Toxicological Effects Of X-Irradiation Exposure To Male Rats And Prophylactic Role Of L-Carnitine

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ABSTRACT

Earlier studies have demonstrated that X-irradiation was tissue-damaging, mutagenic and carcinogenic agent. The aim of the present work was to evaluate the antioxidant effects of L-carnitine (LC) against X-irradiation-induced oxidative damage to blood, liver and testes of rats after total body exposure. To achieve the ultimate goal of this study, forty adult male rats were divided into four equal groups include: control group, group received X-irradiation (45 KV, 100 MA for 0.8 sec. at 120 cm distance) twice weekly for nine weeks, group orally administered with LC (500 mg/ kg b.w.) twice weekly for nine weeks, and group received orally LC (500 mg/ kg b.w.) twice weekly for nine weeks and 1 h after each dose; rats were irradiated with X-rays (45 KV, 100 MA for 0.8 sec. at 120 cm distance). Animals were sacrificed 24 h after the last irradiation. Results revealed that X-irradiation caused significant decrease in RBCs count, Hb content, PCV% and the activity of SOD, CAT, G6PD and GSH, TA contents, whereas, it caused significant increase in MDA and H_2O_2 levels indicating a stress for blood, liver and testes. X-irradiation also caused significant decrease in epididymal sperm cell count, live sperm percentage, and motility percent in addition to a significant elevation in the total sperm abnormalities. Administration of LC prior to X-irradiation resulted in significant improvement in all tested parameters towards the normal values of the controls. It could be concluded that LC exhibited a protective action against the toxic effects of X-irradiation and it had the ability to scavenge free radicals resulted from it.

INTRODUCTION

X-irradiation is a tissue-damaging agent with major mutagenic and carcinogenic effects; it is widely used as a diagnostic and therapeutic tool beside its uses in industrial purposes *(1)*. Radiotherapy is known to be one of the most common and important methods for cancer treatment *(2)*. The biological effects of ionizing radiation (X-rays, γ-rays) resulted from energy deposition in irradiated cells and subsequent acute generation of short-lived free radicals *(3)*, through the decomposition of cellular water including superoxide radical (O2 • −), hydrogen peroxide (H2O2) and hydroxyl radical (OH•) *(4)*, which can cause damage in most major cellular macromolecules: DNA, proteins and lipids *(5)*. These effects take place in tumor as well as normal cells when exposed to radiation *(6)*.

Oxidative stress, associated with ROS is believed to be involved in the pathophysiological role in many diseases including irradiation-induced tissue injury *(7)*. However, organisms have protective systems against free radical reactions, for example, endogenous antioxidants and antioxidative enzymes, all aerobic organisms are susceptible to oxidative stress simply because semireduced oxygen species are produced by mitochondria during respiration *(8)*. Free radicals direct measurement *in vivo* is difficult, thus, ongoing oxidative damage has generally been analyzed indirectly by measurement of secondary products of oxidation *(9)*.

Extensive research work has been carried out on chemical protection against radiation, like Cysteine, Cysteamine, 2-MPG, WR-2721 and AET but application of these compounds is limited in radiotherapy owing to their high

toxicity at optimum dose level *(10)*. A world wide hunt is on to find suitable detoxifying agent against radiation.

Carnitine, is a quaternary amine (β-hydroxy-γ-N-trimethyammonium butyric acid-M.W. 161.2), and is known as a vitamin like and amino acid like substance. Synthetic carnitine occurs as both D & L isomers; however, only LC is physiologically active *(11)*. L-carnitine is an essential cofactor in mitochondrial respiration playing an important role in the transfer of long-chain fatty acids from cytosol to mitochondria for subsequent β oxidation *(12)*. L-carnitine has been found to have an antiperoxidative effect on several tissues *(13,14)*. L-carnitine has a protective effect on lipid peroxidation by reducing the formation of hydrogen peroxide *(15)*, it could also improve antioxidant status, inhibit free radical production and showed free radical scavenging activity as well *(16)*, in addition to protecting cells from ROS and decreases damage to the cell membrane *(12; 13,17)*. There is also good evidence that carnitine plays an important role in the maturation and maintenance of spermatozoa within the epididymal duct *(18)*.

This study has been initiated to investigate on mechanism-based the possible protective effects of LC against X-irradiation-induced oxidative damage in blood, liver and testes.

