The Protective Role of L-Carnitine Against Cisplatin Induced Testicular Toxicity in the Adult Albino Rat

Original Article

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ABSTRACT

Introduction: Testicular damage is one of the most deleterious effects whenever cisplatin (CP) is employed in cancer treatment. CP results in male infertility. L-carnitine (LC) is a vitamin-like substance with antioxidant activity. L-carnitine improves spermatogenesis, sperm maturation and motility.

Aim of the Work: Assess the protective role of LC in cisplatin-induced testicular toxicity.

Material and Methods: Forty adult male albino rats (200-250 grams each) were divided into four groups. The first group is control. Group II: LC was intraperitoneal (IP) injected (500 mg/kg/d) for fifteen days. Group III: CP was injected with only one dose of 7 mg/kg CP (IP) on the 6th day. Group IV: LC plus CP received treatment of both groups II and III. Semen analysis was performed to estimate sperm count and motility. Testicular sections were examined under light microscopy using H&E, PAS, and anti-inducible nitric oxide synthase (iNOS) stainings. A transmission electron microscope was also done.

Results: Cisplatin caused a significant decline in the body weight, absolute and relative testicular weight, sperm count, sperm motility, seminiferous tubular diameter, and germinal lining height in relation to other groups, with a significant improvement in the CP plus LC group. Specimens from CP-treated animals showed thick and destructed seminiferous tubules (ST) basal lamina, obvious disorganized and atrophied ST, spermatogenic arrest, and atrophied Leydig cells. LC improves these changes with variable preservation of spermatogenic cells. Inducible NOS staining in the CP-treated group showed a moderate positive reaction, other groups showed a negative or weak reaction. Transmission electron photomicrography of the CP group showed large cytoplasmic vacuoles, small distorted mitochondria, and large lipid droplets in most spermatogonic cells; abnormally shaped spermatozoa.

Conclusion: L-carnitine partially attenuates CP-related histological changes of the testicular tissue and improves the quality parameters of the sperm, ensuring the protective effect of LC.

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Key Words: Cisplatin, iNOS, L-carnitine, testicular toxicity.

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INTRODUCTION

Cisplatin (CP) is an anticancer agent based on platinum[1]. It is one of the famous chemotherapeutic agents. It has a powerful role in the management of many types of cancer types such as ovarian, testicular, and colorectal malignancies[2]. It was first described by Michel Peyrone in 1844[3] and has been approved by the United States FDA since 1978[4]. One of the most dangerous side effects is testicular injury whenever CP is employed in cancer chemotherapy[5]. It causes gonadal toxicity, which can be temporary or permanent in male patients[6], by inducing abnormalities in sperm motility and count[2,7].

The mechanisms underlying CP-induced testicular injury involve generating oxygen-containing reactive species and lipid peroxidation, which inhibits protein synthesis and DNA damage. These changes lead to mitochondrial dysfunction and programmed cell death (PCD)[8,9].

New strategies have been developed to prevent the toxic effects of CP by using antioxidants that protect against cell damage by neutralizing the free radicals toxic effect[10]. L-carnitine (LC) is a derivative of amino acid and structurally resembles a vitamin-like molecule[11]. The major role of LC is in transporting fatty acids with long chains out of the cytosol and into the mitochondria and facilitating their oxidation, which is critical for the production of energy occurring within the mitochondria[12,13]. In addition, It prevents the creation of reactive oxygen species (ROS) and is used under different pathological conditions associated with increased oxidative stress to ameliorate oxidative damage[14]. L-isoform of carnitine is synthesized endogenously and taken from the outside of red meat, fish, poultry, and milk[15]. Additionally, it significantly influences the sperm formation process, it was found in large amounts in the testis and epididymis[16].

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AIM OF THE WORK

This study was done to evaluate any potential protective effects of LC on testicular toxicity induced by CP in adult albino rats.

MATERIAL AND METHODS

Ethical approval

The study protocol was accepted by the Ethics Committee of the Faculty of Medicine, Helwan University, Egypt. (Serial number: 23-2021).

Drugs

Cisplatin was commercially supplied by Mylan S.A.S, France in the form of a vial (50 mg/ 50 ml) and L-carnitine was commercially supplied by EVA Pharma as ampoules (1gm/ 5ml).

Animals

Our study used forty albino male rats weighing 250-300 gm each. The rats were obtained and the experiment was done at the animal house, Department of Pharmacology, Faculty of Medicine, Al Azhar University for boys, Cairo, Egypt. They adapted for two weeks before the experiment, the rats were housed in different cages with free access to food and water ad libtum.

Experimental Design

Forty adult male albino rats (average 250-300 grams each) were separated into four equal groups. **Group I:** Control injected daily 2ml intraperitoneal (IP) of normal saline for 15 days. **Group II:** LC was IP injected (500 mg/ kg/d) for 15 days[17]. **Group III:** CP was injected with one dose IP of 7 mg/kg CP at the 6th day[2]. **Group IV:** LC plus CP received a combination of treatments as both groups II and III.

Necropsy

The rats were weighed on the first day and on the last day of the experiment and then sacrificed under ether anesthesia. The testes were extracted and weighed. Each rat's epididymides were evaluated for sperm count and motility.

Sperm collection and parameters

The semen was obtained by squeezing the epididymis in a sterile watch glass and examined in accordance with the approach used by Bearden and Fluquary, 1980[18] for assessment the sperm motility and count[19].

Histopathological study

The right testis was fixed in formaldehyde solution, dehydrated, cleared in xylol embedded in paraffin wax, and finally cut into serial 5 µm-thick sections by automatic processing machine at Histology Department, Faculty of Medicine for boys, Al-Azhar University. Examination was done under a light microscope with exposure to the following stains: Hematoxylin and Eosin staining[20], Periodic Acid-Schiff Staining (PAS) reaction: for evaluation of the basement membrane[21], Immunohistochemical staining by using primary antibody for detection of inducible nitric oxide synthase (iNOS)[22]. It is a rabbit polyclonal antibody (Lab Vision Corporation Laboratories, CA, USA), ready-to-use 7ml kit, catalog number (RB-9242-R7). A positive control section for iNOS gives a brown cytoplasmic reaction.

Transmission Electron Microscopic Study

The left testes were cut into small parts (1 mm^3) and fixed in glutaraldehyde. The semithin sections and then ultrathin sections were stained and prepared[24]. The examination was done at the Regional Center for Mycology and Biotechnology, Al-Azhar University.

Morphometric Study

The measurements were performed using the image analyzer Leicia Q500 MC program at the Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University.

The mean values of seminiferous tubules (ST) diameters were analyzed in five random ST in five nonoverlapping fields in five randomly selected rats in each group. The round ST was randomly selected for the measurement. The Height of seminiferous epithelium was measured from the same tubules used to measure seminiferous tubules[23].

Statistical Analysis

This was done by using SPSS software (25.0). The results are presented as mean ± standard deviation. Analysed data were done using a one-way analysis of variance test (ANOVA). The accepted level of significance when the $P\ value \leq 0.05$.

