shrunken and contained blood vessels. Leydig cells were arranged into small clumps and had spherical or polyhedral shapes with rounded or oval vesicular nuclei and pale vacuolated cytoplasm (Fig. 3).

Ultrastructural examination of the testicular specimens of the rats that received L-carnitine and CIS revealed that most of the seminiferous tubules were lined with spermatogenic and Sertoli cells like those of the control group apart from some

residual cytoplasmic vacuolations. Type B spermatogonia with a rounded nucleus and peripheral chromatin condensation were seen resting on a basement membrane. The pachytene primary spermatocytes showed a large, rounded nucleus and the cytoplasm contained many mitochondria and endoplasmic reticulum. The round spermatids with rounded nuclei were covered by acrosomal caps and perinuclear

vesicle. The cytoplasm contained many mitochondria and free ribosomes. Sertoli cells with a large nucleus and prominent nucleolus were observed resting on a basement membrane. The cytoplasm contained mitochondria and endoplasmic reticulum. Leydig cells had an oval nucleus with peripheral chromatin condensation. The cytoplasm contained mitochondria, lipid droplets and free ribosomes (Fig. 6).

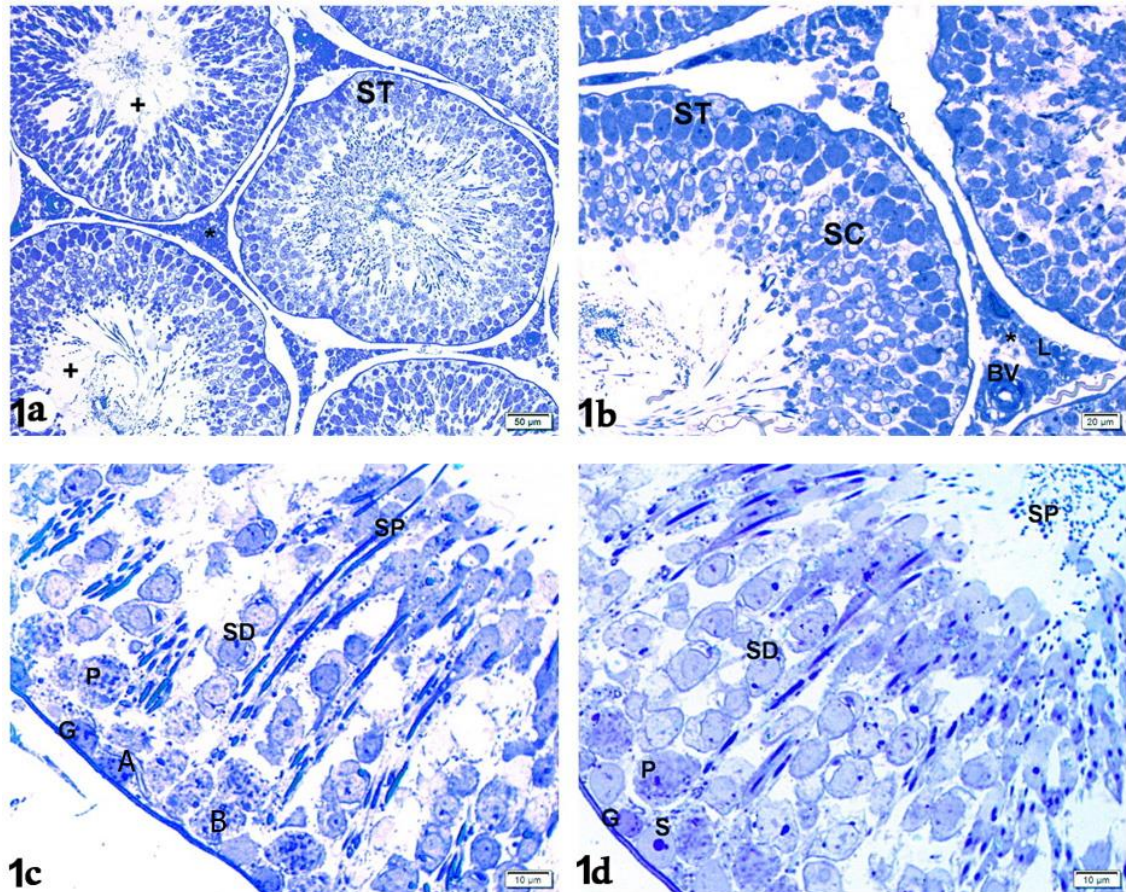


Fig. 1: A photomicrograph of a semithin section of a control albino rat testis (group A) showing;

1a- A group of rounded or oval seminiferous tubules (ST) with patent lumina (+) and interstitial tissue (*) in-between the tubules.

