Original Article

Protective Effect of L-Carnitine and Vitamin E on Cyclophosphamide Induced Toxicity on Rat Testes L-Carnitine & Vitamin E Effects on Testis Toxicity

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ABSTRACT

Background: Cyclophosphamide (CP) was a chemotherapeutic agent, known to cause male reproductive toxicity. Some antioxidant agents were found protective against CP toxicity e.g L-Carnitine and Vitamin E. L-carnitine (LC) was commonly used in treatment of male infertility. While Vitamin E (Vit E) was an essential nutrient for reproduction.

Aim of the work: This study was designed to assess, by anatomical and histological examination, the possible protective effects of LC and Vit E on the testes of CP treated albino rats.

Material and Methods: Rats were categorized into: Control group I (GP Ia receiving intraperitoneal saline, gp Ib and gp Ic receiving LC and Vit respectively), experimental group II receives intraperitoneal CP, and Protected group III (GP IIIa, GP IIIb receiving CP with LC and Vit E respectively). After two weeks, the rats were sacrificed, testes were dissected, and examined grossly then histologically using light and Electron microscopy.

Results: GP II showed a significant reduction in the mean value of the testicular weight and dimensions in comparison with GP I. The mean value for GP IIIa and IIIB was significantly increased in comparison with GP II. Histological changes in GP II revealed intracellular vacuolization and sloughing of the spermatogonial cells. Decreased number of sperms was encountered. Both GP IIIa and GP IIIB showed improvement. However, Gp IIIa was better than Gp IIIb.

Conclusion: LC and Vit E could be used as protective agents against CP-induced reproductive toxicity, but the protective effect of LC was better than Vit E.

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Key Words: Albino rats, cyclophosphamide, electron microscope, L-Carnitine, semi-thin section, spermatogenesis, ultrastructure, Vitamin E.

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INTRODUCTION

Infertility is a problem affecting about 10% of couples worldwide, among which male factor represents 50 %. Most of these cases are related to sperm dysfunction such as oligo-astheno-teratozoospermia (OAT).[1-3]

Cyclophosphamide (CP) is one of the most common alkylating drugs. It is the drug of choice for some breast and pulmonary cancers and is used in the treatment of chronic and acute leukemia, multiple myeloma, and lymphomas. It is also an immunomodultory and immunosuppressant agent.[4-7]

CP undergoes a metabolic activation by hepatic microsomal cytochrome P450.[8,9] Phosphoramide mustard and acrolein are its two active metabolites. The antineoplastic effects of CP are related to phosphoramide mustard, while acrolein is linked to toxic side effects such as apoptosis, oncosis and necrosis.[4]

The capacity of CP to interfere with normal cell division in all rapidly proliferating tissue provides the basis for its therapeutic effects and also many of its toxic properties.[10] CP could disrupt the redox equilibrium of tissues and exerts cytotoxicity by changing the activities of enzymes and levels of non-enzymic antioxidants.[5,11] Lipid

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peroxidation has been suggested to be closely related to CP-induced testicular damage.[4,12,13]

L-carnitine (LC), the L-isomer form of carnitines, is a small water- soluble molecule. It is synthesized from lysine and methionine.[14-16] It was identified as an essential nutrient of multifunction for the body.[3,17] However, 75 % of LC present in human tissues is mainly of exogenous origin from meat, poultry and fish in diet while only 25% in the body.[3, 15,18]

LC exhibits a wide range of biological activities and has been recently shown to act as an important antiapoptotic mediator.[19-21] It acts as a shuttle of the activated long-chain fatty acids from the cytosol into the mitochondria. So it has a pivotal role to generate ATP for cellular energy production.[3,16,20,22] Studies have shown carnitine concentration to be reduced in seminal fluid of infertile patients, and improvement of sperm motility and viability of spermatozoa was obtained by treatment with LC.[16,23]

Vitamin E (Vit E) was discovered by Evans and Bishop who described it as an essential dietary factor in animal nutrition which is especially important for normal reproduction.[24] One form, α tocopherol, the most abundant form in nature, has the highest biological activity, highest bioavailability, with the body preferentially absorbing and using this form.[25,26]

Vit E has been detected in varying compositions in all plants. The richest sources are latex lipids followed by edible oils originating from plants. Vegetables and seed oils are the main sources of the tocopherols whereas animal products are generally poor sources.[24,27]

α tocopherol acts as an antioxidant to protect the cells from the damage by free radicals by preventing lipid peroxidation.[26,28] It allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions.[29-31]

The requirement for vit E for normal testicular function is well established. It is vital for the maintenance of mammalian spermatogenesis and that counteracts the testicular oxidative stress induced by pro-oxidant exposure.[32,33]

AIM OF THE WORK

The aim of the present work is to study the toxic effect of cyclophosphamide on testis of adult male albino rats, to detect the protective effects of L- Carnitine and Vitamin E on these treated testes and to compare between both effects, using anatomical and histological examination.

MATERIAL AND METHODS

A) Chemicals:

1. Cyclophosphamide

Cyclophosphamide (Endoxan®, Germany) was in the form of 1 g vials. The crystalline white powder was freshly prepared in 0.9% sodium chloride saline solution.

2. L-Carnitine.

L-Carnitine tartarate (L-Carnitine®, Egypt), the crystalline white powder (350 mg\capsule) was dissolved in 0.9% sodium chloride saline solution.

3. Vitamin E

Alpha- tocopheryl acetate (Vitamin E®, Egypt), soft gelatin capsules (1G) were dissolved in corn oil.