RESULTS

Body and testicular weights

The control and LC group revealed normal body weight gain during the two weeks of our study with non-significant differences when compared with their initial weight. CP group recorded a significant reduction in body weight versus their initial weight. Similarly, the LC plus CP group also experienced a significant reduction in their weight when compared to their starting body weight. There was no variance Between the control and LC groups in both absolute and relative testicular weights. When compared to the control group, the absolute and relative testicular weights of the CP group were significantly low. However, when compared to the CP group, the CP plus LC group showed a significant increase in these parameters, but it was still significantly lower than the control group (Table 1).

Sperm count and motility

Between the control and LC groups, there was no considerable difference in the mean percentage of sperm motility and count. When comparing the CP group to the

control, the CP group's mean percentage of sperm motility and count were significantly lower. CP plus LC group showed a significant enhancement in sperm counts and motility when compared to the CP group, but not when compared to the control and LC groups (Table 2).

Light Microscopy

H&E-stained sections

Control group: The testicular sections showed the normal thickness of the tunica albuginea with normal-shaped ST having the natural order of spermatogonia (SG) cells, SCs cells were resting on a normal basement membrane. Normal structures of primary spermatocytes (PScs), spermatids, and spermatozoa. The interstitium showed a normal vascular structure and typical Leydig cells (Figures 1,2). LC group: Sections from the testes of this group showed similar results as the control group (Figure 3). CP group: showed tunica albuginea with congested subcapsular blood vessels and most STs have lost their normal architectural style. There are numerous atrophic ST and disturbances in the various phases of sperm production. Spermatogenesis was aborted at either the PScs or the spermatogonial phases. Separation and dispersion of the lining spermatogenic cells. Wide interstitial space with atrophied Leydig cells was seen (Figures 4,5,6). CP plus LC group: showing an improvement in many of the seminiferous tubule structures. Exhibited a regular outline and lines with the different stages of spermatogenic cells. But separated by relatively wide areas of interstitial tissue containing loose vascular C.T with few Leydig cells. However, there are still some atrophied ST and areas of spermatogenic cell loss (Figures 7,8).

PAS-stained sections

The testicular tissue showed a normal regular intact basement membrane in the control group (Figure 9) and LC group (Figure 10). But it was thick, irregular, and destructed in some areas in the CP group (Figure 11). In CP plus LC group: BM showed more regularity and integrity than CP group (Figure 12).

Immunohistochemical sections

The immunostained sections of the control group revealed a negative immunoreactivity in the germinal lining for INOS except a weak positive cytoplasmic reactivity of sporadic cells, negative cytoplasmic reactivity interstitial Leydig cells (Figure 13). LC group revealed a negative cytoplasmic reactivity for iNOS in germinal lining and Leydig cells (a picture almost comparable with that of control group) (Figure 14). Sections of CP group displayed moderate positive cytoplasmic reactivity in the remaining intact germinal lining and in the interstitial cells of Leydig. (Figures 15,16). Sections of the CP plus LC group revealed a weak to moderate positive cytoplasmic reactivity in the germinal lining and more apparent on spermatids and weak positivity in the interstitial cells of Leydig (Figure 17).

The control group showed that ST was lined with different germ cells resting on regular thin lamina propria containing myoid cells. Spermatogonia were characterized by an oval nucleus, exhibited a basal nucleolus with dispersed chromatin, endoplasmic reticulum, rounded mitochondria, few lysosomes, and some cytoplasmic vacuoles. Sertoli cells (SCs) had nuclei with euchromatic appearance and indentations, dominant nucleolus, and numerous mitochondria. SC exhibited lateral cytoplasmic processes. Primary spermatocytes had rounded nuclei with even chromatins. Numerous cytoplasmic mitochondria and a few cytoplasmic vacuoles were noticed. Many rounded spermatids revealed spherical nuclei and elongated spermatids with an acrosomal cap. Well-formed spermatozoa with well-formed acrosomal cap (Figures 18,19,20).

LC group: showed almost the same findings as the control group.

CP group: The testicular sections showed marked destructive changes in the majority of the spermatogenic cells. SG were resting on thick irregular tunica propria having nuclei with clumped chromatin, large cytoplasmic vacuoles, small elongated mitochondria, numerous lysosomes and lipid droplets. PScs with large cytoplasmic vacuoles and small distorted mitochondria were detected and SCs appeared loosely separated from BM and showed a vacuolated cytoplasm with nuclei showing loss of the indentation, prominent nucleolus, and small elongated mitochondria. Markedly degenerated round and elongated spermatids and a few well-formed spermatozoa with absent acrosomal caps were also seen (Figures 21,22,23).

CP plus LC group: ST showed SG resting on thick tunica propria, nuclei with dispersed chromatin, rounded mitochondria, many lipid droplets, and some residual cytoplasmic vacuolations. PScs with a nucleus showing clumped chromatin on the periphery, lipid droplets, residual cytoplasmic vacuoles, and small ovoid mitochondria were detected. SCs with elongated indented nuclei with prominent nuclei were observed resting on a BM. The cytoplasm contained many ovoid mitochondria with few cytoplasmic vacuolation. Many elongated and few distorted spermatids with acrosomal caps and well-formed spermatozoa with well-formed acrosomal caps were seen (Figures 24,25,26,27).

Morphometric Results

Statistical analysis of the mean ST diameter and germinal epithelial height in the CP group demonstrated a significant decline in the mean ST diameter and germinal tissue height in correlation to the control and LC groups. When compared to the CP group, CP plus LC exhibited a significant increase in tubular diameter as well as germinal epithelial height. There was no variance in the seminiferous tubular diameter or the germinal epithelial height between the control and LC groups (Table 3).

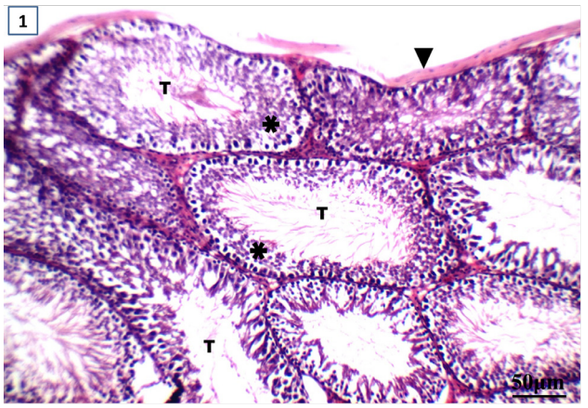

Fig. 1: Photomicrograph of the control group showing: Apparently normal tunica albuginea (arrowhead), adequately sized seminiferous tubules (T) with normal germinal lining (*) (H&E X 200).

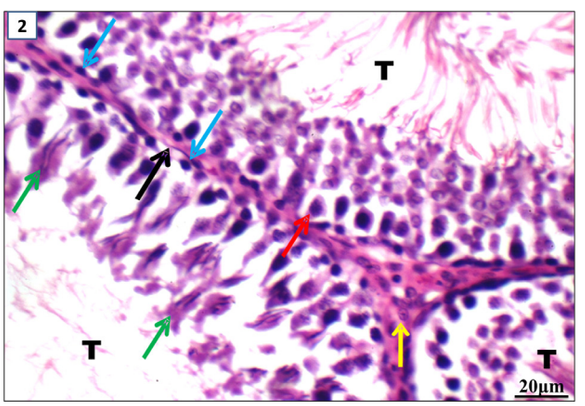

Fig. 2: Photomicrograph of the Control group showing seminiferous tubules (T) with normal BM (black arrow), spermatogonia (blue arrows), primary spermatocytes (red arrow), and many spermatozoa (green arrows), and normal interstitial Leydig cells (yellow arrow) (H&E X 400).

