Histological Study on the Possible Protective Role of L-Carnitine Against Testicular Ischemia-Reperfusion Injury Induced in Rabbits

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Original Article

ABSTRACT

Introduction: The torsion of the testis is a surgical emergent situation which is common; however there are no effective measures to avoid damage of the testis after untwisting this torsion. L-carnitine (LC) is an essential micronutrient with strong anti-apoptotic, anti-inflammatory and antioxidant potencies that can reverse oxidative stress induced testicular damage.

Aim of the Work: To evaluate the potential protective role of LC against the testicular damage induced by ischemia-reperfusion (IR) injury in rabbits.

Materials and Methods: 50 adult male rabbits weighing 1300-1600 g were divided into 5 groups (10 rabbits per group). Acute IR testicular injury was induced in rabbits by twisting the left spermatic cord 720º for 2 h. Group 1: Control group and divided into two subgroups (subgroup A negative control and subgroup B positive control); Group 2: Sham-operated; Group 3: Ischemia group; Group 4: IR group (testicular IR was induced without LC); Group 5: IR+LC testicular IR was induced and LC was given by intra-peritoneal injection at a dose of 500 mg/kg immediately after torsion. At the experimental end, tissue samples of left testes were collected from all rabbits of the five groups and evaluated biochemically and histologically.

Results: Ischemia-reperfusion resulted in disturbance of the oxidant/antioxidant status markers, loss of normal architecture of spermatogenic cells, apparent increase in the number of caspase-3 positive spermatogenic cells as well as abnormal fragmented chromatin material in addition to lack of halos around the sperm nuclei. However, the intra-peritoneal administration of LC immediately after torsion significantly improved these changes caused by IR on the testes.

Conclusion: Administration of LC reduced the testicular damage induced by the ischemia-reperfusion.

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Key Words: Ischemia-reperfusion injury, L-carnitine, rabbits, testes.

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INTRODUCTION

Acute ischemia is a sudden cessation of the arterial blood supply of a specific organ that when prolonged may lead to death of the affected tissues. Therefore, the reperfusion in an appropriate time is essential for avoiding tissue necrosis. However, reperfusion causes more tissue damage leading to ischemia-reperfusion (IR) injury. The neutrophil cells are responsible for the IR injury because when the ischemic tissue is re-oxygenated, the neutrophils produce large amounts of reactive oxygen species (ROS) that lead to more tissue damage[1].

During prolonged ischemia, adenosine tri-phosphate (ATP) levels and the intracellular PH decrease due to anaerobic metabolism with subsequent mitochondrial destruction and cell death. On reperfusion, the oxygen level is restored leading to overproduction of ROS and the pro-inflammatory neutrophils infiltrate the ischemic tissues producing more tissue damage[2].

Testicular torsion is an emergency surgical condition resulting from spermatic cord twisting and referred to 'acute scrotum'. It leads to acute testicular ischemia thus; it needs early surgical intervention for restoration of

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testicular blood flow (untwisting). However, inappropriate treatment may lead to reperfusion injury with more functional and structural testicular damage and subsequent infertility[3]. Testicular torsion may lead to loss of the testis if not corrected within 4 hours[4]. That condition is common in young ages before 25 years old affecting one per each four thousands male[5].

Ischemia-reperfusion injury leads to an oxidative stress-mediated testicular damage which results from the imbalance between mitochondrial oxygen demands and oxygen supply. This causes an excessive ROS production with a subsequent DNA fragmentation of the spermatogenic cells, Sertoli cells as well as the interstitial Leydig cells[6]. Thus, before surgical correction of the testicular torsion, antioxidants should be taken to prevent the testicular reperfusion injury.

L-carnitine (LC) is a naturally occurring antioxidant ingested in food and also synthesized inside the body from trimethyl-lysine that is formed by methylation of the essential amino acids lysine[7]. It is present in large amounts in the milk products and red meats but, it can also be found in fish, fruits, vegetables, seeds, nuts and wheat[8]. It is a water-soluble compound and its uptake inside the cells is facilitated by a number of transporters on the plasma cell membrane such as cation transporter-3, cation transporter-2 and carnitine transporter-2[9]. It is found in high concentrations in the testis and epididymal fluid[10]. Therefore, it is important in the formation, metabolism, maturation and motility of the sperm[11].

L-carnitine shares in the maintenance of the cell viability as it is involved in the lipid and cellular metabolism. It promotes the transportation of long chain fatty acids from the cytoplasm into the mitochondria for the degradation and conversion into metabolic energy by β-oxidation. Consequently, it prevents the intracellular accumulation of the long chain fatty acids which cause injury to cells[12]. Moreover, LC has numerous biological activities including anti-inflammatory, antioxidant and anti-apoptotic capacities[13,14] hence; it has the ability to improve the tolerance against IR injury[15].

AIM OF THE WORK

The current study aimed to evaluate the potential protective role of LC against the testicular damage induced by the ischemia-reperfusion injury in rabbits.

MATERIALS AND METHODS

Chemicals

L-carnitine powder. TITAN BIOTECH. The drug was obtained from Sigma Aldrich Company; Cairo, Egypt. 5 gm of LC were dissolved in 50 mL saline and injected intra-peritoneal at a dose of 500 mg/kg immediately after torsion.

Experimental animals

Fifty adult male rabbits of about 7 months old and weighing 1300–1600 g were bought from the Animal Health Research Institute in Dokki, Egypt. They were put in plastic cages in suitable laboratory conditions in the animal house of the research center at the Faculty of Medicine, Ain Shams University. The rabbits were bred according to the ethics of the Experimental Research Center in the Ain Shams University with a code number [RE (201)23]. The rabbits were kept for one week for acclimatization in their new environment before surgery and received a standard diet and tab water. At the end of the week spent for acclimatization, the fifty rabbits were divided randomly into 5 groups (10 animals per group).