B) Experimental Animals:

36 adult male albino rats, obtained from Animal House of Physiology Department, Faculty of Medicine, University of Alexandria.

The body weight of the rats ranged from 120 gm to 200 gm. The animals were housed in stainless steel cages under the following conditions: 12 h dark/light cycle, 22 ± 2º C temperature, relative humidity and good ventilation. All animals were cared for in accordance with Guidelines for care and use of animals, approved by Local Ethics Committee of Faculty of Medicine, University of Alexandria.

After two weeks of acclimatization, they were randomly divided into different groups as follows:

• **Group I:** (control group) 18 rats as a control group were further subdivided into three equal

subgroups 6 animals each; **Subgroup Ia:** (negative control group) 6 rats received standard diet, free access to water with intraperitoneal (i.p.) injections of saline for a week, **Subgroup Ib:** (positive control group) 6 rats received oral L- Carnitine (2.1 mg/kg) for 2 weeks.[16,22] and **Subgroup Ic:** (positive control group) 6 rats received oral Vitamin E (36 mg/kg) for 2 weeks.[16]

• **Group II:** (experimental group) 6 rats received intraperitoneal cyclophosphamide at a daily dose of 20 mg per kg body weight for 7 days.[16]

• **Group III:** (protected group) 12 rats received cyclophosphamide concomitant with L- Carnitine or vitamin E.[16] **Subgroup IIIa:** 6 rats received intraperitoneal cyclophosphamide weight for 7 days, and were given oral L- carnitine for one week before the use of cyclophosphamide and then simultaneously one and half hour before its injection and **subgroup IIIb:** 6 rats received intraperitoneal cyclophosphamide for 7 days, and were given oral Vitamin E for one week before the use of cyclophosphamide and then simultaneously one and half hour before its injection.

METHODS

A) Gross anatomical Study:

Scarification of the animals was done by decapitation after light ether anesthesia, 24 hours after the last drugs administration. The abdominal wall was incised, the testes were dissected, then washed with saline and examined as regards color, vascularity, and any morphological abnormality. The length and width of each testis were measured using a Vernier Swiss Caliber. The testes were weighed using mini scale.

B) Statistical analysis of the data[34]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range, mean, standard deviation and median. Significance of the obtained results was judged at the 5% level (*P value less than 0.05*). F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons

C) Histological Study:

It included light microscopic and electron microscopic studies. After gross anatomical examination, both testes of each scarified albino rat were dissected out carefully, cut into 1x1mm pieces and immediately immersed in 3% glutaraldehyde solution for two hours. It was then transferred to 4F1G (4% para formaldehyde- 1% glutaraldehyde) solution to be fixed at 4ºC for 48 hours.

Light microscopic study: Semithin sections were obtained from blocks prepared for the electron microscopic examination, and were stained with toluidine blue stain for light microscopic examination.

Electron microscopic study: The fresh specimens of the testes were processed for transmission electron microscopy and were examined and photographed by TEM (Jeol JEM-2100) at the Electron Microscopy Unit, Faculty of Agriculture, Mansoura University.

RESULTS

No alterations were observed in the appearance or the behaviour of the treated rats in all groups, one rat died after the third cyclophosphamide injection in group II. Rats of group IIIb suffered from decreased appetite. One rat died at the fourth day.

Gross anatomical results:

Examination of the testes of different groups externally, showed apparent reduction in the size, and some increase in the vascularity of group II rats (the experimental group) in comparison with the rats of the control group.

The normal weight of the control group was within range (1.34-1.42 gm), the mean weight was 1.4 gm. The mean of group II (experimental group) was significantly reduced than the control group (1 gm), while the means of the protected groups (IIIa and IIIb) were significantly more than that of the experimental group (1.25 gm and 1.28 gm respectively), and non-significantly related to the range of the control group. (Fig. I)

As regards the diameters, the normal range length was (1.27- 1.38 cm), the mean was 1.3 cm. The width was (0.64- 0.74 cm), the mean was 0.7 cm.The means of length and width in group II (experimental group) were significantly less than the control group (length 0.75 cm), (width 0.34 cm), while the means of the protected groups (IIIa and IIIb) were significantly more than that of the experimental group (II) (length IIIa 1.1, IIIb 1.08 cm), (width IIIa 0.56, IIIb 0.55 cm), and non-significantly related to the range of the control group. (Fig. II & III)

Histological results:

Histological sections of testes of the rats of the control group (group I) revealed normal architecture of seminiferous tubules, lined by multiple layers of the stratified germinal epithelium, at different stages of spermatogenesis. The lining epithelium consisted of Sertoli and spermatogenic cells. Several layers of early spermatids with rounded nucleus, were observed. Process of tail formation were also noticed where the cytoskeletal manchettes extend to form the tail regions of the maturing sperm. The lumen of seminiferous tubules revealed cut section in the spermatozoal tails. (Fig IV, VIII)

The positive control groups (Ib and IC) that received LC and Vit E respectively showed similar results as the normal control group (Ia).

Examination of histological sections of testes of group II rats revealed destruction of the normal architecture of some seminiferous tubules in comparison with the control. Widely separated seminiferous tubules were seen with irregular basement membrane. Most seminiferous tubules have shown intracellular vacuolation, wide intercellular spaces and disorganization of the germinal epithelium. Primary spermatocytes were found in an abnormal position near the basement membrane. Moreover, the lumen of the seminiferous tubules contained diminished number or complete absence of sperms. Cut sections through spermatozoal tails revealed disrupted cristae and abnormal disorganization of mitochondrial sheath. (Fig V, IX, X)

Histological sections of rats of group IIIa and IIIb revealed less degenerative changes than those toxic changes in seminiferous tubules by cyclophosphamide, with evident preservation of the histological structure of Sertoli cells and spermatogonia. Only their cytoplasm showed some vacuolations. Middle pieces of the tails were seen exhibiting more or less normal structure. Group IIIa showed more protective features on the seminiferous tubules than group IIIb. (Fig VI, VII, XI, XII).