MATERIAL AND METHODS

Chemicals

L-carnitine®:(a product of Sigma-Tau Pharmaceuticals, Pomezia, Roma, Italy). All chemicals used in this study were of analytical grade.

Kits

Malondialdehyde, hydrogen peroxide, reduced glutathione, superoxide dismutase, catalase, total antioxidant kits were purchased from *Biodiagnostic* ® Company.

Glucose-6-phosphate dehydrogenase kit was obtained from *Biorex diagnostic*® Company.

Irradiation

Whole-body X-irradiation was performed at the National Centre for radiation, Kafr El-sheikh, Egypt. Animals were irradiated with a dose of 45 KV, 100 MA for 0.8 S at a distance of 120 cm twice weekly for nine weeks.

Experimental animals

Forty adult albino male rats (150-200 gm b. w.) obtained from Animal House Colony, Faculty of Vet. Med. Zagazig University. The animals were provided with standard diet and water *ad libtum*, kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained in well ventilated animal house with 12 hours light and dark cycle.

Experimental design

A total of 40 rats were randomly divided into four groups of 10 animals each. The first group served as control. Rats in the second group were received X-irradiation with a dose of 45 KV, 100 MA for 0.8 S at 120 cm distance, twice weekly for nine weeks. Animals in the third group were orally administered with LC (500 mg / kg b.w.) twice weekly for nine weeks *(19)*. The fourth group received oral dose of LC (500 mg / kg b.w.) twice weekly for nine weeks and 1 h after each administration; animals received a dose of whole-body X-irradiation (45 KV, 100 MA for 0.8 S at 120 cm distance).

Twenty-four hrs after the last irradiation, animals were anaesthetized with ether. Two blood samples were collected from each animal via the retro-orbital venous plexus after fasting for 12 h. The first one was collected in heparinized vacutainer tubes for hematological studies according to standard techniques described by ***Feldman et al., (20)*** and determination of H2O2 *(21)*, GSH *(22)*, SOD *(23)*, TA *(24)*, CAT *(21)* and G6PD *(25)*. The other sample was collected in plain centrifuge tube, left to clot then centrifuged at 5,000 rpm for 10 min to separate serum in order to determination of MDA *(26)*.

Testes and liver were surgically removed from the anesthetized rats, washed in ice cold 1.15% KCl and were then taken for

homogenization in a Teflon glass homogenizer while maintaining the unit in ice bath. The homogenate was centrifuged at 10,000 rpm for 20 min; the supernatant was collected and used for determination of GSH *(22),* SOD *(23)* and MDA *(26),* in addition to determination of CAT *(21)*, H_2O_2 *(21)* and TA *(24),* in the liver tissue.

The epididymal contents were collected to investigate the sperm cell concentration, live and dead sperms, sperm motility and the incidence of abnormal sperms *(27).*

Statistical analysis

The obtained data were statistically analyzed using the GLM procedure of SAS computer program, according to the method described by **SAS,** *(28).*

RESULTS

Biochemical profile

X-irradiation, induced oxidative stress in the blood represented by a significant decrease in the activity of SOD, CAT, G6PD and GSH, TA contents (47, 46, 36, 34 and 28%) respectively, and a significant increase in MDA and H_2O_2 levels (98 and 44%) respectively, compared to control group. Administration of LC prior to X-irradiation resulted in amelioration of the adverse effects on the activity of SOD, CAT, G6PD and GSH, TA contents and MDA and H_2O_2 levels, by 87, 83, 83, 71, 71, 90 and 86% respectively, as compared to irradiated group, however, LC-induced non-significant change (Table-1).

The activity of SOD, CAT and GSH, TA contents were significantly decreased in liver tissues of X-irradiated group (54, 42, 46 and 29%) respectively, in addition to significant increase in MDA and H_2O_2 levels (123 and 69%) respectively, compared to control group. Pre-administration of LC resulted in correction of the adverse effect of X-irradiation on the activity of SOD, CAT and GSH, TA contents, and MDA and H_2O_2 levels, by 93, 81, 87, 72, 92 and 88% respectively, as compared to irradiated group, however, LC-induced non-significant change (Table-2).

Measuring the activity of SOD and GSH content of testes tissue in X-irradiated group showed significant decrease (49 and 45%), beside significant increase in MDA level (105%), compared to control group. Administration of LC 1h before X-irradiation exposure provide amelioration of the adverse effect of X-irradiation on the activity of SOD and GSH content and MDA levels, by 84, 80, and 90% respectively, as compared to irradiated group, however, LC-induced non-significant change (Table-3).