1b- Parts of seminiferous tubules (ST) lined by spermatogenic cells at different stages of development (SC). Notice the presence of interstitial tissue (*) containing Leydig cells (L) and blood vessel (BV).

1c&1d- A portion of seminiferous tubule lined by Sertoli cells (S), spermatogonia (G), type A spermatogonia (A), type B spermatogonia (B), pachytene primary spermatocytes (P), round spermatids (SD) and mature sperms (SP).

(Toluidine blue, a X200, b X400, c&d X1000)

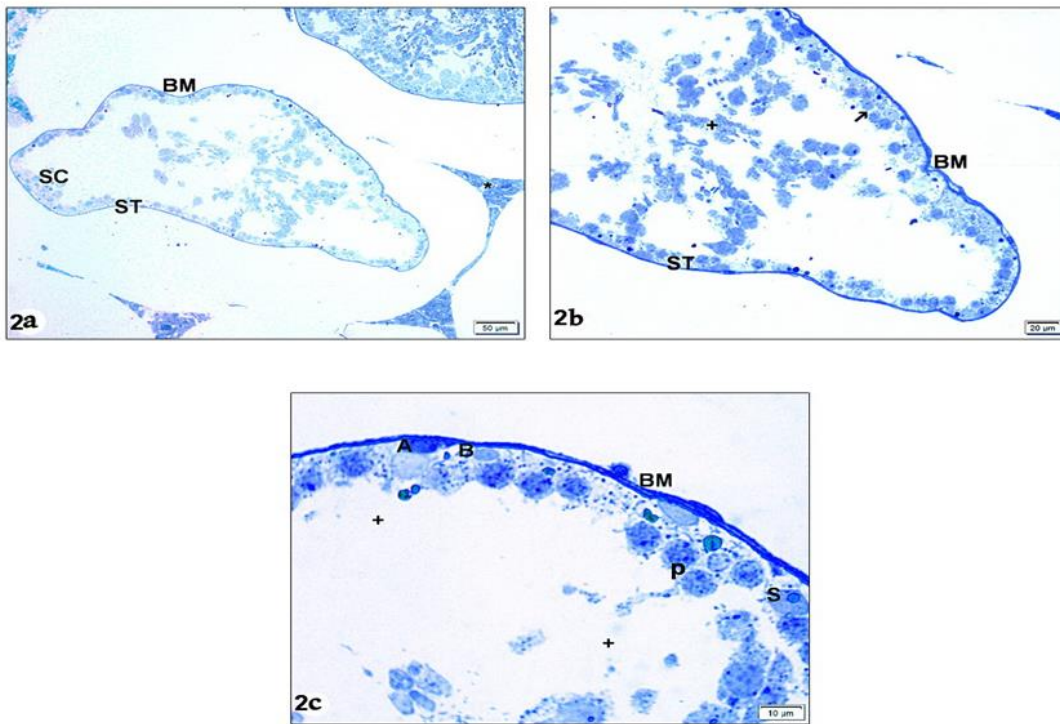


Fig. 2: A photomicrograph of a semithin section of a CIS-treated albino rat testis (Group B) showing;

2a- Apparently shrunken seminiferous tubules (ST) with an irregular basement membrane (BM). Obvious reduction of the spermatogenic cells (SC) with appearance of wide intercellular spaces in-between. Notice the shrunken interstitial tissue (*).

2b- A magnified part of figure (2a) revealing a deformed seminiferous tubule (ST), an irregular basement membrane (BM), degenerated germ cells (+) and separated germ cells from each other (arrow).

2c- A portion of a seminiferous tubule with an irregular basement membrane (BM), type A spermatogonia (A) with vacuolated cytoplasm, type B spermatogonia (B) losing contact with the basement membrane, pachytene primary spermatocytes (P) with vacuolated cytoplasm. Sertoli cells (S) appear separated from the basement membrane. The tubule shows marked loss of cellularity (+).