Experimental design

- **Group 1** (Control group): This group was divided into two subgroups (5 rabbits in each subgroup):
    - Subgroup A (negative control): The rabbits were left without any intervention and were sacrificed with the corresponding ischemia group after 2 hours.
    - Subgroup B (positive control): The rabbits received a single dose of LC by intraperitoneal injection at a dose of 500 mg/kg. The rabbits were sacrificed with the corresponding groups after 12 hours.
- **Group 2** (Sham-operated group): The scrotum was incised and the testis was brought to the outside then the scrotum was sutured without IR induction. Then, the rabbits were sacrificed and orchidectomy was done after 12 hours[8].
- **Group 3** (Ischemia group): The testicular ischemia was induced through incising the scrotum and twisting the left testis 720° in the clockwise direction then keeping it twisted by suturing its tunica albuginea with the surrounding tissues for 2 h only[16]. Then, the rabbits were sacrificed immediately after the 2 h of ischemia and orchidectomy was done without the reperfusion.
- **Group 4** (IR group): Testicular IR was induced then the rabbits were sacrificed 12 h after reperfusion[17].
- **Group 5** (IR+LC group): Testicular IR was induced and LC was given by intra-peritoneal injection at a dose of 500 mg/kg immediately after torsion then the rabbits were sacrificed 12 h after the reperfusion[17]. After completing the experimental procedures, tissue samples were obtained from the left testes for biochemical and histological investigations in the control and different treated groups.

Ischemia-reperfusion induction

The surgical and invasive interventions on the rabbits were done only after complete general anesthesia[4]. The operation was performed under complete aseptic

conditions and after complete sedation of the rabbits by general anesthesia using ketamine hydrochloride injected into the peritoneal cavity at a dose of (50 mg/kg)[18]. All layers of the scrotum were incised on the left side then; the left testes were twisted in the clockwise direction 720°[16,17] and kept twisted by suturing the tunica albuginea with the surrounding tissues for 2 hours (Figure 1). After ischemia for 2 hours, the spermatic cord was untwisted to induce reperfusion then, the tunica vaginalis and the scrotum were re-sutured again[4]. 12 hours after detorsion, the rabbits were re-anesthetized again and the left testes were excised then, the rabbits were euthanized after biopsy with intra-peritoneal overdose of ketamine hydrochloride[19].

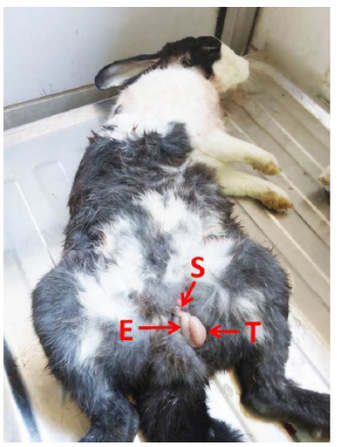

Fig. 1: A completely anaesthetized rabbit with a twisted left spermatic cord (S) 720º in clockwise direction for an induction of ischemia in the left testis (T) and epididymis (E).

Biochemical analysis

The biochemical analysis procedures were done at the biochemistry department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Left testes samples were homogenized for analysis and assessment of the activity of superoxide dismutase (SOD) and the levels of Malondialdehyde (MDA) and reduced glutathione (GSH) to assess the degree of oxidative stress[19]. GSH levels were calculated using colorimetric method (Reduced Glutathione (GSH) colorimeteric Assay kit, E-BC KO3O-S, E lab science, United Kingdom) .MDA levels were calculated using colorimetric method (TBA method). (MDA colorimeteric Assay Kit, MBS2540407, My Biosource, Canada). SOD activity was measured using ELISA technique by the use of (Mouse Superoxide Dismutase-1 ELISA Kit, ab285309, abcam, United Kingdom).

Histological evaluations

The left testes were immediately preserved through Bouin's solution for 6 hours then, paraffin sections 5µ thick were prepared for the following stains:

a. Hematoxylin and eosin staining: for assessment of the histological structure of the testes[20].

b. mmunohistochemical staining using active caspase-3 antibody: for detection of spermatogenic cells apoptosis[21]. It is a Rabbit Polyclonal antibody (ABclonal company, Catalog No.: A2156). The caspase-3 showed in early stages of apoptosis nuclear reaction and later it showed brown cytoplasmic reaction. Positive control: was the human tonsil using Caspase-3 antibody. Negative control: Additional specimens of testes were processed in the same way like the positive control but omitting the step of primary antibody.

Sperm DNA fragmentation assay

Sperm DNA fragmentation assay procedure was performed in Faculty of Medicine, Al-Azhar University, Cairo, Egypt. The test kit was utilized to grade the chromatin quality in sperm with various halo size. The kit was purchased from Egypt-based Biodiagnostic Company, Cairo. Samples of seminal fluid were collected from the caudal epididymis region immediately after sacrificing the rabbits and diluted with PBS. Subsequently, 1% agarose was added to get a 0.7% agarose concentration. To make the micro gel as well as sperm solidification, few drops of the prepared mixture were put on a super-coated agarose glass slide (0.65%), covered with a glass coverslip (22x22 mm), and kept at 4 °C in a refrigerator for 5 min. After solidification, the coverslips were removed carefully, and the slides were immersed horizontally for 7 min. at room temperature in a fresh denaturation solution (0.08 N HCl). To stop the denaturation reaction, the slides were then transferred to an alternative lysis buffer (0.4 m Tris, 0.4 m DTT, 50 mm EDTA, 0.3% SDS, and 1% Triton X-100) and incubated for 25 min at room temperature. After carefully cleaning the slides using distilled water, they were gradually dehydrated in ethanol at progressively increasing concentrations (70, 90, and 100%). Then the slides were stained by Giemsa after dehydration, examined using the bright field microscope (Zeiss Axioskope 2 plus; Carl Zeiss, Gottingen, Germany) to detect the size of the halos of the sperm nuclei that indicate the degree of DNA dispersion. Four different dispersion patterns were observed; (i) big halos, (ii) moderate-sized halos, (iii) small halos and (iv) no halos. The big and moderate-sized halos indicated intact sperms with normal DNA while the small and absent halos indicated significantly fragmented DNA of the sperm nuclei[22,23].