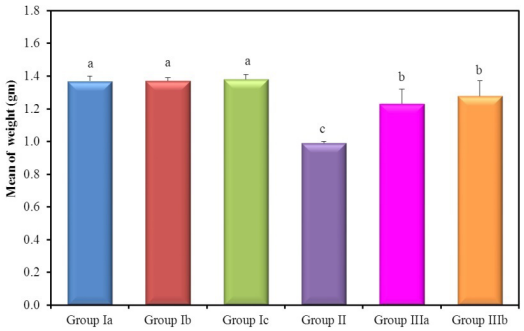

Fig. I: Comparison between the studied groups according to the mean weight (gm).

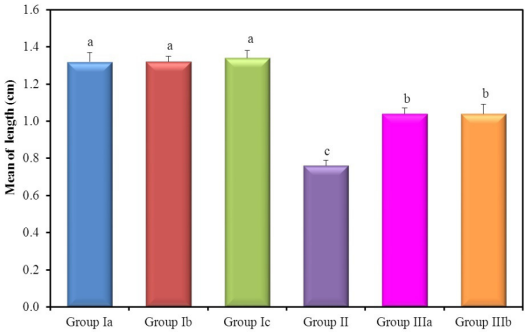

Fig. II: Comparison between the studied groups according to the mean length (cm).

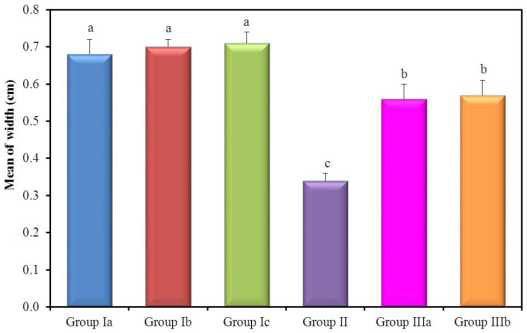

Fig. III: Comparison between the studied groups according to the mean width (cm).

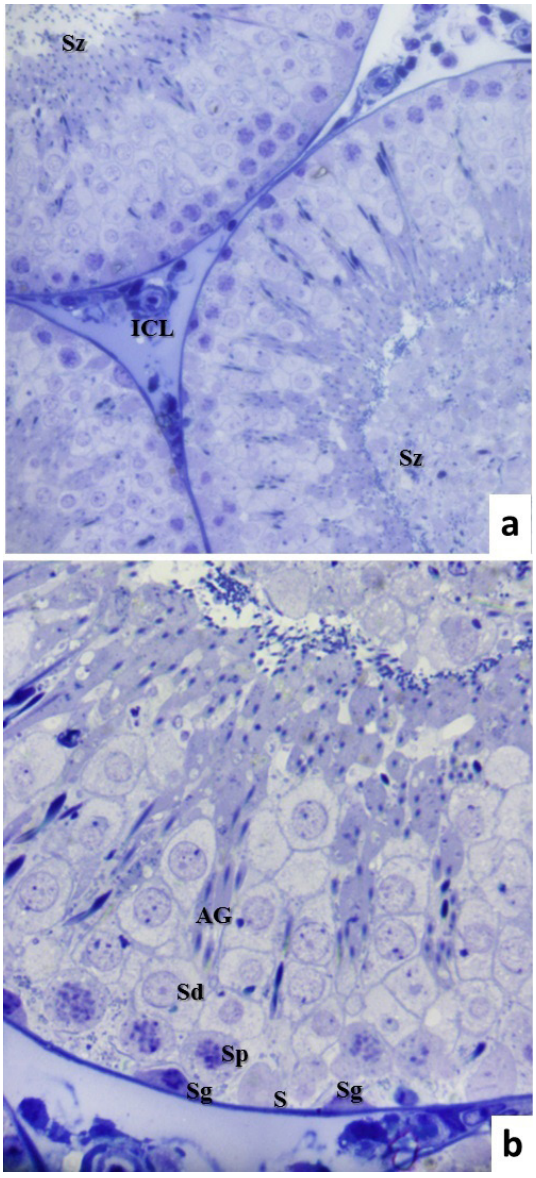

Fig. IV: (a,b): Light photomicrographs of control group rats showing regular cross sections of seminiferous tubules with regular layers of spermatogenic cells lying on regular basal lamina. Sertoli cell (S); spermatogonia (Sg); spermatocyte (Sp); spermatid (Sd); acrosomal granule (AG); spermatozoa (Sz); interstitial cells of Leydig (ICL). Semi-thin section. Toluidine blue stain. (Mic. Mag. a X 400,b X 1000)