Hematological picture

X-irradiation, induced significant decrease in the RBCs count, Hb content and PCV% (27, 24 and 20%) respectively, compared to control group. Administration of LC prior to X-irradiation resulted in amelioration of these adverse effects by 81, 79 and 80% respectively, as compared to irradiated group, however, LC-induced non-significant change (Table-4).

Semen pictures

X-irradiation-induced significant decrease in epididymal sperm cell count, live sperm percentage, and motility percent (36, 29, and 34%) respectively in addition to a significant elevation in the number of abnormal shape of sperm (173%) when compared to control group. Administration of LC prior to irradiation resulted in amelioration of the adverse effect of X-irradiation on the epididymal sperm cell count, live sperm percentage, motility percent and number of abnormal shape of sperm by 78, 77, 76 and 90% respectively, as compared to irradiated group, however, LC-induced non-significant change (Table-5); (Figs. 1 , 2).

Table 1. Effect of X-irradiation (45 KV, 100 MA for 0.8 S at a distance of 120 cm) twice weekly for 9 weeks; L-carnitine (500 mg / kg b.w.) orally twice weekly for 9 weeks and their combination on the levels of malondialdehyde (MDA), hydrogen peroxide (H2O2) and the activities of superoxide dismutase (SOD), catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD) and the contents of reduced glutathione (GSH), total antioxidant (TA) in rat blood. (n=10). Data are represented as (mean ± SD).

Treatment	MDA (mmol/L)	H_2O_2 (mM/L)	SOD (U/ml)	CAT (U/ml)	G6PD (mU/g Hb)	GSH (mg/100ml)	TA (µmol/L)
Untreated-control	220.20 ± 6.45 (100%)	81.7±3.2 (100%)	0.0064 ± 0.0002 (100%)	0.52±0.04 (100%)	7648±218 (100%)	170.6 ± 4.21 (100%)	2.38±0.31 (100%)
Irradiation	436.13 ±7.42 [a] (198%)	118.0±.5.1[a] (144%)	0.0034 ± 0.0001[a] (53%)	0.28±0.01[a] (54%)	4885±136[a] (64%)	112.4 ± 4.54[a] (66%)	1.72±0.11[a] (72%)
LC	206.71 ± 5.2[b] (94%)	75.11±3.7[b] (92%)	0.0066 ± 0.0001[b] (103%)	0.56±0.03[b] (108%)	7712±254[b] (101%)	180.7 ± 5.11[b] (106%)	2.45±0.72[b] (103%)
LC + irradiation	239.00 ± 8.3[b] (110%)	86.45±3.3[b] (106%)	0.0060 ± 0.0001[b] (94%)	0.48±0.02[b] (92%)	7200±198[b] (94%)	153.5 ± 4.42[b] (90%)	2.19±0.21[b] (92%)

Values in the same column with different superscripts vary significantly at differ $p < 0.05$.

Table 2. Effect of X-irradiation (45 KV, 100 MA for 0.8 S at a distance of 120 cm) twice weekly for 9 weeks; L-carnitine (500 mg / kg b.w.) orally twice weekly for 9 weeks and their combination on the levels of malondialdehyde (MDA), hydrogen peroxide (H2O2) and the activities of superoxide dismutase (SOD), catalase (CAT) and the contents of reduced glutathione (GSH), total antioxidant (TA) in rat liver tissue. (n=10). Data are represented as (mean ± SD).

Treatment	MDA (mmol/g)	H_2O_2 mM/g	SOD U/mg	CAT U/g	GSH (mg/g)	Total antioxidant (µmol/g)
control	17.41 ± 1.3 (100%)	40.51±2.1 (100%)	0.0068 ± 0.0001 (100%)	0.50±0.03 (100%)	87.4 ± 3.11 (100%)	2.30±0.21 (100%)
Irradiation	38.83 ± 2.3[a] (223%)	68.53±.3.1[a] (169%)	0.0031 ± 0.0001[a] (46%)	0.29±0.01[a] (58%)	47.0 ± 2.01[a] (54%)	1.63±0.13[a] (71%)
LC	16.34 ± 1.0[b] (94%)	38.24±2.3[b] (94%)	0.0070 ± 0.0001[b] (103%)	0.53±0.03[b] (106%)	89.3 ± 4.09[b] (102%)	2.35±0.22[b] (102%)
LC + irradiation	19.10 ± 1.2[b] (110%)	43.82±3.0[b] (108%)	0.0065 ± 0.0001[b] (96%)	0.46±0.02[b] (92%)	82.5 ± 3.09[b] (94%)	2.11±0.15[b] (92%)

Values in the same column with different superscripts vary significantly at differ $p < 0.05$.