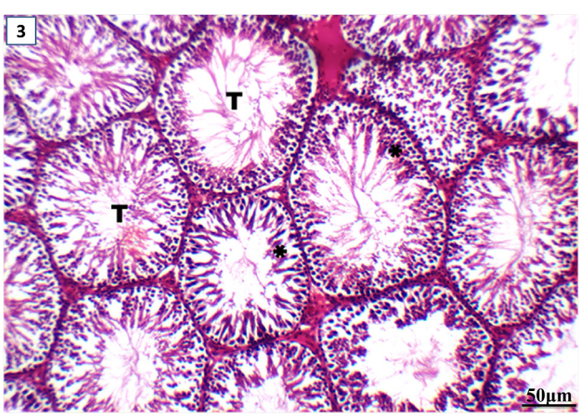

Fig. 3: Photomicrograph of the L-carnitine group showing normal-sized seminiferous tubules (T) with normal germinal layer and complete spermatogenesis (*) (H&E X 200).

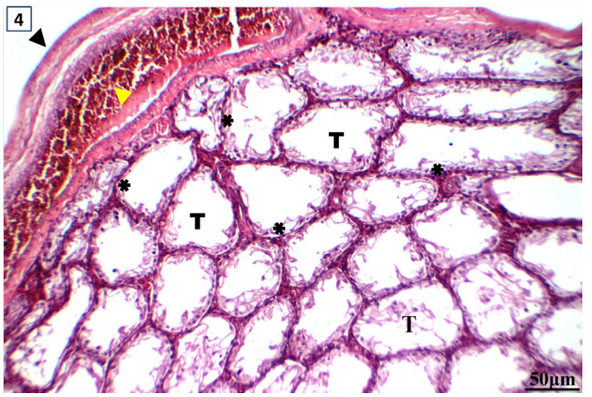

Fig. 4: Photomicrograph of the CP group, showing thick tunica albuginea (black arrowhead) with congested sub-capsular blood vessels (yellow arrowhead), small-sized empty seminiferous tubules (T) with markedly atrophied germinal lining (*) (H&E X 200).

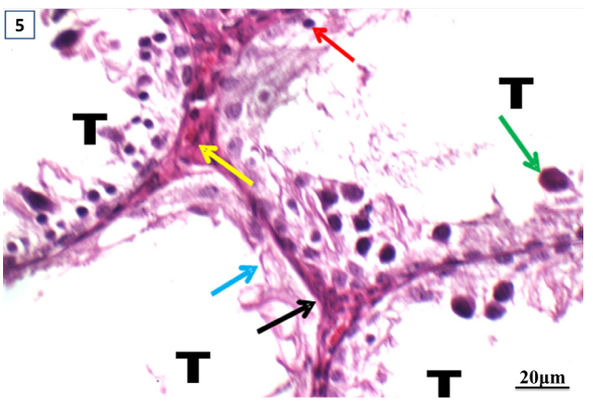

Fig. 5: Photomicrograph of the CP group, showing thick BM (black arrow), seminiferous tubules (T) with no germinal lining (blue arrow) with apoptotic germinal cells (red arrows), few scattered primary spermatocytes (green arrow), and few Leydig cells (yellow arrow) (H&E X 400).

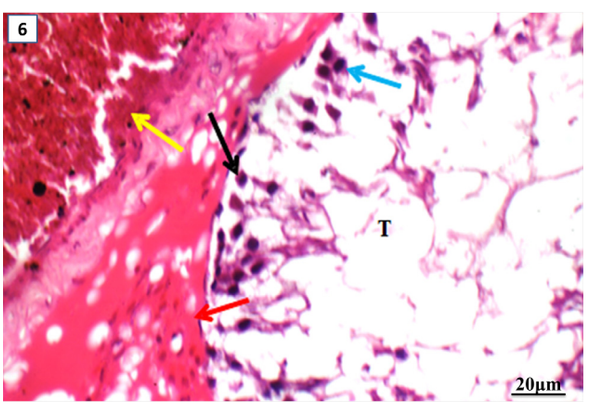

Fig. 6: Photomicrograph of the CP group showing seminiferous tubules (T) with scattered spermatogonia (black arrow) and scattered primary spermatocytes (blue arrow), congested blood vessels (yellow arrow), and interstitial edema (red arrow) (H&E X 400).

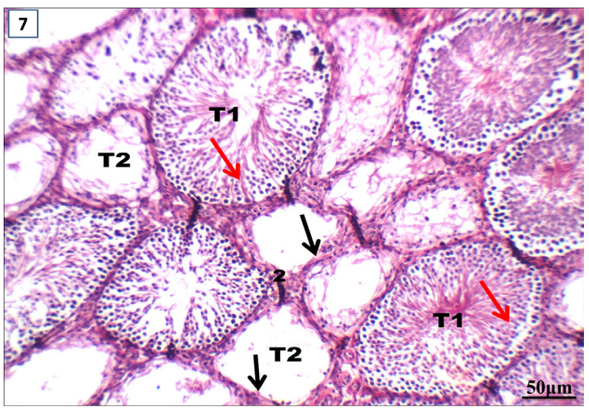

Fig. 7: Photomicrograph of the CP plus LC group showing normal-sized tubules (T1) with complete spermatogenesis (red arrows) and others were atrophied seminiferous tubules (T2) with disruption of spermatogenesis (black arrows) (H&E X 200).

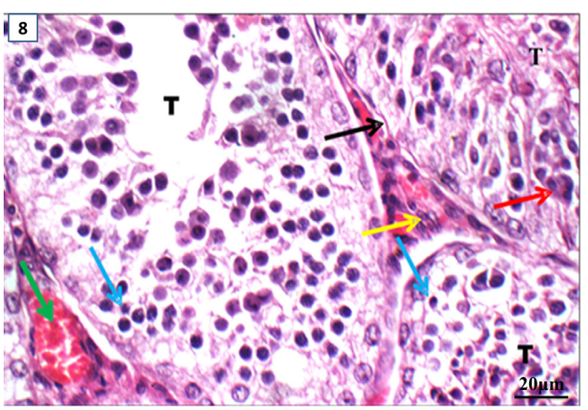

Fig. 8: Photomicrograph of the CP plus LC group, showing BM (black arrow), average-sized tubules (T) with scattered apoptotic germinal cells (blue arrows), scattered giant cells (red arrow) and congested interstitial blood vessels (green arrow) with interstitial edema and Leydig cells (yellow arrows) (H&E X 400).

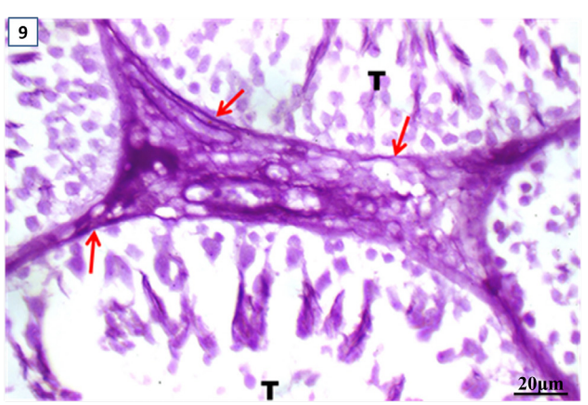

Fig. 9: Photomicrograph of the Control group, showing seminiferous tubules (T) with intact regular basement membrane (red arrow) (PAS X 400).