Morphometric study and Statistical analysis

These were carried out at the Histology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt by Leica "Qwin 500C" image analyzer computer system (Cambridge, England). The caspase-3 immune positive cells were counted in 10 non-overlapping fields in each specimen under the ×400 lens.

Analysis of the obtained measurements was done through Statistical Package of Social Sciences (SPSS) with the assistant version 7.7. One-way analysis of variance (ANOVA) was performed for mean comparisons between the control and treated groups. The differences were considered statistically significant when probability (*P) value* is < 0.05[24].

RESULTS

Biochemical results

The differences between the two subgroups of the control group 1 and the sham operated group 2 were non-significant in the levels of the oxidant/antioxidant status markers; MDA, GSH and SOD in the testicular tissue. The ischemia operated group 3 showed a significantly elevated level of MDA, a significantly depressed GSH content and SOD activity in comparison to group 1. In the IR group 4, there were more significantly elevated level of MDA, more significantly decreased GSH content and SOD activity in comparison to group 1. In the IR+LC group 5, treatment with LC before the testicular reperfusion resulted in a significantly decreased level of MDA and a significantly elevated GSH content and SOD activity in comparison to the IR without treatment group 4. (Table 1, Figures 2,3)

Histological results

a. Hematoxylin and eosin stained sections: (Figures 4,5)

The 2 subgroups of control and sham operated groups showed normal histological pattern of the testis with tunica albuginea around it. The sections showed regular seminiferous tubules that were surrounded by a connective tissue layer called basal lamina that consisted mainly of flat myoid cells. These tubules were lined by several layers of rounded spermatogenic cells that had central rounded nuclei and acidophilic cytoplasm. These cells were arranged from peripheral to central as following spermatogonia, primary spermatocytes and spermatids. The spermatogonia were observed resting on the basal lamina. The primary spermatocytes revealed large rounded nuclei, while the spermatids were seen towards the tubular lumen that showed completely formed sperms. Sertoli cells were irregular with pale cytoplasm and basal nuclei with prominent nucleoli. The interstitial cells of Leydig were observed between the seminiferous tubules (Figures 4,5 (A, B)).

Testicular sections from the ischemia group showed thickening of tunica albuginea. Most seminiferous tubules were distorted. Separation between the spermatogenic cells was observed and some cells showed vacuolated cytoplasm. In addition, certain seminiferous tubules showed severe destruction of the spermatogenic epithelium. The interstitial tissue showed marked congestion of the blood vessels with vacuolated Leydig cells (Figures 4,5C).

In the IR group, the testicular sections showed a thick separated tunica albuginea. Many seminiferous tubules were severely distorted and showed exudates inside the tubules and others showed a marked loss of the spermatogenetic cells. There was separation between the cells and some cells were shed off inside the lumen. Some cells showed dark fragmented nuclei and scattered apoptotic cells were also observed. The interstitial tissue showed congested blood vessels (Figures 4,5D).

As regards the IR + LC group, the testicular section showed an apparently normal tunica albuginea. Some seminiferous tubules showed separation between the spermatogenic cells however; some tubules showed spermatogonia with deep nuclei resting on the basal lamina and spermatozoa at the lumen were noticed. The interstitial tissue showed congested blood vessel (Figures 4,5E).

b. Immuno-histochemical results for caspase-3: (Figure 6)

In the 2 subgroups of control and sham groups, the spermatogenic cells of the seminiferous tubules showed a negative reaction while the Leydig cells showed a positive cytoplasmic reaction (Figures 6 A,B). In the ischemia group, some spermatogenic and Leydig cells showed positive nuclear reaction (Figure 6 C). In the IR group, most of the spermatogenic and Leydig cells showed apparent increase in the number of caspase-3 positive cells (Figure 6 D). As regards the IR + LC group, some spermatogenic and Leydig cells showed nuclear positive reaction (Figure 6 E)

The mean count of caspase-3 positive cells in all groups

Morphometric analysis showed an insignificant difference between the 2 subgroups of control and the sham groups in the mean count of the spermatogenic and Leydig cells. In the ischemia and IR groups, the mean count of the caspase-3 positive spermatogenic and Leydig cells was significantly elevated than in the control group. However, the IR+LC group showed a significant reduction in the mean count of caspase-3 positive spermatogenic cells with an insignificant change in the mean count of the interstitial cells in comparison to the IR group (Table 2, Figure 7).

c. Sperm DNA fragmentation assay (Figure 8)

This assay detected normal large and full halos around the sperm nuclei with a normal DNA in both the 2 subgroups of control and sham groups (Figures 8 A,B) respectively. Moreover, the ischemia group exhibited medium sized halos around the sperm nuclei with a normal non-fragmented chromatin material (Figure 8 C). In the IR group (Figure 8 D), an abnormal fragmented chromatin material and lack of halos around the sperm nuclei. IR + LC treated group showed apparent full halos around the sperm nuclei with a normal non-fragmented chromatin material (Figure 8 E).

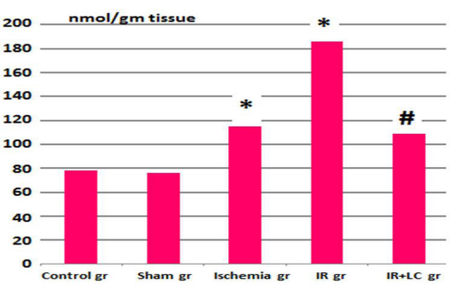

Fig. 2: The mean values of MDA level in all groups.
* significant in comparison to the control
# significant in comparison to the IR group