(Toluidine blue, a X200, b X400, c X1000)

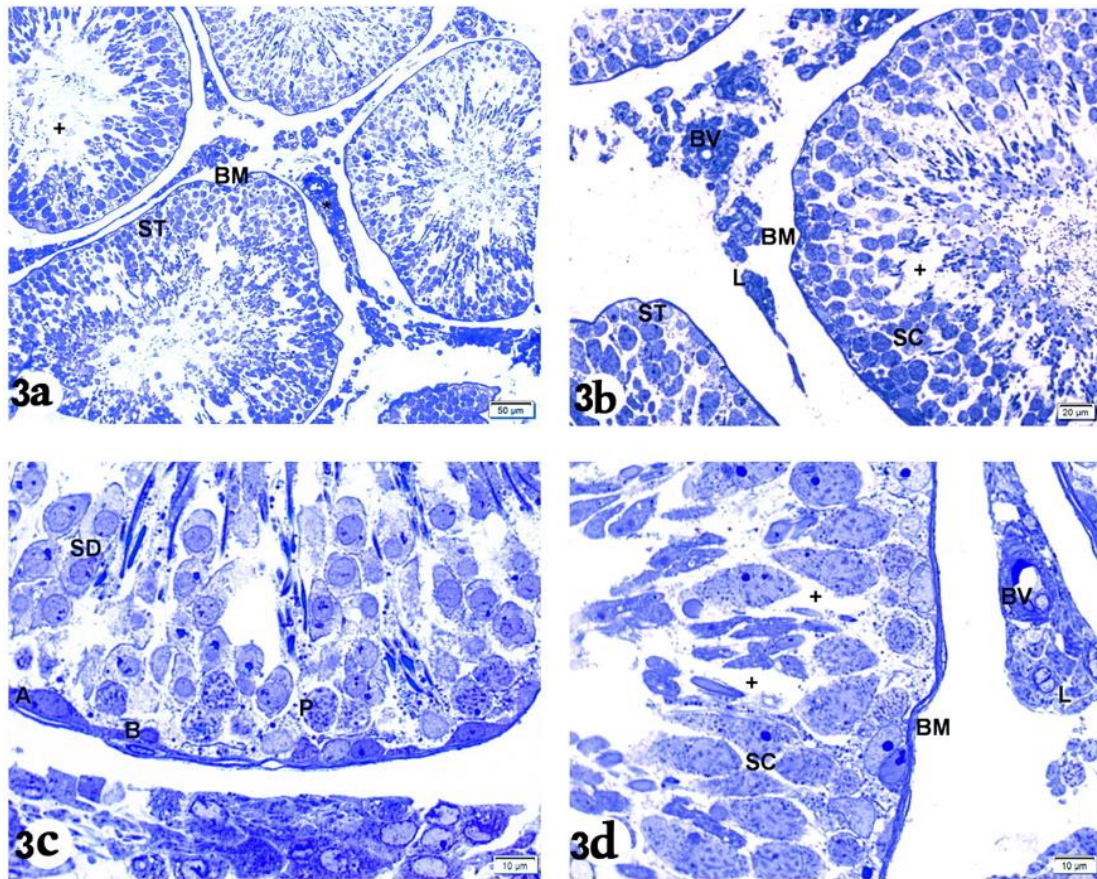


Fig. 3: A photomicrograph of a semithin section of a CIS+L-carnitine-treated albino rat testis (Group C) showing;

3a- A group of seminiferous tubules (ST) with patent lumen containing spermatozoa (+) and residual irregular basement membranes (BM). The interstitial tissue (*) appears less shrunken among the tubules.

3b- Seminiferous tubules (ST) with residual irregular basement membranes (BM) and empty spaces (+). The spermatogenic cells are organized (SC). Leydig cells (L) are arranged into small clumps. Notice the blood vessel (BV) in the less shrunken interstitial tissue.

3c- Type A spermatogonia (A), type B spermatogonia (B), pachytene primary spermatocytes (P) and round spermatids (SD) like the control ones.

3d- A seminiferous tubule with a residual irregular basement membrane (BM) and empty spaces (+). The spermatogenic cells are well-organized (SC). Leydig cells (L) are arranged into small clumps and have spherical or polyhedral shape with rounded or oval vesicular nuclei and pale vacuolated cytoplasm. Notice the blood vessel (BV) in the less shrunken interstitium.