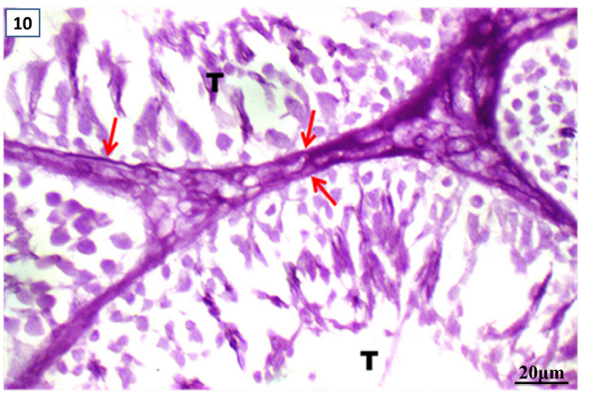

Fig. 10: Photomicrograph of the LC group: showing seminiferous tubules (T) with intact regular basement membrane (red arrow) (PAS X 400).

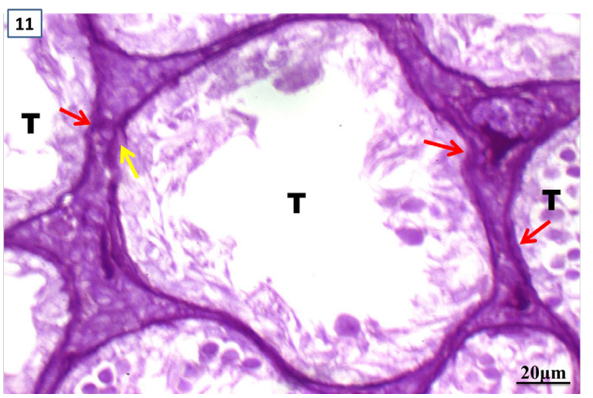

Fig. 11: Photomicrograph of the CP group, showing seminiferous tubules (T) with thick irregular basement membrane (red arrow) disrupted part of BM (yellow arrow) (PAS X 400).

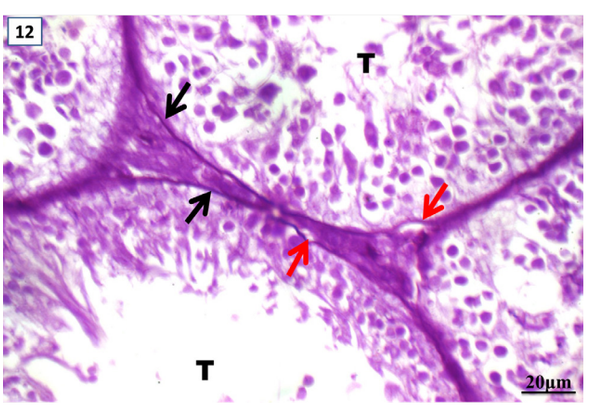

Fig. 12: Photomicrograph of the CP plus LC group, showing seminiferous tubules (T) with regular thin parts (black arrows) of the basement membrane and irregular thick in other parts (red arrows) of the basement membrane (PAS X 400).

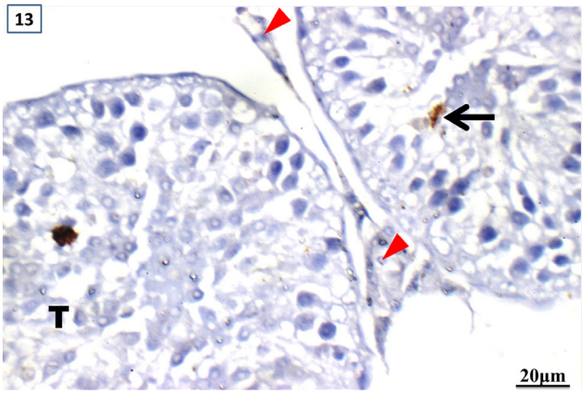

Fig. 13: Photomicrograph of the Control group, showing seminiferous tubules (T) with weak positive cytoplasmic reactivity for iNOS in sporadic germinal lining (black arrow), and negative in Leydig cells (red arrow) (iNOS immunostain X 400).

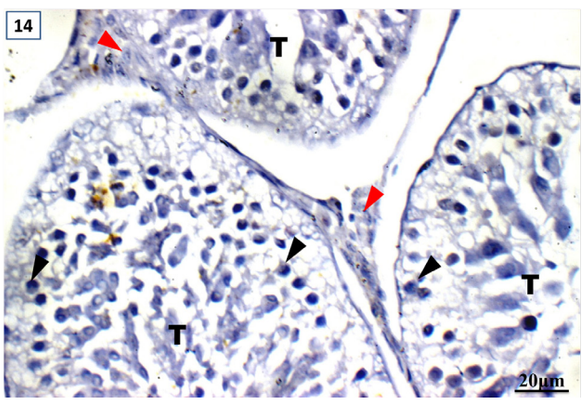

Fig. 14: Photomicrograph of the LC group, showing seminiferous tubules (T) with negative cytoplasmic staining for iNOS in the germinal lining (black arrowhead), and negative reactivity in Leydig cells (red arrowhead) (iNOS immunostain X 400).

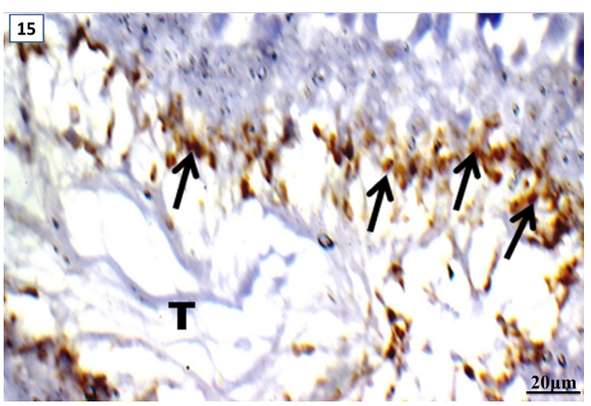

Fig. 15: Photomicrograph of the CP group, showing seminiferous tubules (T) with positive cytoplasmic reactivity for iNOS in the germinal lining (black arrow) (iNOS immunostain X 400).

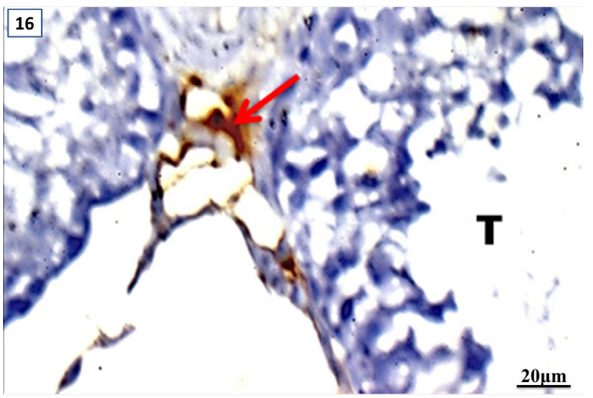

Fig. 16: Photomicrograph of the CP group, showing seminiferous tubules (T) with moderate cytoplasmic reactivity in Leydig cells (red arrow) (iNOS immunostain X 400).