Table 3. Effect of X-irradiation (45 KV, 100 MA for 0.8 S at a distance of 120 cm) twice weekly for 9 weeks; L-carnitine (500 mg / kg b.w.) orally twice weekly for 9 weeks and their combination on the levels of malondialdehyde and the activity of superoxide dismutase and the content of reduced glutathione in rat testes tissue (n=10). Data are represented as (mean ± SD).

Treatment	MDA (mmol/100 g)	SOD (U/mg)	GSH (mg/g)
Untreated-control	190.41 ± 4.12 (100%)	0.0037 ± 0.0001 (100%)	123.9 ± 3.51 (100%)
Irradiation	389.62 ±6.22 [a] (205%)	0.0019 ± 0.0001[a] (51%)	68.4 ± 2.31[a] (55%)
LC	189.15 ± 4.35[b] (99%)	0.0040 ± 0.0001[b] (108%)	125.3 ± 4.11[b] (101%)
LC + irradiation	210.72 ± 6.13[b] (111%)	0.0034 ± 0.0001[b] (92%)	112.4 ± 4.14[b] (91%)

Values in the same column with different superscripts vary significantly atdiffer $p < 0.05$.

Table 4. Effect of X-irradiation (45 KV, 100 MA for 0.8 S at a distance of 120 cm) twice weekly for 9 weeks; L-carnitine (500 mg / kg b.w.) orally twice weekly for 9 weeks and their combination on RBCs count, Hb level and PCV % in rat blood (n=10). Data are represented as (mean ± SD).

Treatment	RBCs (10^9)	Hb (mg/dL)	PCV%
Untreated-control	5.80±0.63 (100%)	16.27 ± 0.51 (100%)	45 ± 2.71 (100%)
Irradiation	4.21 ±0.34 [a] (73%)	12.34 ± 0.41[a] (76%)	36 ± 2.54[a] (80%)
LC	5.95 ± 4.35[b] (103%)	17.2 1± 0.62[b] (106%)	46 ± 3.11[b] (102%)
LC + irradiation	5.52 ± 6.13[b] (95%)	15.40 ± 0.42[b] (95%)	43 ± 3.56[b] (96%)

Values in the same column with different superscripts vary significantly atdiffer $p < 0.05$.

Table 5. Effect of X-irradiation (45 KV, 100 MA for 0.8 S at a distance of 120 cm) twice weekly for 9 weeks; L-carnitine (500 mg / kg b.w.) orally twice weekly for 9 weeks and their combination on semen picture of male rats (n=10). Data are represented as (mean ± SD).

Parameter	Sperm cell conc. X 10^6	Live sperms %	Motility %	Abnormality %
Untreated-control	61.42±2.3	93.51±2.11	90.72± 2.71	4.43 ± 0.51
Irradiation	39.53±1.60^a	66.73±1.23^a	59.62±1.21^a	12.11±0.83^a
LC	64.21±2.54^b	95.23±2.35^b	93.40±2.33^b	3.11±0.35^b
LC + irradiation	56.5±2.36^b	87.31±2.32^b	83.13±2.72^b	5.21±0.61^b

Values in the same column with different superscripts vary significantly atdiffer $p < 0.0$