(Toluidine blue, a X200, b X400, c&d X1000)

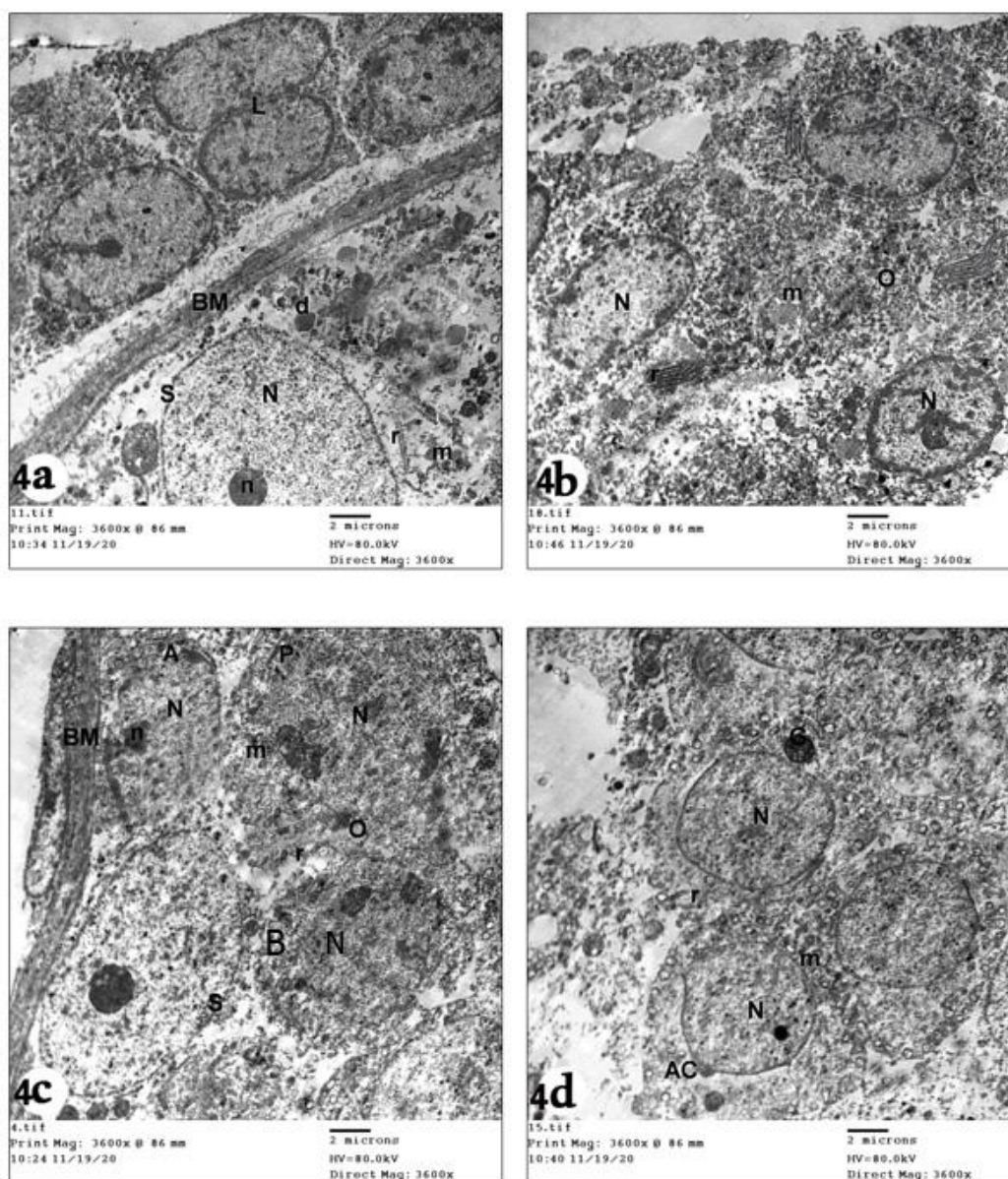


Fig. 4: An electron photomicrograph of a control albino rat testis (Group A) showing; 4a- Sertoli cell (S) resting on a regular basement membrane (BM). Its euchromatic nucleus (N) is oval with a prominent nucleolus (n). Its cytoplasm shows multiple mitochondria (m), endoplasmic reticulum (r) and lipid droplets (d). The figure also shows multiple Leydig cells (L) in the interstitium.

4b- Multiple spherical or polyhedral Leydig cells with rounded or oval vesicular nuclei (N) with peripheral rim of chromatin material and fine indentations. The pale vacuolated cytoplasm is rich in mitochondria (m), endoplasmic reticulum (r) and free ribosomes (O).