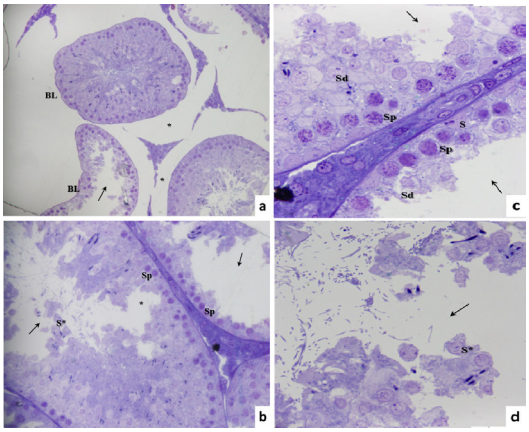

Fig. V: (a, b, c, d): Light photomicrographs of group II rats depicting irregular basal lamina (BL), wide inter-tubular spaces (*), defective spermatogenesis (↑) and sloughed spermatogenic cells (S*). spermatocyte (Sp); spermatid (Sd). Semi-thin section. Toluidine blue stain. (Mic. Mag. a X 200,b X 400, c & d X 1000)

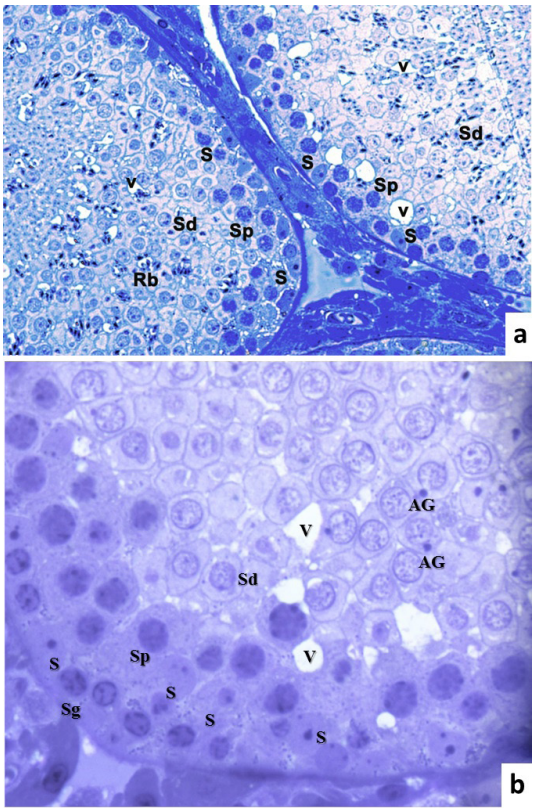

Fig. VI: (a, b): Light photomicrographs of seminiferous tubules of group IIIa rat showing restoration of spermatogenic activity (↑), with some vacuolations (V) in between spermatogenic cells. Sertoli cell (S); spermatogonia (Sg); spermatocyte (Sp); spermatid (Sd); acrosomal granule (AG); residual bodies (Rb). Semi-thin section. Toluidine blue stain. (Mic. Mag. a X 400, b X 1000)

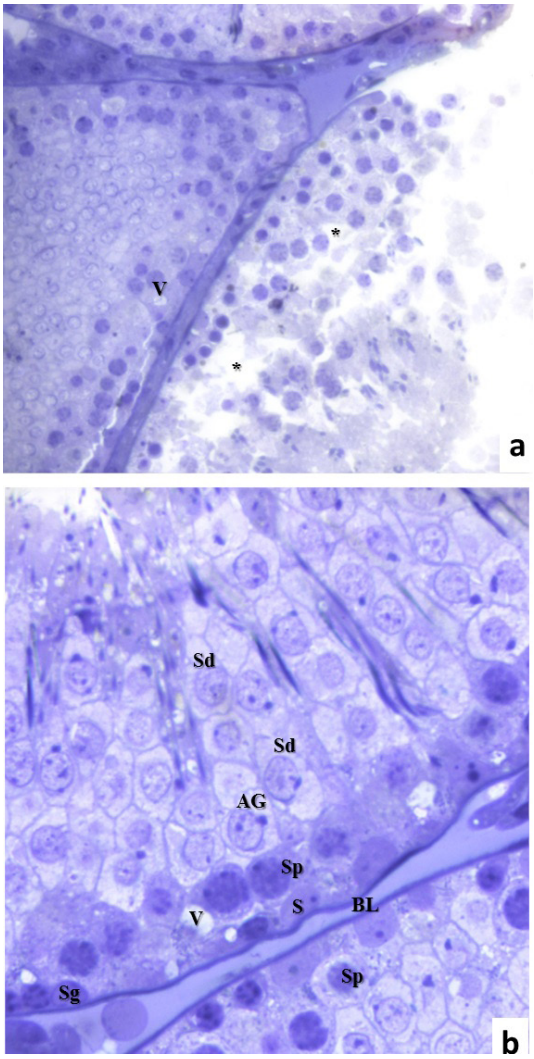

Fig. VII: Light photomicrographs of group IIIb rat revealing some seminiferous tubules with wide intercellular spaces (*), persistent vacuolations (V) and detachment of germinal epithelium. Sertoli cell (S); spermatocyte (Sp); spermatid (Sd); acrosomal granule (AG); basal lamina (BL). Semi-thin section. Toluidine blue stain. (Mic. Mag. a X 400, b X 1000)