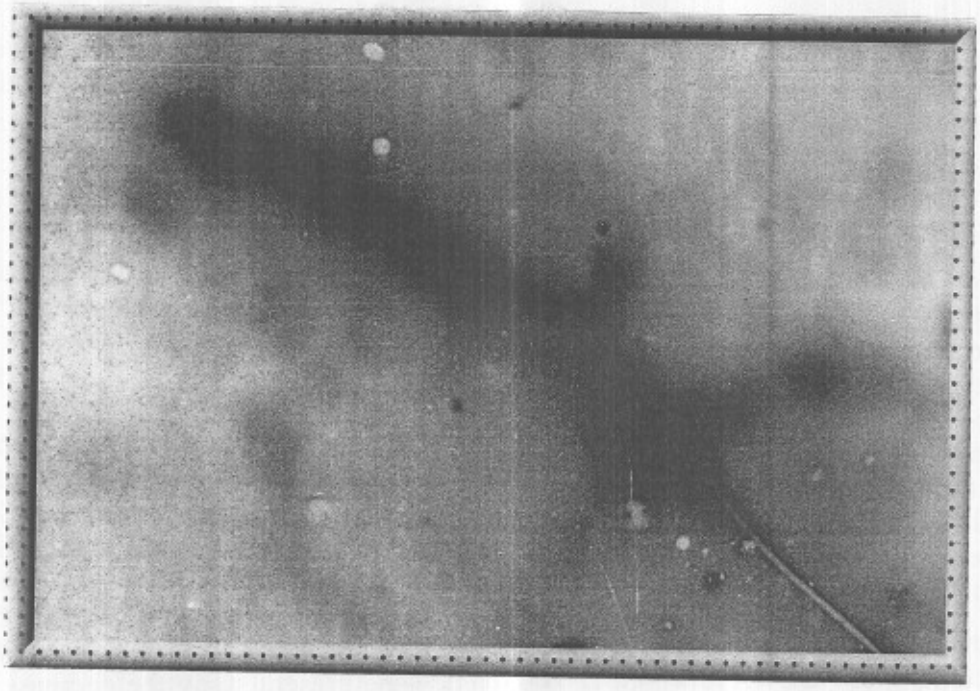

Fig. 1. A photograph of rat spermatozoa from albino rat received X-irradiation at dose of 45 KV, 100 MA for 0.8 S at a distance of 120 cm twice weekly for 9 weeks, showing abnormalities in the form of absence of head.

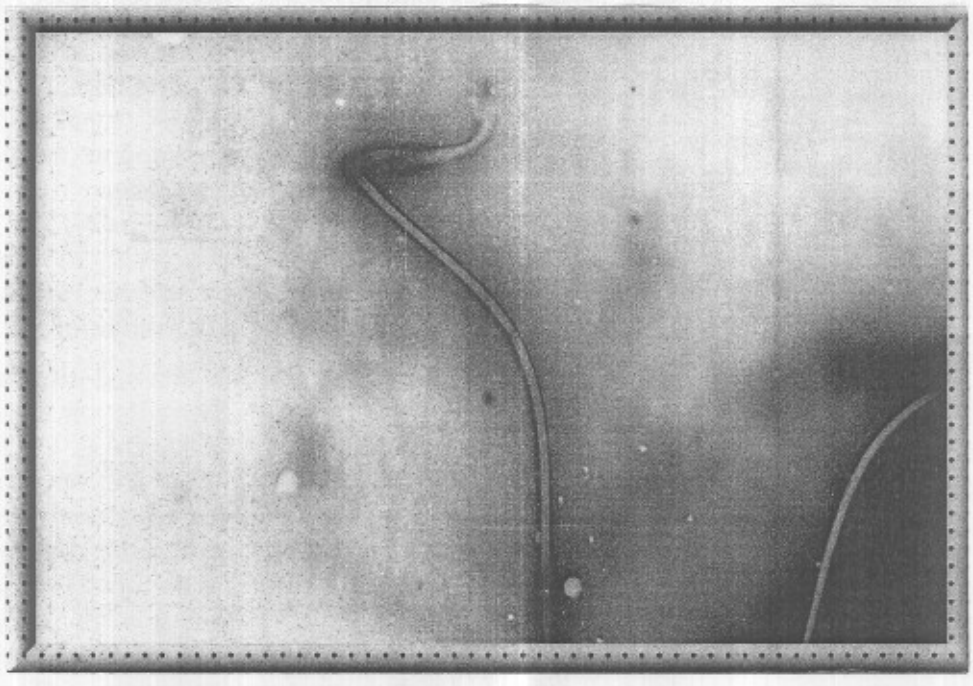

Fig. 2. A photograph of rat spermatozoa from albino rat received X-irradiation at dose of 45 KV, 100 MA for 0.8 S at a distance of 120 cm twice weekly for 9 weeks, showing abnormalities in the form of Brocken neck

DISCUSSION

The present study was conducted to elucidate the possible protective role of LC on the antioxidant system; hematological parameters and semen quality of rats exposed to X-rays.