4c- A type A spermatogonium (A) exhibiting wide contact with the basement membrane (BM) and has an oval basal nucleus (N) with a prominent nucleolus (n). A type B spermatogonium (B) appears smaller with less contact with the basement membrane than type A. Its nucleus (N) is spherical and contains more than one nucleolus with multiple chromatin masses attached to the inner aspect of the nuclear membrane. A pachytene primary spermatocyte (P) has rounded nucleus (N) with evenly chromatins. Mitochondria (m), endoplasmic reticulum (r) and free ribosomes (O) are present in the cytoplasm. Notice the presence of a Sertoli cell (S).

4d- Multiple rounded spermatids with spherical nuclei (N). The acrosomal cap (AC) spreads over one pole of the nucleus. The cytoplasm shows an acrosomal granule (G), multiple mitochondria (m) and endoplasmic reticulum (r). (X 3600)

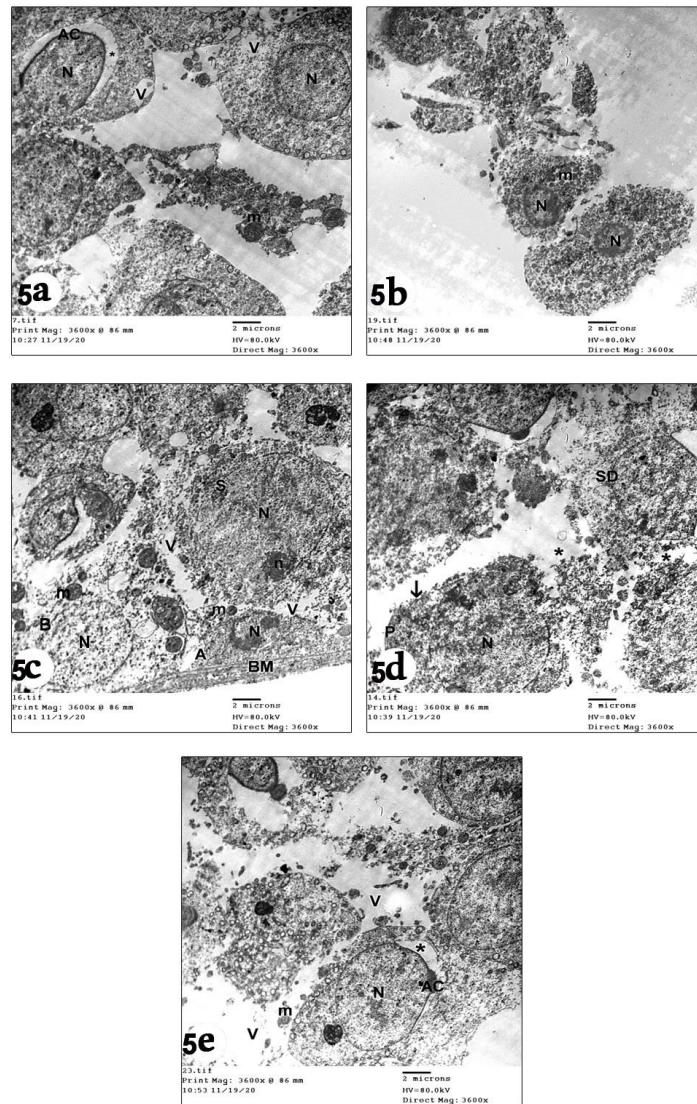


Fig. 5: An electron photomicrograph of a CIS-treated albino rat testis (Group B) showing;

5a- Many rounded spermatids that are separated by wide intercellular spaces. The nuclei (N) are rounded and covered by acrosomal caps (AC). There are many vacuoles in the cytoplasm (V) and a huge perinuclear vesicle (*). There is a central cellular debris including damaged mitochondria (m).

5b- Leydig cells with shrunken nuclei (N). The cytoplasm is rarified and contains damaged mitochondria (m).

5c- Type A spermatogonia (A) resting on a basement membrane (BM) and containing oval nucleus (N) is shrunken. Type B spermatogonia (B) with rounded nucleus (N) and severely vacuolated cytoplasm (v) contain swollen mitochondria (m). Sertoli cell (S) appears loosely separated from the basement membrane and contains rounded nucleus (N) with prominent nucleolus (n) and vacuolated cytoplasm (V).

5d- A pachytene primary spermatocyte (P) with a rounded nucleus (N) that shows an interruption of the nuclear envelope (arrow). The cytoplasm is severely rarified. Notice the loss of integrity (*) between the primary spermatocyte and adjacent spermatids (SD).