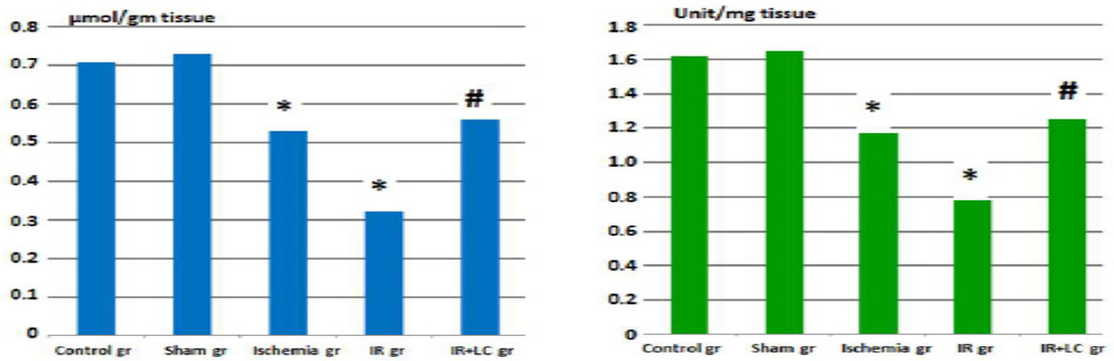

Fig. 3: The chart to the left represents the mean values of GSH level in all groups. The chart to the right represents the mean values of SOD level in all groups

* significant in comparison to the control

significant in comparison to the IR group

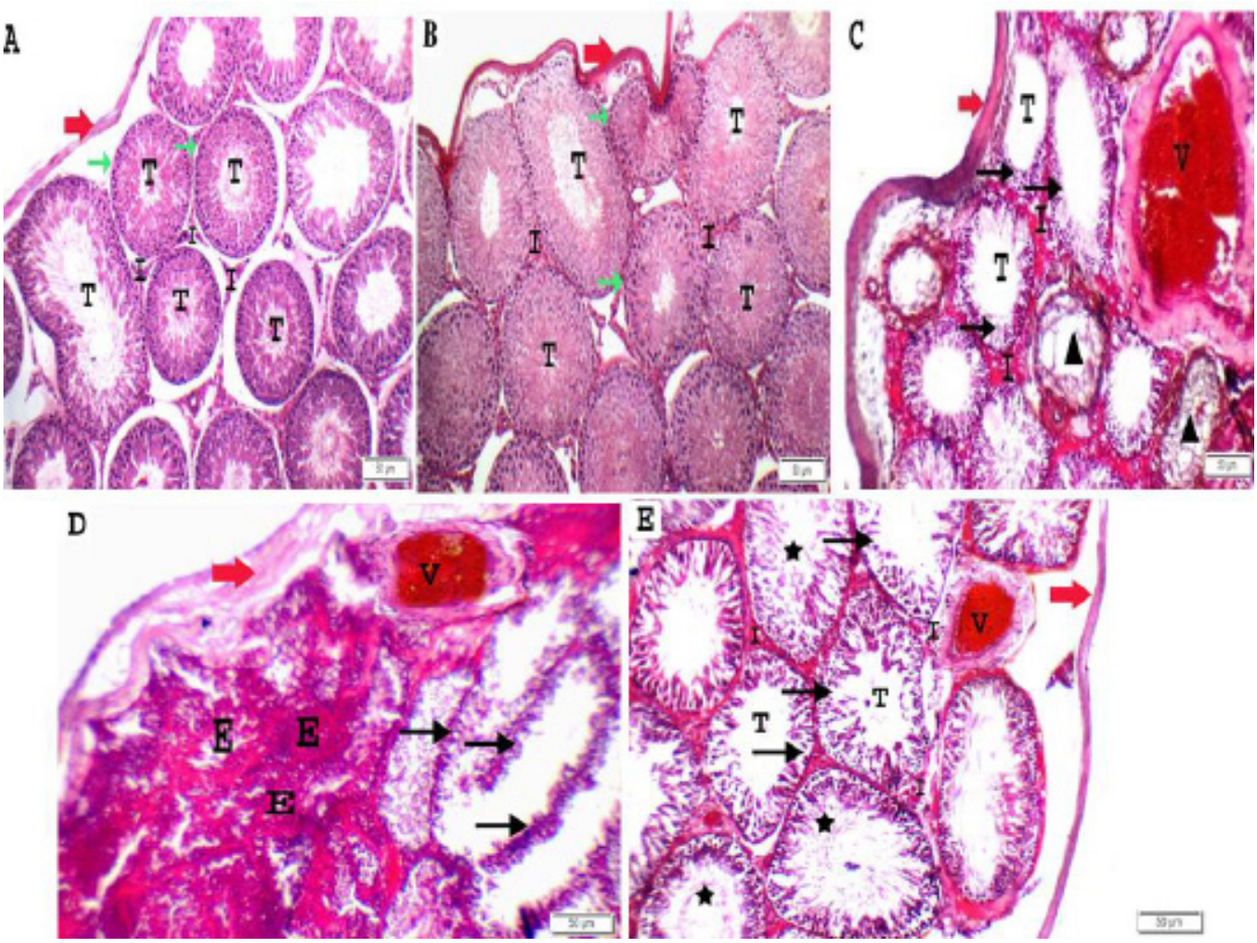

Fig. 4: Photomicrographs of the testes of all groups. The photomicrographs [A] and [B] represent the 2 subgroups of control and sham groups respectively and show that, the testes are surrounded by tunica albuginea (red arrows). The sections show regular seminiferous tubules (T) that are surrounded by a connective tissue layer called basal lamina (green arrows). Tubules are lined by several layers of spermatogenic cells. The tubules are separated by interstitial tissue (I). In the ischemia group [C] there is a thickening of tunica albuginea (red arrow), most seminiferous tubules (T) are distorted (black arrows) and some tubules show severe destruction of the spermatogenic epithelium (triangles). The interstitial tissue (I) shows a marked congestion of the blood vessels (V). In the IR group [D], the testicular section shows a thick separated tunica albuginea (red arrow), many seminiferous tubules show exudates (E) inside the tubules and others show a marked loss of the spermatogenetic cells (black arrows). The interstitial tissue shows congested blood vessel (V).In the IR + LC group [E], the testicular section shows apparently normal tunica albuginea (red arrow). Some seminiferous tubules (T) show a separation between the spermatogenic cells (black arrows) and some tubules show spermatozoa at their lumen (stars). The interstitial tissue shows a congested blood vessel (V). (H&E, X 200)