Blood is considered as early models for conducting studies on oxidative stress enzymes as these are highly prone to oxidative reactions because of relatively high oxygen tensions, the presence of hemoglobin, and a plasma membrane rich in polyunsaturated lipids *(29)*. One of the most important fields of radiobiology is the digestive system including liver *(1 , 30)*. Male germ cells are highly susceptible to ROS attack produced by any agent or xenobiotic *(31)*.

In the current study, significant decreases in the activity of SOD and GSH contents accompanied with significant increase in MDA level were demonstrated in the blood; liver and testes post X-irradiation when compared to control. The activity of CAT and TA contents were significantly decreased in the blood and liver of X-irradiated group in addition to significant increase in H_2O_2 levels compared to control group. The activity of G6PD was significantly decreased in the blood post irradiation compared to control group.

Evidence at hands indicates that, exposure to ionizing radiation causes radiolysis of water in tissues leading to generation of ROS which are known to affect the antioxidant defense systems *(4)*. The most important antioxidant defense system include, superoxide dismutase which is the first line of defense against oxygen derived free radicals and functions by dismutating two superoxides (O2 -) ions into H2O2 *(32)*; catalase enzyme which degrades hydrogen peroxide into water *(33)*; glucose-6 phosphate dehydrogenase which recycled NADPH via the pentose phosphate pathway *(34)* and glutathione which plays a crucial role in the detoxification process *(35)*. In keeping with this line, it is worthy to mention that the reduction of the antioxidant parameters in the present study could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by X-irradiation *(32, 36)*.

One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides (LPO) in tissues *(37)*. In this study, MDA is used as a marker of the rate of LPO which is accepted as tissue chain reaction *(38)*. The elevated levels of lipid peroxidation and hydrogen peroxide in the present investigation are indicative of the oxidative damage caused by X-irradiation *(39)*, as various studies have shown that free radicals induced by ionizing radiations have a damaging effect on lipids *(40)*, since the ionizing radiation abstracts hydrogen from a molecule to form a radical *(41)*. Moreover, in the present study the decrease in the activity of CAT, (which degrades hydrogen peroxide) in irradiated group suggests an increase in this oxygen species in addition to the production of H2O2 during oxidative stress *(31, 42)*.

The decrease in the activity of SOD, CAT, G6PD and the contents of GSH, TA and the increase in MDA and H_2O_2 levels post-irradiation as recorded in the present study are in agreement with those recorded by several investigators *(5, 32, 42-45)*. They recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides after whole body irradiation.

Our results showed that administration of LC prior to X-irradiation causes a significant inhibition of SOD, CAT, G6PD and GSH, TA depletion. Thus, LC treatment effectively protected against oxidative damage *(46)*, as LC may protect against the damage produced from X-irradiation by the up-regulation of antioxidant enzymes; modulation of cellular antioxidants levels and by scavenging free radicals generated by ionizing radiation *(47)* due to LC is considered as a direct antioxidant *(48)*. LC can also act as a chelator by decreasing the concentration of cytosolic iron, which plays a

very important role in free radical chemistry *(15)* in addition to LC, has been able to neutralize the accumulation of free radicals to great extent *(48, 49).*

The present results illustrate that LC pre-treatment significantly lowered radiation-induced lipid peroxidation in terms of malondialdehyde and hydrogen peroxide. The inhibition of lipid peroxidation in biomembranes can be caused by antioxidants. ***Yasui et al., (50)*** Reported that LC has antioxidant activity towards oxidative stress via an inhibition of the increase in lipid hydroperoxidation. On the other hand, it has been *(51)* earlier documented that the protective effect of LC on NADPH-induced lipid peroxidation might be mediated through antioxidant mechanisms.

Previous studies showed that LC supplementation enhanced the dismutation of O2 • – by increasing the level of SOD activities, protect DNA from cleavage induced by H2O2 UV-photolysis *(52);* and increase the activity of catalase substantiating *(38),* in addition to restoring the glutathione and MDA levels in rats exposed to irradiation. *(35).* The higher CAT activity after the treatment with LC might indicate an improvement in the removal of hydrogen peroxides before they produce new ROS. In addition, the reduced production of lipid peroxides together with the increase in GSH content observed after the treatment with LC might contribute to the enhancement in the total antioxidant status (TA) found in these rats. Therefore, all these effects of LC would lead to an amelioration of oxidative status of X-irradiation. Taken together, all these results indicate that the treatment with LC leads to an enhancement in tissue antioxidant defense and a reduction in the systemic oxidative process *(53).*

It would be worthwhile studying the effect of LC supplements to improve antioxidant enzyme activity in radiation-treated cancer patients, in the hope of reducing radiation-induced toxicity *(47).*

The present study clearly revealed that X-irradiation induced significant decrease in the RBCs count, Hb content and PCV% compared to control group. Administration of LC prior to X-irradiation revealed abolishing of these adverse effects as compared to irradiated group.