5e- Multiple round spermatids with rounded nuclei (N) covered by acrosomal cap (AC) and perinuclear vesicle (*). The cytoplasm is severely vacuolated (V) and contains damaged mitochondria (m). (X 3600)

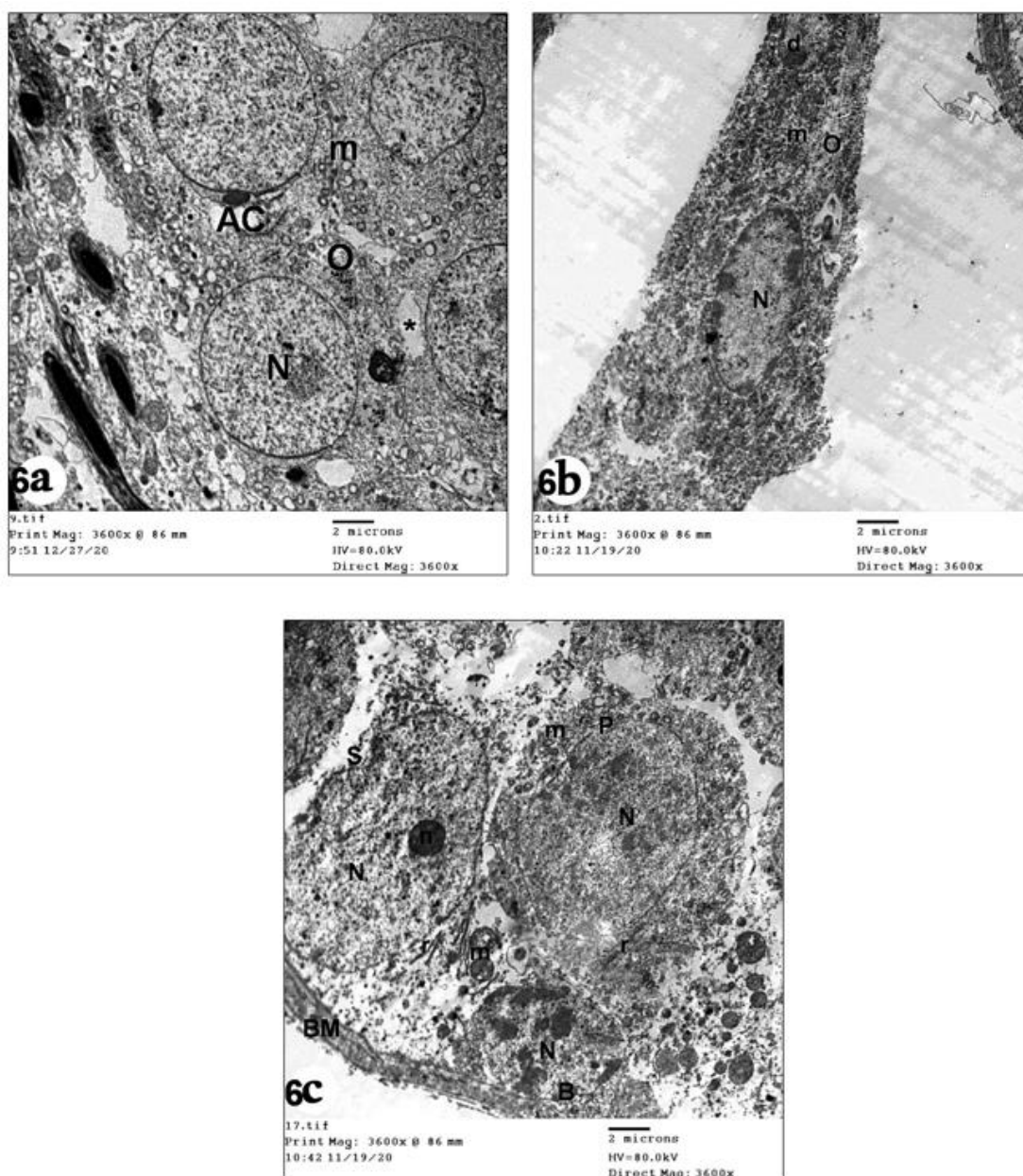


Fig. 6: An electron photomicrograph of a CIS+L-carnitine-treated albino rat testis (Group C) showing;

6a- Multiple round spermatids with rounded nuclei (N) covered by acrosomal cap (AC) and perinuclear vesicle (*). The cytoplasm contains many mitochondria (m) and free ribosomes (O).