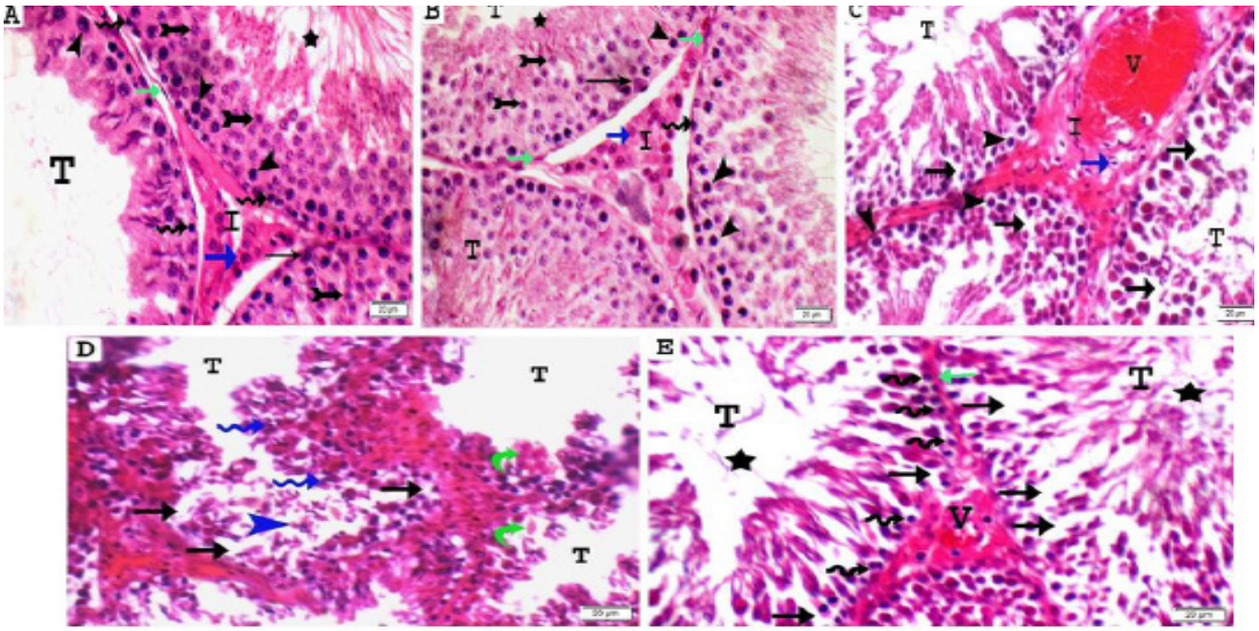

Fig. 5: Photomicrographs of the testes of the five groups. The photomicrographs [A] and [B] represent the 2 subgroups of control and sham groups respectively and show seminiferous tubules (T) that are surrounded by a connective tissue layer called basal lamina that consists mainly of flat myoid cells (green arrows). The spermatogonia (zigzag arrows), primary spermatocytes (arrow heads) and spermatids (bifid arrows) are observed. Spermatozoa are detected within the lumen (stars). Sertoli cells are irregular with pale cytoplasm and basal nuclei with prominent nucleoli (black thin arrows). Interstitial cells of Leydig (blue arrows) are observed in the interstitial tissue (I). In the ischemia group [C] the seminiferous tubules (T) show a separation between the spermatogenic cells (black arrows) with vacuolated cytoplasm in some of them (arrow heads).The interstitial tissue (I) shows a marked congestion of the blood vessels (v) with vacuolated Leydig cells (blue arrow). In the IR group [D], the seminiferous tubules (T) are severely distorted and show a marked loss of the spermatogenetic cells; separations are seen between the spermatogenic cells (black arrows) with shedding off some cells into the lumen of the tubules (blue arrow head).Some cells show dark fragmented nuclei (blue zigzag arrows) and scattered apoptotic cells are also seen (green curved arrows). However, in the IR + LC group [E], some seminiferous tubules (T) show a separation between the spermatogenic cells (black arrows). Some tubules show spermatogonia with deep nuclei (zigzag arrows) resting on the basal lamina (green arrow) and spermatozoa at the lumen (stars) are noticed. The interstitial tissue shows a congested blood vessel (V). (H&E, X 400)