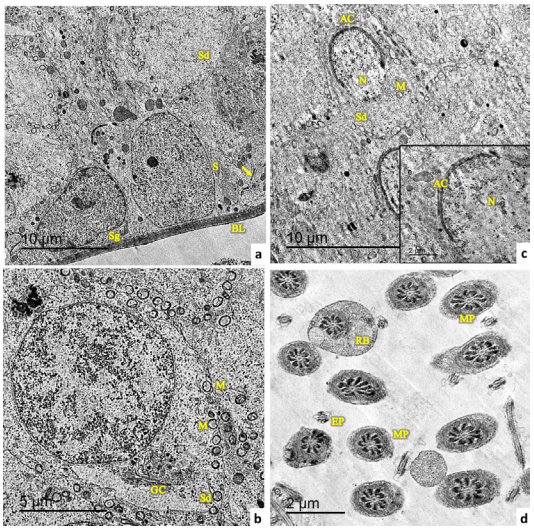

Fig. VIII: (a, b, c, d): Electron photomicrographs of control rat seminiferous tubules showing, Sertoli cell (S) and spermatogonium (Sg) lying on a regular basal lamina (BL) and inter-Sertoli tight junctions (↑). Figure b shows spermatids (Sd) with peripheral regularly arranged mitochondria (M). Figure c illustrates acrosomal cap (AC) which extends over the anterior pole of the nucleus (N). Figure d of spermatozoal tail showes middle pieces (MP), end pieces (EP) and residual bodies (RB). (Mic. Mag a X 10.000, b X 20.500, c X 13.500, d X 34.000)

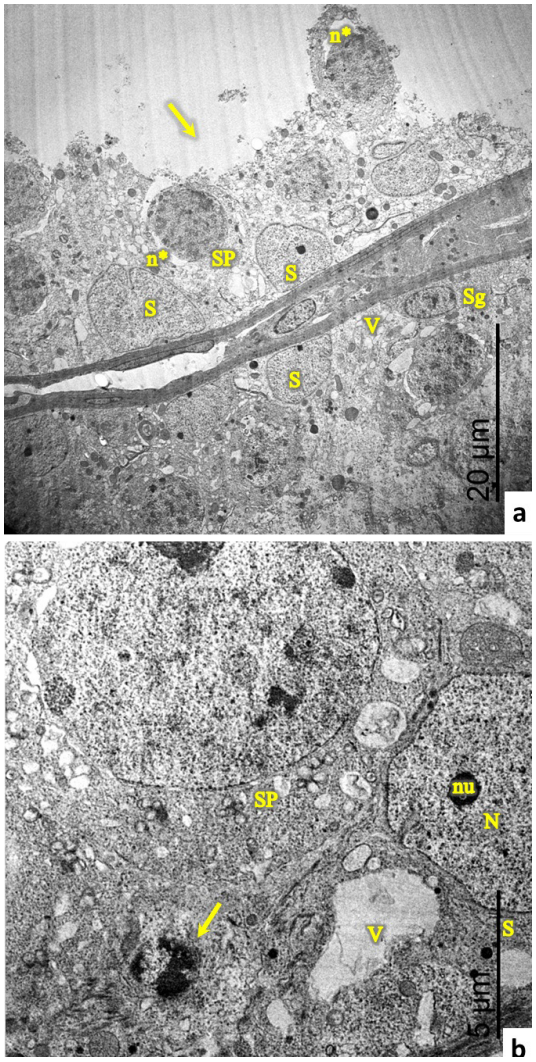

Fig. IX: (a, b): Electron photomicrographs of group II rat seminiferous tubule showing persistent vacuolations (V) and wide inter-cellular spaces (*). Notice defective spermatogenesis (↑) and degenerating cell (▲). Sertoli cell (S); spermatogonia (Sg); spermatocyte (Sp); nucleus (N); nucleolus (n); perinuclear cistern (n*). (Mic. Mag a X 5.000, b X 17.000)

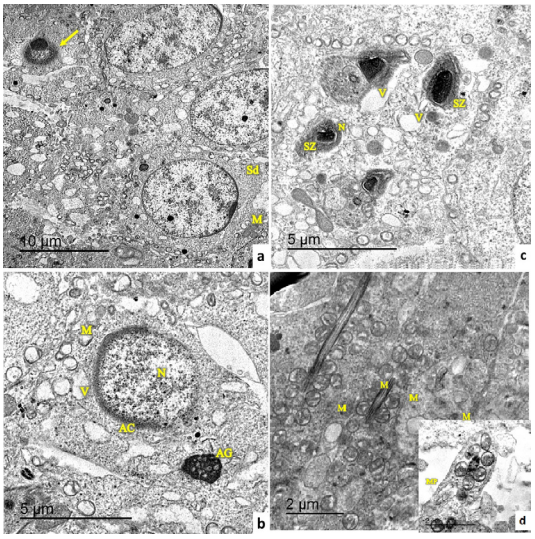

Fig. X: (a, b, c, d): Electron photomicrographs of group II rat seminiferous tubule revealing a degenerated spermatid (↑) in figure a, while figure b shows spermatid with dilated Golgi complex (GC), vacuolated mitochondria (M) and multiple vacuolations (V). figures c illustrates spermatozoal heads (SZ) with irregular nuclear outline. Figure d shows middle pieces with disorganized mitochondria (M) with disrupted cristae. Spermatid (Sd); acrosomal cap (AC); acrosomal granula (AG); nucleus (N); (Mic. Mag a X 10.000, b X 25.500, c X 25.500 , d X 34.000)