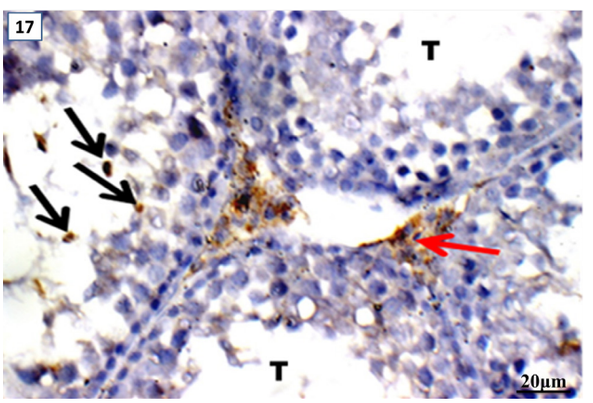

Fig. 17: Photomicrograph of the CP plus LC group, showing seminiferous tubules (T) with weak to moderate positive cytoplasmic reactivity for iNOS in the germinal lining (black arrow) more apparent on spermatids, and weak positivity in Leydig cells (red arrow) (iNOS immunostain X 400).

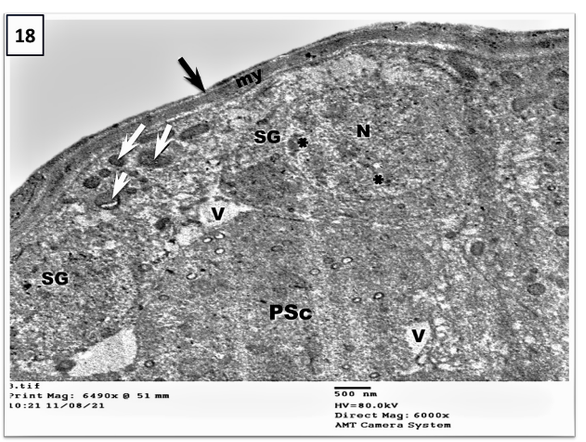

Fig. 18: An electron photomicrograph of the control group showing the ST with spermatogonia (SG) resting on average regular thin lamina propria (black arrow) contained myoid cells (My). Spermatogonium (SG) was characterized by an oval nucleus (N) with dispersed chromatin (*), rounded to ovoid mitochondria (white arrows), and primary spermatocyte (PSc) with few cytoplasmic vacuoles (V). (X 6000).

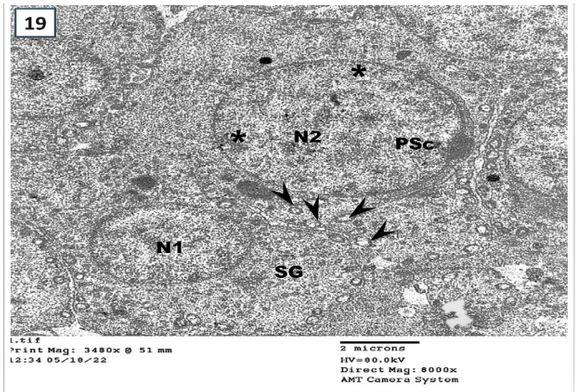

Fig. 19: An electron photomicrograph of the Control group showing normal spermatogonia (SG) with a prominent nucleus (N1) and well-formed primary spermatocyte (PSc) with oval nucleus (N2) with dispersed chromatin (black asterisks), peripheral arranged numerous rounded cytoplasmic mitochondria (black arrowheads). (X 8000).

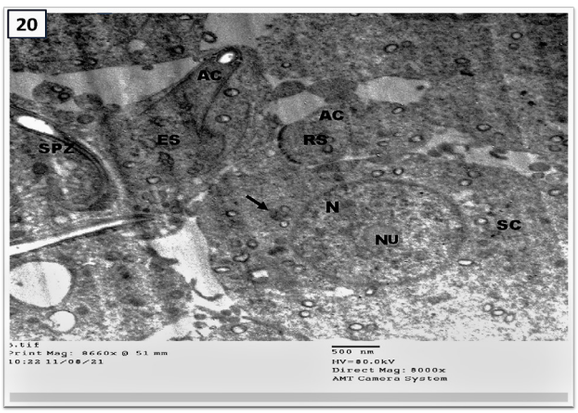

Fig. 20: An electron photomicrograph of the Control group showing ST with rounded spermatids (RS) and elongated spermatids (ES) with acrosomal cap (AC) surrounding Sertoli cell (SC) with oval nucleus (N) showing prominent nucleolus (Nu) and rounded mitochondria (black arrow) well-formed spermatozoa with acrosomal cap (SPZ). (X 8000).

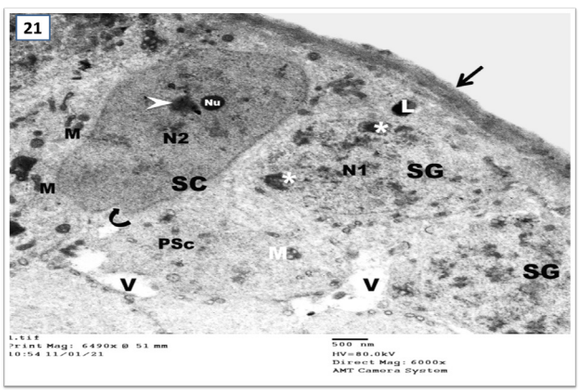

Fig. 21: An electron photomicrograph of CP group showing Sertoli cell (SC) resting on thick irregular BM (black arrow), with a large nucleus (N2) with minor indentation (curved arrow) and prominent nucleus (Nu), numerous lysosomes (white arrowheads), elongated distorted mitochondria (white M), and lipid droplet (L). Primary spermatocytes (PSc) with small destructed mitochondria (black M) and cytoplasmic vacuole (V). (Mag:6000X)

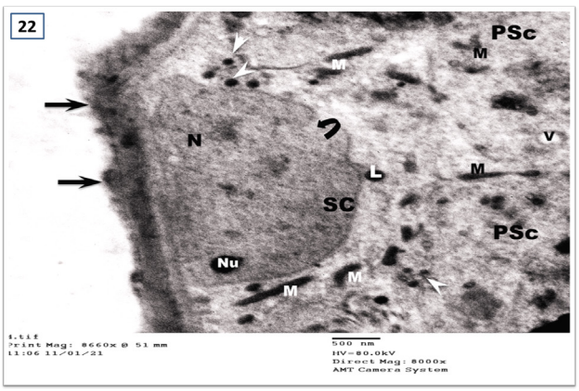

Fig. 22: An electron photomicrograph of CP group ST showing thick BM (black arrow). Sertoli cells (SC) with elongated nuclei with small indentation (black curved arrow), prominent nucleus (N), and clumped chromatin (white arrow head) and small elongated mitochondria (black M) with lipid droplet (L). Primary spermatocytes (PSc) with small rounded mitochondria (white M) and large cytoplasmic vacuoles (V) were also seen. (X 8000).