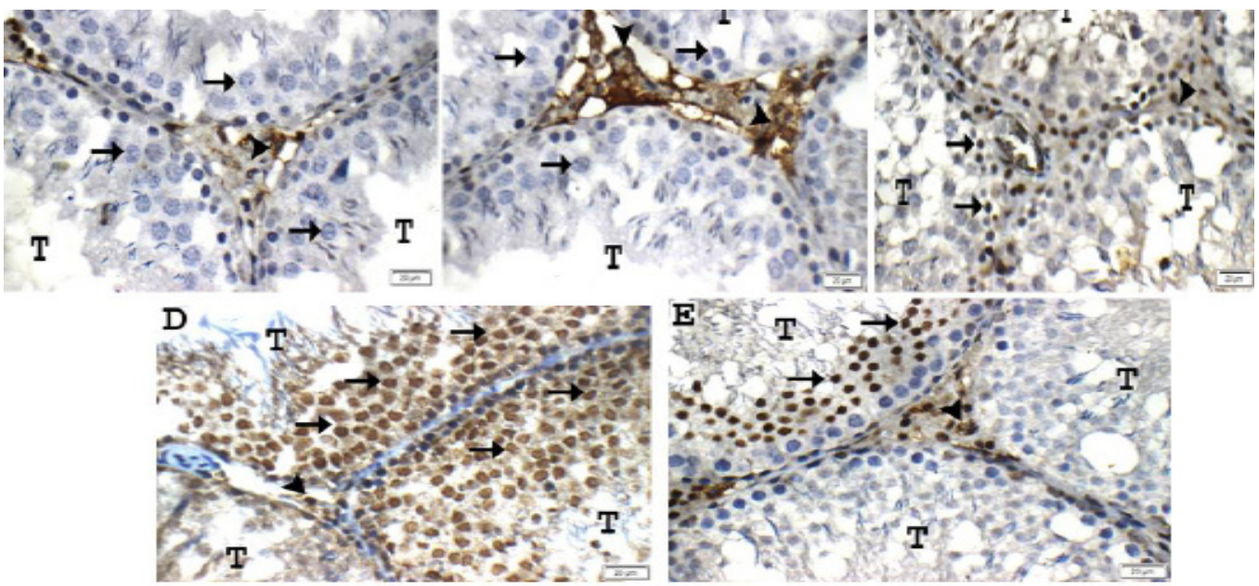

Fig. 6: Photomicrographs of the testes of the five groups. In the 2 subgroups of control [A] and sham [B] groups, the spermatogenic cells (arrows) in the seminiferous tubules (T) show a negative caspase-3 reaction while the Leydig cells (arrow heads) show a positive cytoplasmic reaction. In the ischemia group [C], some spermatogenic cells (arrows) and some Leydig cells (arrow heads) show a positive nuclear reaction. In the IR group [D], the testicular sections show apparent increase in the number of caspase-3 positive cells in most of the spermatogenic cells (arrows) and in Leydig cells (arrow heads). However, in the IR + LC group [E], some spermatogenic cells (arrows) and Leydig cells (arrow heads) show nuclear positive reaction. (Caspase-3 immunohistochemical staining, X 400)

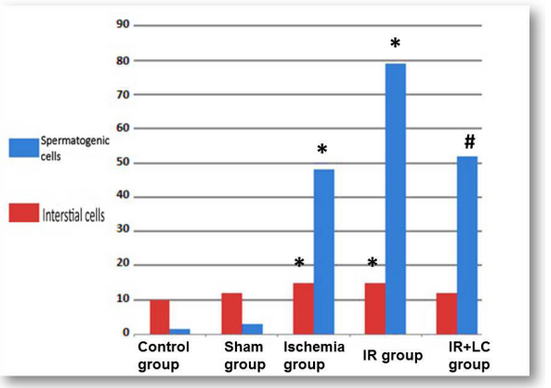

Fig. 7: A histogram representing the mean count of caspase-3 positive cells at high power field (X400) of control and treated groups.

* Significant in comparison to the control

Significant in comparison to the IR group

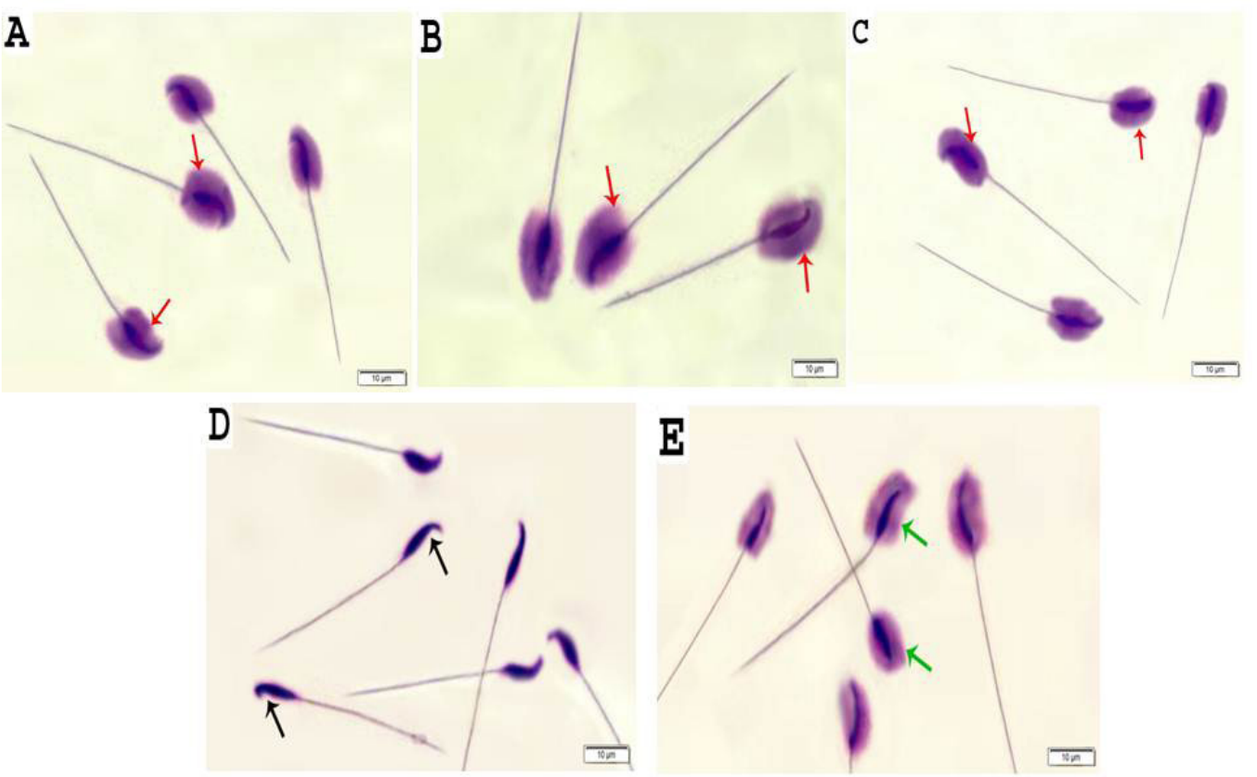

Fig. 8: Photomicrographs of sperm chromatin quality by sperm DNA fragmentation assay from the 2 subgroups of control [A] and sham [B] groups showing normal large and full halos around the sperm nuclei with a normal DNA (red arrows), ischemia [C] group exhibiting medium sized halos around the sperm nuclei with a normal non-fragmented chromatin material (red arrows), IR group [D] detecting abnormal fragmented chromatin material and lack of halos around the sperm nuclei (black arrows), IR + LC [E]treated group demonstrating apparent full halos around the sperm nuclei with a normal non-fragmented chromatin material (green arrows). (Giemsa stain, X 1000).