6b- A Leydig cell with oval nucleus (N) that has peripheral chromatin condensation. The cytoplasm contains mitochondria (m), lipid droplets (d) and free ribosomes (O).

6c- A Sertoli cell (S) with large nucleus (N) and prominent nucleolus (n) resting on a basement membrane (BM). Its cytoplasm contains mitochondria (m) and endoplasmic reticulum (r). A type B spermatogonium (B) has rounded nucleus (N) with peripheral chromatin condensation. The pachytene primary spermatocyte (P) shows large rounded nucleus (N) and its cytoplasm contains many mitochondria (m) and endoplasmic reticulum (r).

(X 3600)

B) Morphometric Results:

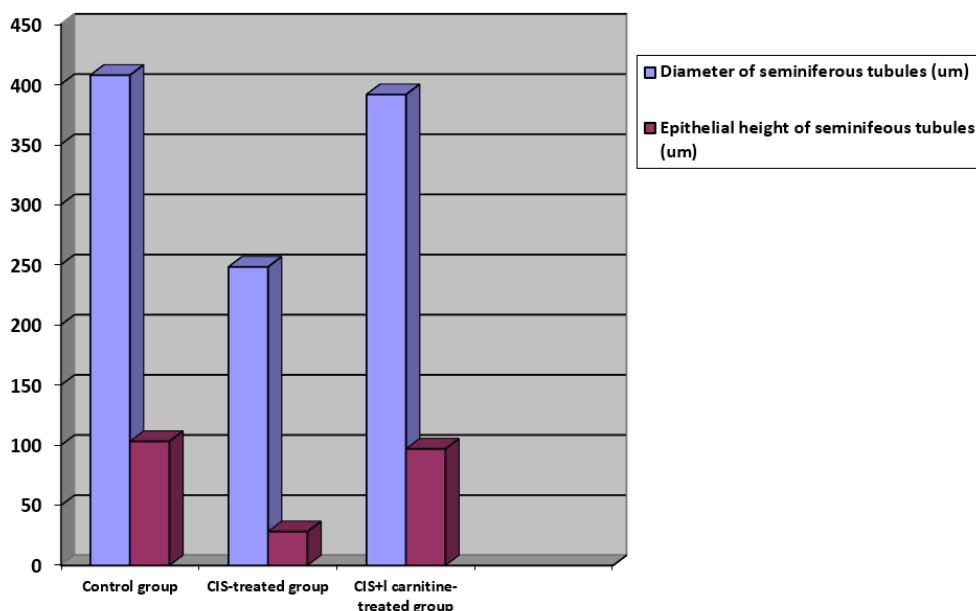
Statistical analysis of the means of the seminiferous tubular diameters and the epithelial heights of the three experimental groups showed that they were significantly decreased in the CIS-treated group in comparison

to the control group. Whereas there was an insignificant difference between the means of the seminiferous tubular diameters and the epithelial heights of the CIS+L-carnitine-treated group when compared to the control (Table 1 and Histogram 1).

Table 1: The means of the seminiferous tubular diameters and their epithelial heights of the different studied groups.

	Control group (mean±SD)	CIS-treated group (mean±SD)	CIS+L carnitine group (mean±SD)
Diameters of the seminiferous tubules (um)	407.6±15.2	248.2±11.4*	391.5±12.2
Epithelial heights of the seminiferous tubules (um)	103.2±9.4	28.2±2.7*	97.0±2.7

*Significant, at p-value <0.05



Histogram 1: Showing the means of the seminiferous tubular diameters and the epithelial heights of the different studied groups.

DISCUSSION

Although cisplatin is widely used as a treatment of numerous cancers, its usage is restricted because of its unfavorable effects on the renal, reproductive, and nervous systems. The affection of the testes is recognized and documented as a consequence of CIS toxicity. Cisplatin was stated to cause apoptosis of the germ cells, sperms, and Leydig cells of the experimental animals (Atessahin *et al.*, 2006; Amin *et al.*, 2012).

The present study revealed that the CIS administration to adult male albino rats resulted in shrunken deformed seminiferous tubules and irregular basement membranes. There was a marked loss of the spermatogenic cells and marked degenerative changes affecting the germ cells. The interstitial tissue was markedly shrunken. These results are in agreement with Abdel-Mohsen *et al.* (2013) who found that Cisplatin had destructive influences on the seminiferous tubules. Certain areas

of the tubules exhibited severe germ cell depletion. Sertoli cells displayed a variable degree of degeneration.