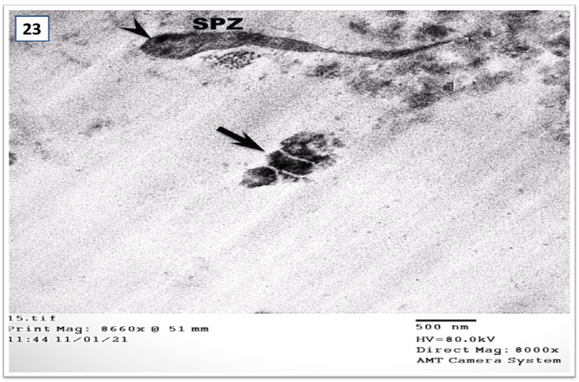

Fig. 23: An electron photomicrograph of the CP group showing well-formed spermatozoa (SPZ) with absent acrosomal cap (black arrow head), and markedly degenerated spermatids (black arrow). (8000X).

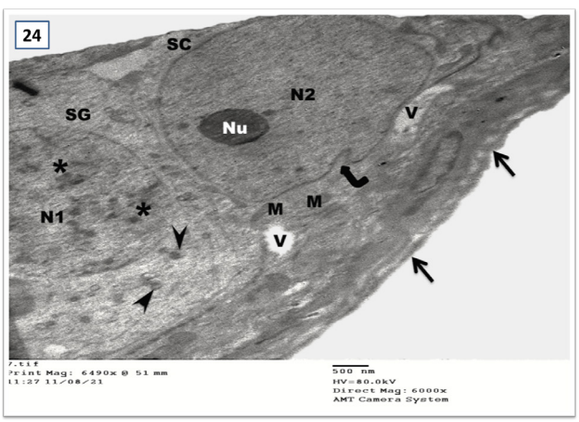

Fig. 24: An electron photomicrograph of the CP plus LC group showing intact irregular BM (black arrows). Seminiferous tubules with spermatogonia (SG) resting on BM, nucleus (N1) showing dispersed chromatin (black asterisks), small cytoplasmic mitochondria (black arrow head). Sertoli cells (SC) with nucleus (N2) showing prominent nucluleos (Nu) and apparent indentation (black curved arrow), small ovoid mitochondria (M), cytoplasmic vaculoe (V). (6000X).

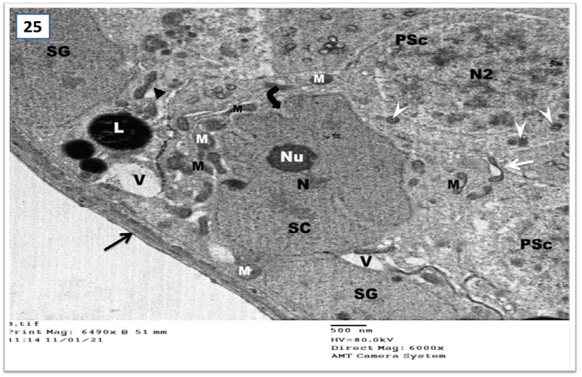

Fig. 25: An electron photomicrograph of the CP plus LC Group showing seminiferous tubules with spermatogonia (SG) resting on average BM (black arrow) with small cytoplasmic vacuoles (V), small elongated mitochondria (black arrow head) and large lipid droplets (L), primary spermatocytes (PSc) with small rounded mitochondria (white arrowheads), Sertoli cells (SC) has nucleus (N) with prominent nuclei (Nu) and some indentations (curved arrow) and many rounded to oval mitochondria (white M)and some elongated mitochondria (black M). (6000X)

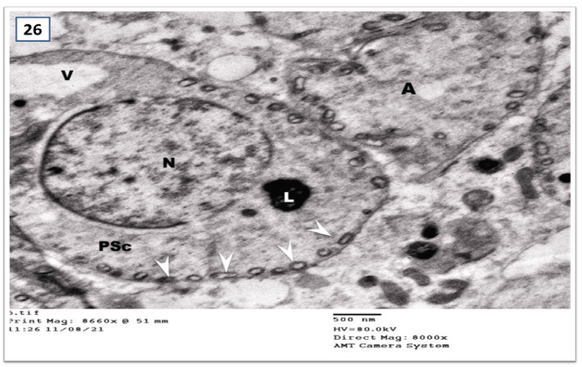

Fig. 26: An electron photomicrograph of the CP plus LC group showing seminiferous tubules with primary spermatocytes (PSc) with rounded euchromatic nucleus (N) showing dispersed chromatin on the periphery, lipid droplets (L), large cytoplasmic vacuoles (V) and peripherally arranged oval cytoplasmic mitochondria (white arrow heads), Atrophied primary spematocyte (A). (8000X).

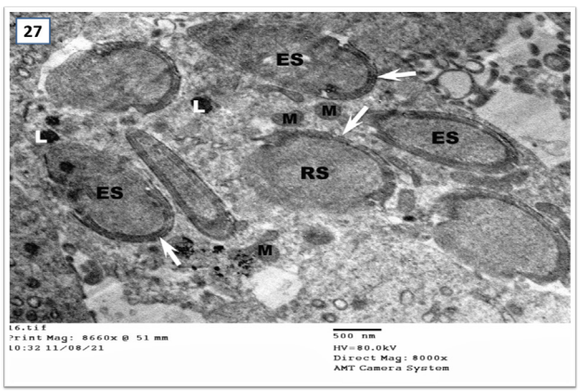

Fig. 27: An electron photomicrograph of the CP plus LC group showing seminiferous tubules with many rounded (RS) and elongated (ES) spermatids with acrosomal cap (white arrows), many rounded mitochondria (M) and few lipid droplets (L). (8000X)

Table 1: Showing the means ± *SD* of body weight change, absolute and relative testicular weight of both testes of the different studied groups

Parameters Groups	CON	LC	CP	CP plus LC
Body weight change (%)	3.04 ± 1.41 [b]	2.07 ± 1.04 [b]	-8.63 ± 2.90[a]	-2.93 ± 0.60[ab]
Absolute Testes weight (gm)	2.67 ± 0.23[b]	2.75 ± 0.28[b]	1.81 ± 0.22[a]	2.12 ± 0.08 [ab]
Relative testicular weight (%)	1.15 ± 0.09 [b]	1.14 ± 0.12[b]	0.85 ± 0.08 [a]	0.96 ± 0.05 [ac]

The significant difference from the control group represented at (P<0.01 [a]), the significant difference from the CP group represented at (P<0.01 [b]) and (P<0.05 [c]); using one-way ANOVA, followed by Tukey–Kramer for multiple comparison.

Table 2: Showing the means ± *SD* of caudal sperm count (10^6) and sperm motility (%) of the different studied group

Parameters	Caudal sperm count (10^6)	Sperm motility (%)
Groups	Mean ± SE	Mean ± SE
Control	67.43 ± 6.86 [c]	82.90 ± 4.07[c]
LC	69.70 ± 6.63 [c]	85.80 ± 3.94 [c]
CP	53.40 ± 7.68 [a]	58.30 ± 6.95 [a]
CP PLUS LC	60.93 ± 6.26 [bc]	71.80 ± 5.51[ac]

The significant difference from the control group represented at (P<0.01 [a]), (P<0.05 [b]), and the significant difference from the CP group represented at (P<0.01 [c]); using one way ANOVA, followed by LSD test.