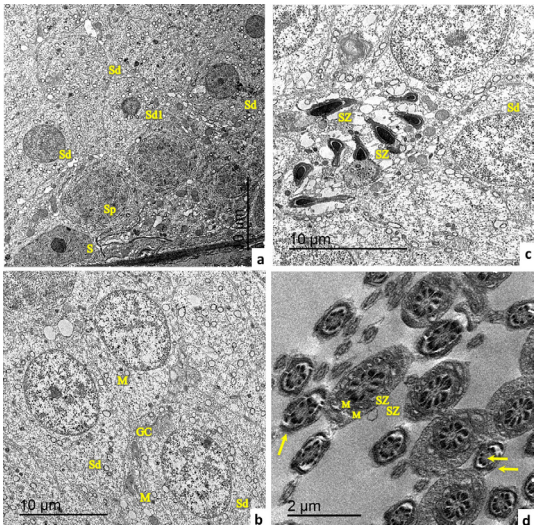

Fig. XI: (a, b, c, d): Electron photomicrograph of group IIIa rat seminiferous tubules. Figure a shows a degenerating spermatid (Sd1) in between normally looking cells. Figures b reveals apparently normal spermatids with irregular mitochondria (M) and dilated golgi complex (GC), while figure c and d show apparently normal spermatozoa (Sz) with other abnormal tails (↑). Sertoli cell (S); spermatocyte (Sp); spermatid (Sd); Mitochondria (M). (Mic. Mag a X 8.500, b X 10.000, c X 13.500 , d X 42.500)

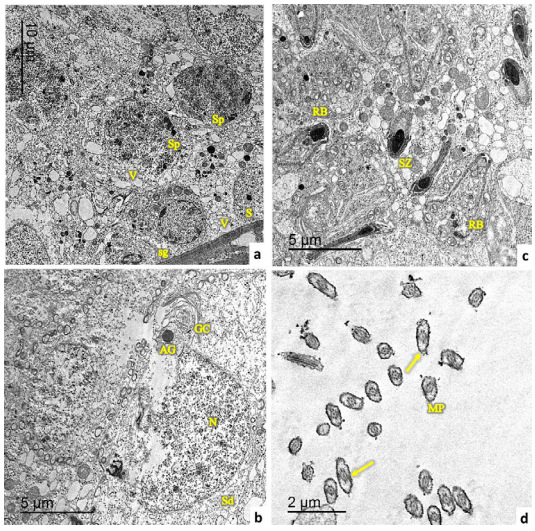

Fig. XII: (a, b, c, d): Electron photomicrographs of group IIIb rat seminiferous tubules illustrating, persistent intercellular vacuolations (V) and wide intercellular spaces (*). Figure (b) reveals an abnormal spermatid (Sd) with irregular nuclear outline (N), dilated Golgi complex (GC) with persistence of the acrosomal granule (AG), while figure c and d show apparently normal spermatozoa (Sz) and abnormal tails (↑). Sertoli cell (S); spermatogonia (Sg); spermatocyte (Sp); spermatid (Sd); residual bodies (RB). (Mic. Mag a X 8.500, b & c X 17.000 , d X 34.000)

DISCUSSION

Gonadal damage is the common side effect of cancer treatment. The exact mechanism of chemotherapy-induced fertility impairment is not fully understood. This may be achieved by generating reactive oxygen species (ROS) to induce the apoptosis.[11,26,35,36]

The human testes are known to be susceptible to toxicity because of highly sensitive and complex cellular composition of the spermatogenic epithelium, with high rate of mitotic activity. Cyclophosphamide (CP) was reported to affect rapidly dividing cells e.g. gastric epithelium, bone marrow, as well as testicular cells.[37]

In the present study, the weight and size of the testis of rats treated with CP were significantly lower than those in the control group. On the other hand, in both the protected groups with LC and Vit E, the mean values of the testicular weight and size were significantly higher compared to GP II, but were still less than those of the control group.

Das *et al*.[38] explained the negative effect of CP on the morphology of the testis as specific induced toxicity on the testis and not the result of its general toxicity. Katoh *et al*.[39] attributed the decrease in the weight of the testis to decrease of the mass of differentiated spermatogenic cells.[40] While according to Bhargavan *et al*.[35], the reduction in the weight of testes in CP treated rats was attributed to atrophy of leydig cells, reflecting the decrease of androgens.

The morphology of testes protected by LC or Vit E may be explained by restoration of spermatogenic cycle, sperm production and/or availability of androgens by antioxidant agents.

The light and electron microscopic examination of the testis of rats which received CP included its spermatogenic and Sertoli cells. These changes were less in the protected group of the current study.

Oyagbemi *et al*.[4,41] attributed the male reproductive toxicity induced by CP to acrolein which leads to inactivation of microsomal enzymes and result in increased ROS generation in the testis.

Elgazar[42] and Al-Attar[43] stated that oxidative stress due to CP may occur as a result of an imbalance between ROS generation and the intracellular capacity for removing ROS, subsequently leading to excessive cell damage. The decrease of activity of SOD and catalase following CP administration in rats has been previously documented in studies by Das *et al*, oyagbemi *et al.*, and Zanchi *et al*.[38,41,44]

Generally, it is accepted that the increased lipid peroxidation in the testis is one of the toxic manifestations of CP[6,13,37,45]. It was reported by Aghaei *et al*. that CP treatment results in high malondialdehyde (MDA) level, which is a by-product of lipid peroxidation; because of the excessive production of free radicals.[4]

The histopathological evidences in the present study of cellular degeneration of individual cells or segments of the seminiferous tubules such cell loss was reflected upon the observed thinned out lining of the spermatocytes layers in many seminiferous tubules, making spermatocytes lying near the basal lamina. The mitochondria in the different cells of the testis were affected organelles as result

of exposure to CP in the present study. There was ultrastructural detection of swollen mitochondria, sometimes with disintegrating cristae in spermatids and spermatozoa. This was explained by Hirai *et al*.[46], as a result of increased oxygen radicals production during NADH oxidation by the outer membrane NADH cytochrome b5 oxidoreductase, while cytoplasmic vacuolations of spermatogenic cells were probably due to the increased cell permeability secondary to the alteration of membrane configuration. The lesion was furthermore attributed to the CP-induced membrane lipid peroxidation.