Table 1: The mean values of MDA level, GSH content and SOD activity in 2 subgroups of control and different treated groups:

Parameters	Control	Sham	Ischemia	IR	IR+LC
MDA± SD nmol/gm tissue	78 ± 6	76 ± 8[a]	115 ± 9[b]	186 ± 13[bc]	109 ± 11[bde]
GSH± SD µmol/gm tissue	0.71 ± 0.18	0.73 ± 0.14[a]	0.53 ± 0.11[b]	0.32 ± 0.8[bc]	0.56 ± 0.15[bde]
SOD± SD Unite/mg tissue	1.62 ± 0.18	1.65 ± 0.21[a]	1.17 ± 0.16[b]	0.78 ± 0.12[bc]	1.25 ± 0.22[bde]

Representation of the data was in mean ± SD (Standard deviation)
a = Insignificant *P-value* when compared to group 1 ($P > 0.05$)
b = Significant *P-value* when compared to group 1 ($P < 0.05$)
c = Significant *P-value* when compared to group 3 ($P < 0.05$)
d = Insignificant *P-value* when compared to group 3 ($P > 0.05$)
e = Significant *P-value* when compared to group 4 ($P < 0.05$).

Table 2: The mean count of caspase-3 positive cells at high power field (X400) of 2 subgroups of control and different treated groups:

Caspase-3 positive cells	Control	Sham	Ischemia	IR	IR+LC
spermatogenic cells	1.66	2.8[g]	48.6[bc]	79.2[bcd]	52.1[bcef]
Interstitial cells	10	12[g]	14.2[ah]	14.4[ai]	12[ghij]

a = Significant *P-value* as compared to the control group ($P < 0.05$).
b = Highly significant *P-value* as compared to the control group ($P < 0.02$).
c = Highly significant *P-value* as compared to the sham group ($P < 0.02$).
d = Significant *P-value* as compared to the ischemia group ($P < 0.05$).
e = Insignificant *P-value* as compared to the ischemia group ($P > 0.05$).
f = Significant *P-value* as compared to the IR group ($P < 0.05$).
g = Insignificant *P-value* as compared to the control group ($P > 0.05$).
h = Insignificant *P-value* as compared to the sham group ($P > 0.05$).
i = Insignificant *P-value* as compared to the ischemia group ($P > 0.05$).
j = Insignificant *P-value* as compared to the IR group ($P > 0.05$).

DISCUSSION

The testicular torsion is an important urgent urological condition resulting from spermatic cord twisting and necessitates rapid correction to re-establish the normal circulation of the testis before occurrence of ischemic testicular necrosis. The prolonged ischemia leads to testicular and sperm damage but reperfusion produces more damage through overproduction of ROS, elevation of the concentration of calcium inside the mitochondria and enhancement of apoptosis rate[25]. It is vital to find a prophylactic agent against testicular IR insult because it is a serious surgical condition responsible for some cases of male infertility[26].

In this study, rabbits were used rather than other experimental animals due to their being frequently used in biomedical research, particularly as a bioreactor for the production of antibodies. In addition to their appropriate size, mild disposition, ease of use, and low care requirements in the laboratory, the rabbits are phylogenetically more similar to humans than rodents. In addition, the rabbits usually act as a distinct species between smaller rodents (mice and rats) and larger animals, such as the dogs, pigs, and monkeys, due to their short life spans, brief gestation periods, high numbers of progeny, low cost (compared to other large animals), and availability of genomics and proteomics[27].

Moreover, the rabbit was chosen in this study because it has several advantages as a non-rodent and second model for assessing the effects of toxic agents on semen quality, fertility, teratology and developmental toxicity. In addition, the rabbit semen can be obtained easily for monitoring many functions and their disruption in *vivo* and in *vitro* is of particular value because of the importance[28].

The present study evaluated the protective role of LC against testicular IR injury biochemically and histologically. In the current work, testicular ischemia was induced by twisting the spermatic cord 720º for 2h due to the fact that it is a sufficient period to induce ischemia then, reperfusion was done by untwisting[1,16].

The sham-operated group aimed to detect the standard levels of the biochemical and histological values. Hematoxylin and Eosin as well as caspase-3 immunostained sections of this group proved apparently normal spermatogenic and interstitial Leydig cells in comparison to the control group. These findings were concurrent with previous histological and immunohistochemical results[8,29].

Moreover, the testicular sections from the ischemia group showed distortion of the seminiferous tubules and destruction of the spermatogenic cells. In addition the caspase-3 immunohistochemical stained sections revealed nuclear reactivity for caspase-3 in spermatogenic cells. These results can be explained by the disturbed oxidant/antioxidant status causing ischemia-induced oxidative stress testicular damage and consequently cells apoptosis[25].

In the group of IR, there was significant IR-induced testicular damage indicated by lost normal histological picture with destruction of the spermatogenic cells as was

indicated by dark fragmented nuclei and apoptotic cells. In addition, marked increase in number of caspase-3 positive spermatogenic cells was an indicator for marked apoptosis and spermatogenesis failure. These histological changes were further confirmed by an elevation of MDA level which is considered as oxidative stress marker produced from lipid peroxidation. In addition, there were decreased GSH content and SOD activity which are considered as the main antioxidants present in the testicular tissue.

These destructive effects of IR were attributable to the IR-induced testicular oxidative stress. In addition, after IR the neutrophil accumulation, proinflammatory, and inflammatory cytokines, necrotic and apoptotic processes in the tissue also accelerate[29] and this was correlated with previous results proved that IR leads to a destruction of the spermatogonia of the seminiferous tubules with increased apoptosis and suppressed spermatogenesis[29].

In the present work, the oxidative stress induced by testicular IR goes in line with the findings observed by[26,30] who detected that, IR caused a testicular damage through an enhancement of the oxidative stress associated with raised MDA and decreased GSH levels in addition to decreased SOD activity.