Table 3: The means ± SD of the seminiferous tubular diameters and their epithelial heights of the different study groups

		Control group	LC group	CP group	CP plus LC group
Diameters of the seminiferous tubules (um)	Mean ± SD	277.06 ± 5.03 [b]	277.71 ± 5.65 [b]	257.40 ± 16.42[a]	269.54 ± 8.81 [a c]
Epithelial heights of the seminiferous tubules (um)	Mean ± SD	66.99 ± 2.97 [b]	66.38 ± 3.18 [b]	58.93 ± 2.22[a]	63.58 ± 6.49 [a c]

The significant difference from the control group represented at (P<0.01 [a]),, the significant difference from the CP group represented at (P<0.01 [b]) and (P<0.05 [c]), using one way ANOVA, followed by LSD test.

DISCUSSION

CP usage causes male infertility[25]. Therefore, to preserve the patient's fertility while they are receiving CP, protective treatments must be used[26].

Regarding the body and testicular weight results; in this study, there was a significant reduction in the body weight, both absolute and

relative testicular weights in the CP group when compared with the control group. These results are in conjunction with Aly and Eid[27] who reported that CP treatment of rats resulted in a significant drop in the rat body and absolute testicular weights as compared to the corresponding control group. Adejuwon *et al*.[28] are in line with these study results including the relative testicular weight as they reported a significant decrease in rats received CP compared with the control. Aly and Eid[27] and Zhang *et al*.[29] explained this decrease in body weight as it may be a result of gastrointestinal toxicity attributed to CP causing malabsorption. Zhang *et al*.[29] explained the drop in the testicular weight in CP rats by a marked parenchymal atrophy and a shrinkage of ST in testicular tissues.

In this study, CP plus LC group, LC ameliorated the violent loss in the rat body and both testicular weights caused by CP, with a considerable rescue of testicular tissues by preserving normal ST morphology and germinal lining epithelium but still, there was a significant reduction when compared to the control or LC groups. These results follow the study by Aboul-Naga *et al*.[30] that reported both CP plus LC groups compared to the control and LC, showed a remarkable drop in the body and testicular weights. Eid *et al*.[5] are not fully agreed with this study as they reported a complete normalization of both the body and testicular weights by LC administration. This may be due to different experiment methodology as CP was injected into rats at 12th day then they scarified after three days only.

Regarding the sperm motility and count results; in our study, Semen collection and examination was accomplished by the same procedure used by Yucel *et al*.[26] who revealed a normal sperm motility and count to demonstrate the effectiveness of this technique in both control and LC groups with no significant difference in between. In contrast, administering CP revealed prominent reduction in sperm motility and count in comparison with control and CP plus LC groups, these CP results in agreement with many studies[7,26,31]. Al-Shahari *et al*.[32] and Jahan *et al*.[31] matched that the loss of germinal epithelium thickness with the inhibition of spermatogenesis was the main cause of sperm count reduction. Eid *et al*.[5] explained the reduction in sperm motility by fatty acid lipid oxidation in the plasma envelope of the sperm, resulting in lack in the sperm plasma envelope fluidity and function.

Treatment with LC partially improved CP-induced sperm abnormalities. This is maintaining Aboul-Naga *et al* results regarding the significant improvement of these parameters with LC usage against CP group[30]. Abd-Elrazek and Ahmed-Farid[16] studied busulfan-induced oligospermia and the protective effect of LC in adult rat. LC significantly showed an improvement in the recorded motility and sperm count, compared to busulfan rats. Such constructive effects of LC may be attributed to the decreased apoptotic cell death and suppression of the oxidative stress [5,17,30]. Abd-Elrazek and Ahmed-Farid[16] explained the improvement in sperm motility in CP plus LC treated rats as LC eliminates the hazardous acyl groups and encourages lipid synthesis with in cell membranes so that it protects the spermatozoa cellular membranes.

The commonest pathological changes noted regarding the testicular toxicity is the loss of germinal epithelium. Therefore, a quantitative assessment of ST diameter and height of the epithelium has been performed in many studies[33]. CP treated group in this study, revealed irregular ST with a valuable decrease of tubular diameter and the height of the epithelium compared to control and LC groups. These results concur with other earlier studies[31,34]. But, treatment with LC in CP group in this study, resulted in a substantial improvement in diameter and epithelial height of ST versus to CP group and get closer to the normal group but still with significant differences, This is not fully agree with Aboul-Naga *et al*.[30] that showed in their study a non-significant difference from the control group, this may because they used low dose of CP 5 mg/kg body weight in their experiment.

Regarding the histological presentation; control and LC groups showed no differences inbetween, as they showed a normal testicular

design with a typical arrangement of differentiating spermatogenic, SCs were resting on intact regular BM with normal Leydig cells in the interstitial spaces. In CP group, specimens showed loss of most testicular tissue's normal architecture, shrunken deformed ST resting on thick irregular BM, germ cell layers shrinkage with disruption of spermatogenesis and wide interstitium with atrophied Leydig cells.

Our histological results are in agreement with Aly and Eid[27] who reported a scattered distorted empty tubule, thick BM and marked atrophy of the germinal lining epithelium in CP group. Bushra and Bastwrous[13] also reported a marked reduction and a degeneration of the spermatogenic cells with lost contact with BM in CP group. In harmony with our results in CP group, Aboul-Naga *et al*.[30] showed shrunken deformed ST, irregular BM and marked loss of the spermatogenic cells with shrunken interstitial tissue. Elrashidy and Hasan[35] confirmed our results regarding CP induced ST distortion, disorganization and dispersion of the spermatogenic cells. El-Amir *et al*.[36] are in accordance with our results regarding CP rats by showing empty seminiferous tubular lumen and many ST were lined only with SG and PScs.

In CP plus LC group, some ST were relatively normal and others partly or completely showed loss of germ cells. Mild irregularity in BM of ST with average thickness BM appearance could be detected. Depletion of germ cells in ST was infrequently observed.This is in accordance with Eid *et al*.[5] who observed that CP plus LC showed advancement in ST shape with maintaining spermatogenesis that was disrupted by CP treatment. Aboul-Naga *et al*.[30] also showed that LC showed a lower degree of disturbance with minor germ cell loss when compared to CP group. Yaman and Topcu-Tarladacalisir[37] discovered a significant decrease in the percentage of atrophied tubules in CP plus LC group compared to CP group. The improvement in ST in CP plus LC group can confirm the significance of using LC against CP to protect the testicular tissues. However, the protective role in this study of LC was partial, as some ST were separated by wide interstitial spaces and lined by few scattered and corrupted spermatogenic cells and this is proved by Bushra and Bastwrous[13] who stated that the spermatogenic cells became more organized but still with residual empty spaces and an irregular BM.

In the current study, moderate positive reactivity of iNOS was detected in CP-treated rats and in CP plus LC group, it showed weak cytoplasmic reactivity. Negative activity was detected for control group and LC groups. This is the same as menthioned by Sherif *et al*.[38]and Ilbey *et al*.[39]. The process by which LC decreased expression of iNOS is unknown, but it could be caused by a reduction in TNF- creation or its clearing impact on nitric oxide[38]. LC tends to decrease iNOS mRNA and protein overexpression, causing a decrease in nitric oxide levels.[40]. Co-treatment with LC protects the testis against the harmful effects of CP and reduces the oxidative stress and prevents mitochondrial injury[37].