Spermatozoa are particularly susceptible to ROS-induced injury because of their high concentrations of polyunsaturated fatty acids and low antioxidant capacity.[43] This explains the spermatozoal changes in the current work.[6]

Apoptosis mechanism supports the histological changes induced by CP such as, degenerating changes like darkening and shrinkage of some spermatids, as well as rarification of their cytoplasmic organelles. This was supported by the study of Makale *et al*.[47] who reported apoptosis in almost all spermatogenic types in CP treated testis.

The mechanism by which both L-Carnitine and Vitamin E protected the testis from the toxic effect of cyclophosphamide, was mainly by their antioxidant properties.

LC prevents the formation of ROS system and decreases damage to the cell membrane.[48] It was also found to attenuate germinal cell damages and prevents decrease in MDA in rats subjected to ischaemia–reperfusion or to irradiation.[11] Topcu-Tarladacalisir *et al*.[48] found that LC also plays a key role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects sperm maturation, motility and the spermatogenic process.

Vitamin E (α tocopherol) is vital for the maintenance of mammalian spermatogenesis as it has been shown to suppress lipid peroxidation in testicular mitochondria and to reverse the detrimental effects of oxidative stress on testicular function.[32] Maneesh *et al*.[31] postulated that Vit E has protected the rat testis from the oxidative stress induced by ethanol, as they found that α-tocopherol reversed the testicular MDA level and restored scavenger system.

Shalaby *et al*.[49] reported that Vit E may execute the antioxidant role by modulating testicular free radical production and/or stimulating testicular androgenesis. Elgazzar[42] and Sabik *et al*.[26] found that vit E can act as a protective against the testicular toxicity caused by CP, and this effect was mainly due to its antioxidant effect.

The present findings confirmed the toxic effect of cyclophosphamide and that this toxicity could be prevented by the administration of LC and Vit E as antioxidant agents before and during the injection of CP. However, LC is a powerful protective agent to CP-treated testis more than Vit E. Also LC has a stimulant effect on spermatogenesis as well as its antioxidant effect. So the choice of an antioxidant against CP-induced testicular toxicity depends on the strength of the agent as well as its mechanism.

CONCLUSIONS

• L-Carnitine and Vitamin E are antioxidant materials that can reduce this toxic effect of cyclophosphamide on the morphology and histology of the testis. Results of L-Carnitine was considered better than Vitamin E.

• Both L-Carnitine and Vitamin E can be used as protective agents against the testicular toxicity of cyclophosphamide and should be used before and/or during its administration.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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ملخص البحث

المقدمة: يعتبر العقم و نقص الخصوبة الان من أهم المشكلات الصحية المهمة التى يواجهها العالم. وعلاج السرطان بالأدوية الكيماوية يساعد على انتشار هذا العقم. و من هذه الكيماويات عقار السيكلوفوسفامايد , فهو يعد من اكثر علاجات السرطان استخداما, و معروف انه يسبب سمية تؤثر على القدرة التناسلية لدى الذكور. وغالبا يتم هذا التاثير عن طريق الخلل فى الاكسدة. لذلك قد تستخدم بعض مضادات الأكسدة للحد من هذه السمية الناتجة عن السيكلوفوسفامايد مثل : ل- كارنيتن و فيتامين هـ.

يعد ل-كارنيتين من المواد الطبيعية واسعة الاستخدام. و يلعب دورا اساسيا فى الانتاج الخلوى للطاقة و عمليات الأيض. كما انه قد يستخدم فى علاج العقم لدى الرجال. أما فيتامين هـ فهو أحد الفيتامينات القابلة للذوبان فى الدهون و هو يعتبر مادة غذائية اساسية للتكاثر حيث أنه يحافظ على حيوية الحيوانات المنوية.

الأهداف: دراسة التأثير السمى لعقار السيكلوفوسفامايد الذى يؤثر على خصية ذكور الجرذان البيضاء البالغة. بالاضافة الى تسليط الضوء على الدور الوقائى المحتمل لمادتى ل – كارنيتين و فيتامين هـ ضد هذه السمية. مع المقارنة بينهما فى هذا الدور.

الطرق والوسائل: قد أجريت هذه الدراسة على ٣٦ جرذ أبيض ذكر بالغ متوسط أوزانهم من ١٢٠ الى ٢٠٠ جرام.

بعد اسبوعين من التأقلم ثم تقسيمهم عشوائيا الى مجموعات مختلفة كما يلى:

المجموعة الأولى (المجموعة الضابطة): و تشمل ١٨ جرذ لاستخدامهم كمجموعة ضابطة و تم تقسيمها عشوائيا الى ٣ مجموعات فرعية:

- المجموعة الفرعية الأولى أ (المجموعة الضابطة السلبية): و تشمل ٦ جرذان وحقن كل منهم بمحلول ملح بجرعة ٢٠ مجم / كجم من وزن الجسم داخل الغشاء البريتونى لمدة أسبوع.
- المجموعة الفرعية الأولى ب (المجموعة الضابطة الايجابية): و تشمل ٦ جرذان وأعطى كل منهم عقار ل-كارنيتين يوميا عن طريق الفم جرعة ٢,١ مجم / كجم من وزن الجسم يوميا لمدة اسبوعين.
- المجموعة الفرعية الأولى ج (المجموعة الضابطة الايجابية): و تشمل ٦ جرذان وأعطى كل منهم عقار فيتامين هـ يوميا عن طريق الفم جرعة ٣٦ مجم / كجم من وزن الجسم يوميا لمدة اسبوعين.