Moreover, the results of the present study were concomitant with Eid *et al.* (2016) who observed that administration of cisplatin in rats had resulted in enormous seminiferous tubular degeneration and reduction in the number of the spermatogenic cell layers.

In addition, the present results are in accordance with the findings of El-Amir *et al.* (2019) who stated that the testes of the rats injected with CIS showed spermatogenic epithelial exhaustion, empty seminiferous tubular lumen and many seminiferous tubules were lined only with spermatogonia and primary spermatocytes.

Also, Mercantepe *et al.* (2018) found cisplatin-induced damage of the germinal epithelial cells and loss of the connection between spermatocytes and spermatogonia. On ultrastructural examination, degenerative changes were detected in spermatogonia, spermatocytes and spermatids.

Almeer and Abdel Moneim (2018) attributed the cisplatin-induced gonadal toxicity to the increased reactive oxygen species production as well as the exhausted enzymatic and non-enzymatic antioxidant defense mechanism of the testis. Cisplatin is well-known that it interrupts the normal balance between the testicular tissue oxidant activity and antioxidant one (Anand *et al.*, 2015).

Moreover, inflammation is also reported to be associated with CIS-induced toxicity as reported by Zhu *et al.* (2017). It initiates the pathways encouraging the appearance of the inflammatory substances as cytokines, including IL-1 β and TNF- α (Eid *et al.*, 2016).

Previous studies suggested that cisplatin had a destructive effect on Sertoli cells leading to a destruction of the blood-testicular barrier leading to the release of hydrolytic enzymes, and consequent the destruction of the

adjacent cells (D'cruz and Mathur, 2005).

Moreover, Cisplatin inhibits the replication of the DNA and the transcription of the RNA via the creation of intra-strand and inter-strand DNA cross-links (Boekelheide *et al.*, 2003).

The electron microscopic examination of the CIS-treated rats' testicular sections of the present study came parallel to the light microscopic results. It revealed widely separated and degenerated spermatogenic cells, vacuolated cytoplasm and swollen mitochondria, and separation of the lining cells from the basement membrane. These findings are characteristic of apoptosis as mentioned by OZgoli *et al.* (2009) and Sajjad (2012).

On the other hand, L-carnitine is reported to be a natural nutrient that is essential for mitochondrial fatty acid oxidation in order to produce ATPs (Tunez *et al.*, 2007). Consequently, it possesses antioxidant properties that play a defensive mechanism against the oxidative stress of many tissues, including the liver, kidney (Cayir *et al.*, 2009) and testis (Eid *et al.*, 2016).

In the present study, it was found that L-carnitine treatment had markedly reversed most of the deteriorative changes induced by CIS. The results of the present study are supported by Eid *et al.* (2016) who stated that L-carnitine had a noticeable protective effect against cisplatin provoked testicular alterations. The results of the current study are also concomitant with the finding of Gawish *et al.* (2011) who observed that the administration of the L-carnitine attenuated the damaging effect of varicocele in the rat testis.

Topcu-Tarladacalisir *et al.* (2009) stated that the L-carnitine administration improved the spermatogenic cell number and the morphological state of the testis and exhibited protection against the

changes obtained because of gamma radiation exposure.

Moreover, L-carnitine is provoked to protect the cells against mitochondrial and nuclear DNA damage. It improves the mitochondrial function by decreasing DNA damage through decreasing the manufacture of the oxidants and augmenting the antioxidant state (Calò *et al.*, 2006). The L-carnitine in the epididymis was found to affect the number, motility, and maturity of the sperms (Ng *et al.*, 2004).

In the present study the morphometric results came in harmony with the histological results where the means of the seminiferous tubular diameters and the epithelial heights showed that they were significantly decreased in CIS-treated group in comparison to the control group. Whereas there was an insignificant difference between the means of the seminiferous tubular diameters and the epithelial heights of the CIS+L-carnitine-treated group when compared to control.

So, it is concluded that L-carnitine treatment affords substantial protective properties against CIS-induced rat testicular damage. Consequently, L-carnitine supplementation appears to be helpful in the clinical management of CIS-dependent regimen cancer patients to avoid its deteriorating effect on the testis.

Conflict of Interest:

The authors announce that they have no individual or financial conflict of interest to reveal.

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