The Transmission electron microscopic examination of CP group rats of our study revealed widely separated and degenerated spermatogenic cells resting on thick irregular BM, large cytoplasmic vacuoles, small distorted or elongated mitochondria and separation of the lining cells from BM with appearance of markedly degenerated spermatids, admixed with few rounded and elongated spermatids with absent acrosomal cap. SCs showed varying degrees of degeneration, as evidenced by the loss of the nucleus's normal indented appearance and distorted small mitochondria with cytoplasmic vacuoles and lipid droplets.

These outcomes are comparable to those described by Bushra and Bastwrous[13] who reported widely separated and degenerated spermatogenic cells, vacuolated cytoplasm, and swollen mitochondria. This study results are in accordance with Mercantepe *et al*.[41] who reported apoptotic SG, spermatocytes and spermatids.

On the other hand, the current study discovered that LC usage in CP plus LC group had significantly inhibited many of the deteriorative effects of CP, and spermatogenia cells became closer to the normal presentation, apart from some cytoplasmic vacuolations and

some distorted mitochondria with small lipid droplets. These results of LC group are supported by Bushra and Bastwrous[30] who stated that LC had a noticeable protective effect against CP provoked testicular alterations. The current study's findings are also consistent with the establishment that LC is provoked to preserve the cells from mitochondrial and nuclear DNA damage.

CONCLUSION & RECOMMENDATIONS

LC could be a reasonable solution for minimizing infertility in people receiving cisplatin treatment. However, further studies should be performed with LC combined with other vitamins and antioxidants for achieving definitive prevention and cure of CP induced testicular toxicity.

CONFLICT OF INTERETS

There are no conflicts of interest.

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الملخص العربى

الدور الوقائي لمادة إل ـ كارنيتين ضد سمية الخصية التي يسببها عقار السيسبلاتين في الفأر الأبيض البالغ

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الخلفية: يعد تلف الخصية أحد أكثر الآثار الضارة التي تحدث عند استخدام عقارالسيسبلاتين في علاج السرطان وقد يؤدى الي العقم عند الذكور. ماده إل-كارنيتين هي مادة تشبه الفيتامينات ولها نشاط مضاد للأكسدة وتحسن من انتاج الحيونات المنويه وزياده معدل الحركه لدي الحيونات المنويه.

الهدف من الدراسة: هذه الدراسة استهدفت تقييم فاعليه التأثير الوقائي لماده ال الكارنتين ضد تسمم الخصية المحدث بواسطة عقار السيسبلاتين.

المواد والطرق: تم إجراء الدراسة على أربعين فأراً من الذكور البالغة و يتراوح وزن الجرذ الواحد ما بين مائتين إلى مائتين وخمسون جرام وتم تقسيمهم إلي أربعة مجموعات: المجموعة الأولى (المجموعة الضابطة): تم حقن الفئران يومياً بـ ٢ مل من المحلول الملحي ٠,٩٪ عبر الحقن البريتوني لمدة خمسة عشر يوماً. المجموعة الثانية (المجموعة المعالجة بإل- كارنتين) تم حقن الفئران يومياً بـ ٥٠٠ مجم/ كجم من وزن الجسم عبر الحقن البريتوني لمدة خمسة عشر يوما. المجموعة الثالثة (المجموعة المعالجة بالسيسبلاتين): تم حقن الفئران بـ ٧ مجم / كجم من وزن الجسم من السيسبلاتين داخل الغشاء البريتوني في اليوم السادس. المجموعة الرابعة: (المجموعة المعالجة سيسبلاتين وإل- كارنتين): تم حقن الفئران بنفس جرعات السيسبلاتين وال- كارنيتين المستخدمه بكلا المجموعتين الثانيه والثالثه معا. تم تسجيل وزن الفئران لجميع الحيوانات في بداية الدراسة وقبل أن يتم التضحية بها وتسجيل وزن الخصية بعد التضحية بالفئران. ثم عمل سحب وتحليل للسائل المنوي مباشره بعد التضحية لقياس نسبه تركيز الحيوانات المنوية ومدي حيويتها وتم عمل شرائح الفحص النسيجي باستخدام عدد من الصبغات الهستولوجية والمناعية وفحصها بواسطة المجهر الضوئي و الإلكتروني النافذ. تم إجراء دراسة لقياس متوسط قيمه قطر الأنابيب المنوية، وارتفاع النسيج الطلائي للأنابيب المنوية.

النتائج: حدث انخفاض في وزن الفأر ووزن الخصيتين والوزن النسبي للخصيتين بشكل ملحوظ بعد إعطاء عقار السيسبلاتين مقارنة بالمجموعة الضابطة. ومع ذلك، أظهر العلاج المشترك مع إل - كارنيتين تحسنًا ملحوظا في وزن الفأر ووزن الخصيتين والوزن النسبي للخصيتين مقارنة بمجموعة عقار السيسبلاتين. وقد اظهر ايضا تحليل السائل المنوي انخفاض متوسط النسبة المئوية لتركيز الحيوانات المنوية وحركتها بشكل ملحوظ في الفئران المعالجة بالسيسبلاتين مقارنة مع المجموعة الضابطة في حين أظهرت مادة إل- كارنيتين تحسنًا ملحوظًا في عدد وحركه الحيوانات المنوية في المجموعة المعالجة بواسطه عقار السيسبلاتين. إعطاء عقار السيسبلاتين لذكور الجرذان البيضاء البالغة أدى إلى ضمور الأنابيب المنوية وانكماش طبقات خلايا الأنابيب المنوية وأظهر علاج إل- كارنيتين انخفاضًا

كبيرًا في النسبة المئوية للأنابيب المتحللة مع تحسن في شكل الأنابيب المنوية و الحفاظ الجزئي على عمليه تكوين الحيوانات المنوية.

بالنسبة لفحص مقاطع الخصية بواسطة الصبغة المناعية أظهرت المجموعات المعالجة بعقار السيسبلاتين تفاعلا إيجابيا ملحوظ في حين المجموعة الضابطة أظهرت تفاعلا سلبيا وعلي الرغم من علاج الكارنتين أدي إلي تفاعل إيجابي أيضا ولكن أقل حده من التي أظهرته مجموعة عقار السيسبلاتين.

وجاء فحص الخلايا المنوية بواسطة المجهر الالكتروني النافذ مؤكدا لنتائج المجهر الضوئي فكشف عن خلايا منوية متدهورة مع فجوات حشوية كبيرة وميتوكوندريا صغيرة مشوهة، ونطف متدهورة بشده مع غياب الغطاء الكروموسومي من ناحية أخرى، وجد أن العلاج بإل- كارنيتين قد حسن ذلك بشكل ملحوظ في العديد من الأثار المدمرة التي أحدثها السيسبلاتين فقد اظهر إل- كارنيتين قدرته علي حماية الميتوكوندريا والحمض النووي.

الاستنتاج والتوصيات: ماده إل- كارنيتين تقلل معظم الاثار المرضية المحدثة بواسطه عقار السيسبلاتين علي الخصيه وتحسن معايير جودة الحيوانات المنوية. لذلك، يُقترح أن تكون ماده إل- كارنيتين مرشح جيد للوقاية من العقم للمرضى الذين يتلقون علاج السيسبلاتين .