In the current study, IR treated group, the reperfusion resulted in an abnormal DNA fragmentation in addition to lack of halos around the sperm nuclei. This was previously explained that the DNA fragmentation and chromatin condensation are the hallmarks of cells apoptosis[31]. In contrast, the administration of LC intra-peritoneal before the reperfusion (IR + LC) ameliorated the reperfusion negative effects as illustrated by apparent full halos around the sperm nuclei with normal non-fragmented chromatin material. These results go in line with the findings of[32,33] who documented that, the antioxidants protects against the oxidative DNA destruction through prevention of the oxidative stress-induced damage of the pyrimidine and purine bases of the DNA strands. In addition, it was proved that, the antioxidants maintain the integrity of DNA molecules through preventing the formation of transverse bonds between the proteins and DNA[32,33].

In the current study the caspase- 3 immunohistochemical stained sections showed positive cytoplasmic reaction in the Leydig cells in the control and sham groups. The other groups showed positive nuclear reaction in the spermatogenic and Leydig cells. This may be consistent with the previous study that mentioned that procaspase-3 processing occurred in both cytoplasmic and nuclear compartments, but at different apoptotic stages. This study added that some cells with normal morphological appearance contained low amounts of active-caspase-3 in the cytoplasm[31].

In addition, the administration of LC significantly ameliorated the IR-induced hazardous effects on the testes as indicated by restoration of some of the histological features of the germinal testicular epithelium towards the normal. Also, the moderate number of caspase-3 positive cells was detected in this group that may be explained by the antioxidant healing potency of LC. Furthermore, LC administration significantly decreased MDA and elevated GSH and SOD levels indicating the LC efficacy in counteracting the IR-induced testicular oxidative stress injury.

The ability of LC to counteract the IR-induced germinal epithelium damage in rabbits detected in the current work was in correlation with previous results. It was proved that LC attenuates the testicular damage induced by IR in rats by ameliorating the IR-induced oxidative stress with the prevention of the formation of cytokines that are strong pro-inflammatory agents and also prevents ROS production[34]. Also, previous findings have proved the LC efficacy in protection against IR-induced insults of other viscera including the kidneys[35], heart[36], intestine[37] and spinal cord[38].

The antioxidant potency of LC and its efficacy to reverse the IR-induced an oxidative stress detected in the present study were attributable to its ability to prevent the excessive oxygen consumption with the subsequent inhibition of excessive ROS production leading to enhanced cell viability[39].

CONCLUSION AND RECOMMENDATION

It is concluded that, administration of LC reduced the reperfusion-induced testicular injury. Therefore, it is recommended to use LC in patients with twisted testes before untwisting for preserving the testis. Also, there is a need for additional researches to learn more and more about LC use and its efficacy in long term cases of testicular torsion.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة هستولوجية للدورالوقائي المحتمل للكارنيتين على ضررالخصية الناشئ من إعادة تدفق الدم عقب نقص التروية المستحدثة في الأرانب

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الخلفية: التواء الخصية هو حالة طوارئ شائعة الحدوث ومع هذا لا يوجد علاج فعال للحفاظ علي وظيفة الخصية بعد فك الإلتواء. يعد الكارنيتين غذاء أساسي له خصائص قوية مضادة لموت الخلايا المبرمج، مضادة للإلتهابات وللأكسدة والذي يمكن بدوره أن يعكس الإجهاد التأكسدي المسبب لتلف الخصية.

الهدف من الدراسة: الهدف من تلك الدراسة هو تقييم الدور الوقائي المحتمل للكارنيتين على ضررالخصية الناشئ من إعادة تدفق الدم عقب نقص التروية المستحدثة في الأرانب.

المواد و الطرق: تم تقسيم خمسين أرنباً بالغاً تتراوح أوزانهم بين ١٣٠٠-١٦٠٠ جرام الي خمس مجموعات (١٠ في كل مجموعة). تم استحداث التواء حاد في الحبل المنوي الأيسر في الأرانب وذلك عن طريق تدويره ٧٢٠ درجة لمدة ساعتين. المجموعة الأولي:المجموعة الضابطة وقسمت إلى مجموعتين فرعيتين أولهما ضابطة سلبية وثانيتها ضابطة إيجابية; المجموعة الثانية: المجموعة الضابطة الجراحية، المجموعة الثالثة: مجموعة نقص التروية. المجموعة الرابعة: (مجموعة إعادة تدفق الدم عقب نقص التروية) تم استحداث التواء في الحبل المنوي للخصية بدون تلقى الكارنيتين;المجموعة الخامسة: مجموعة إعادة تدفق الدم عقب نقص التروية مع تلقي حقن الكارنيتين، تم استحداث التواء في الحبل المنوي للخصية متبوعاً مباشرة بتلقي حقن الكارنيتين داخل الغشاء البريتوني بجرعة مقدارها ٥٠٠مجم\كجم مباشرة بعد الإلتواء. في نهاية التجربة تم الحصول علي عينات الخصى اليسرى وذلك لإستخدامها فى التحاليل البيوكيميائية والنسيجية في جميع المجموعات.

النتائج: تسبب نقص الدم في الخصيتين في اضطراب في دلالات حالة التأكسد وفقدان الشكل الطبيعي للخلايا المولدة للحيوانات المنوية مع زيادة عدد الخلايا الايجابية ضد كاسبيس -٣ بالإضافة إلى وجود تكسير في الحمض النووي للخلايا ونقص في الهالات المحيطة لأنوية الحيوانات المنوية. ومع ذلك فإن الحقن بالكارنيتين داخل الغشاء البريتوني مباشرة بعد الإلتواء قد أدي إلي تحسن التأثيرات السلبية التى سببها نقص الدم في الخصيتين.

الإستنتاج: نستنتج أن العلاج بالكارنيتين قد أدي الي تقليل الاصابة بإعادة تدفق الدم عقب نقص التروية وذلك بعد استحداث التواء فى الحبل المنوي للخصية.