• المجموعة الثانية (المجموعة المعالجة): و تشمل ٦ جرذان و أعطى كل منهم جرعة ٢٠ مجم / كجم من وزن الجسم من عقار السيككلوفوسفامايد عن طريق حقنه داخل الغشاء البريتونى يوميا لمدة أسبوع.

• المجموعة الثالثة (المجموعة الوقائية): تتكون من ١٢ جرذا تم استخدامهم كمجموعة وقائية تلقت السيكلوفوسفامايد مع ل-كارنيتين او فيتامين هـ.

- المجموعة الثالثة الفرعية أ (المجموعة الوقائية): و تشمل ٦ جرذان و كل منهم تم حقنه داخل الغشاء البريتونى بعقار السيكلوفوسفامايد بجرعة ٢٠ مجم / كجم من وزن الجسم يوميا لمدة أسبوع و تم اعطاءهم ل- كارنيتين عن طريق الفم بجرعة٢,١ مجم / كجم من وزن الجسم لمدة اسبوع قبل السيكلوفوسفامايد .
- المجموعة الثالثة الفرعية ب (المجموعة الوقائية): و تشمل ٦ جرذان و كل منهم تم حقنه داخل الغشاء البريتونى بعقار السيكلوفوسفامايد بجرعة ٢٠ مجم / كجم من وزن الجسم يوميا لمدة أسبوع و تم اعطاءهم فيتامين هـ يوميا عن طريق الفم بجرعة ٣٦ مجم / كجم من وزن الجسم لمدة اسبوع قبل و أثناء حقن السيكلوفوسفامايد.

فى نهاية التجربة مات جرذان فى المجموعتين الثانية و الثالثة الفرعية و تم فصل رأس الجرذان و شرحت الخصى وتم فحص الشكل الخارجى لها و وزنها بالميزان الحساس و قياس أبعادها باستخدام مقياس فيرنييه. و تم تقطيعهم بعناية الى قطع صغيرة بحجم ١مم x ١مم وضعهم فى محلول الجلوتيرالديهايد ٣ ٪ لمدة ساعتين. بعد ذلك تم نقلهم الى محلول ٤ ف ١ ج لتثبيتها فى درجة حرارة ٤ سيليزيوس لمدة ٤٨ ساعة لفحصها بالميكروسكوب الالكترونى.

تم تجهيز الخصى للشرائح الهيستولوجية و تم صبغتها بصبغة التولويدين الزرقاء من اجل فحص الشرائح شبه الرقيقة باستخدام المجهر الضوئى. بينما تم فحص العينات فائقة الرقة باستخدام المجهر الالكترونى النافذ.

النتائج التشريحية:

بالنسبة للفحص الخارجى للخصية , تبين ان المجموعة المعالجة التى تلقت السيكلوفوسفامايد اظهرت انتقاص ذودلالة احصائية فى الوزن و المقاييس مقارنة بالمجموعة الضابطة و ان فى كل من المجموعتين الوقائيتين اللتين تلقتا ل- كارنيتين و فيتامين هـ كانت قيمة الأوزان و الابعاد افضل من المجموعة المعالجة و لكن اقل من وزن و مقاييس المجموعة الضابطة.

النتائج الهيستولوجية:

قد أظهر الفحص المجهرى للعينات شبه الرقيقة للمجموعة المعالجة تشوه بعض الأنابيب الناقلة للمنى, و اظهر بعضها تعرجا فى الغشاء القاعدى الخاص بها. بعض المناطق كشفت زيادة المسافات بشكل كبير بين القنوات الناقلة للمنى و البعض الاخر اظهر مسافات بين الخلايا المنوية وبعض بؤر الفجوات بالسيتوبلازم. كما ظهرت بعض الانابيب مع خلايا منوية منفصلة و ساقطة فى تجاويفها و بعض النطف ظهرت

فى غير موقعها الطبيعى قريبا من الغشاء القاعدى. و علاوة على ذلك ظهرت بعض الانابيب خالية من الحيوانات المنوية. كما ظهرت فجوات بالسيتوبلازم فى خلايا ليدج.

كشف فحص المجهر الالكترونى للانابيب الناقلة للمنى للمجموعة الثانية عن تغيرات تحلليه شملت معظم الخلايا المنوية. كما لوحظ مسافات واسعة بين الخلايا و فجوات بالسيتوبلازم. واظهرت الخلايا المنوية بعض التغيرات مثل :انوية منكمشه كثيفة و توسعات حول النواة. كما اظهرت النطف بعد التغيرات حيث كانت قريبة من الغشاء القاعدى و كشفت عن قبعات اجسام طرفية متعرجة وتغير فى مواقع الميتوكوندريا حول أطراف الخلية. كانت هناك ايضا اشكال مشوهة و نقص فى أعداد الحيوانات المنوية. و اظهرت المقاطع العرضية فى ذيول الحيوانات المنوية سوء تنظيم فى أغشية الميتوكوندريا. أما خلايا ليدج فقد أظهرت أيضا فجوات بالسيتوبلازم.

و بفحص الانابيب الناقلة للمنى للمجموعة الثالثة الوقائية , لوحظ احتفاظ بعض الانابيب بشكلها الطبيعى بينما اظهرت البعض بؤر الفجوات بين الخلايا المنوية. و قد أظهرت المجموعة الوقائية أ و التى تلقت ل-كارنيتين تغيرات ضمورية اقل من التى ظهرت فى المجموعة الوقائية ب التى تلقت فيتامين هـ.