Previous studies recorded that magnetic fields (MF) decreased splenocyte viability, blood cell count, as well as mitogen-induced lymphocyte proliferation, and LC could ameliorate these adverse effects of MF *(54).* The most plausible explanation for this pattern is that LC plays an important role in erythrocyte membrane phospholipids turnover *(55),* in addition to LC has been shown to reduce hemolysis and improve *in vitro* survival in red blood cells *(13, 56).* L-carnitine enhances NADPH recycling by G6PD via the pentose phosphate pathway, which is particularly important in red blood cells and protecting erythrocytes and low-density lipoproteins against peroxidation induced by ROS *(49).*

The current study clearly indicated that X-irradiation-induced significant decrease in epididymal sperm cell count, live sperm percentage, and motility percent in addition to a significant elevation in the total sperm abnormalities when compared to control group. Administration of LC prior to irradiation resulted in amelioration of these adverse effects as compared to irradiated group.

These results can be cleared by the fact that, membrane of mammalian spermatozoa is rich in high unsaturated fatty acid and is sensitive to oxygen–induced damage mediated by lipid peroxidation in addition to the excessive generations of ROS increase the number of abnormal spermatozoa *(35).* Exposure to fractionated doses of magnetic field caused a significant decrease in sperm count, motility, daily sperm production, and LDH-X activity. Moreover, a marked testicular histopathological change was observed after exposure to fractionated doses of magnetic field, pretreatment with LC caused a significant

recovery of testes damage induced by magnetic field. *(57),* due to the fact that spermatozoa and epididymal epithelial cells actively absorb free carnitine which is stored as acetylcarnitine in the distal epididymis *(58),* also maturing spermatozoa require a carnitine-dependent energy source in order to traverse and survive in the distal epididymis *(18).* L-carnitine would stimulate testosterone production in addition to spermiogenesis was arrested in some seminiferous tubules as a result of carnitine deficiency *(59).*

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الملخص العربي

التأثير السمي لتعرض ذكور الجرذان لأشعة اكس و الدور الوقائي لمادة إل- كارنيتين

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أوضحت الدراسات السابقة أن أشعة اكس مادة محطمة للأنسجة و محدثة للطفرات و مسرطنة. أجريت هذه الدراسة لتقييم دور إل-كارنيتين في حماية الفئران من الآثار السمية لأشعة اكس علي الدم و الكبد و الخصية. قسم عدد ٤٠ فار ابيض إلى أربعة مجاميع متساوية، مجموعة ضابطة سالبة و وأخرى ضابطة موجبة تعرضت لأشعة اكس(٤٥ كيلو فولت- ١٠٠ مللي أمبير- لمدة ٠،٨ ثانية علي مسافة ١٢٠ سم) مرتين أسبوعيا لمدة تسع أسابيع. المجموعة الثالثة تجرعت عن طريق الفم إل-كارنيتين (٥٠٠ ملجم/كجم من وزن الجسم) مرتين أسبوعيا لمدة تسع أسابيع. المجموعة الرابعة تجرعت عن طريق الفم إل-كارنيتين (٥٠٠ ملجم/كجم من وزن الجسم) مرتين أسبوعيا لمدة تسع أسابيع و بعد ساعة من كل جرعة تعرضت لأشعة اكس (٤٥ كيلو فولت- ١٠٠ مللي أمبير- لمدة ٠،٨ ثانية علي مسافة ١٢٠ سم. تم ذبح جميع الحيوانات بعد مرور ٢٤ ساعة من أخر تعرض إشعاعي و أوضحت النتائج أن أشعة اكس تسبب انخفاض ملحوظ في عدد خلايا الدم الحمراء و كمية الهيموجلوبين و حجم الخلايا المتراصة بالأضافة لإحداث انخفاض ملحوظ في نشاط السوبر اوكسيد ديسميوتاز و الكاتاليز و جلوكوز٦فوسفات دي هيدروجيناز و انخفاض ملحوظ في كمية الجلوتاثيون وإجمالي المواد المؤكسدة كذلك يسبب ارتفاع ملحوظ في الليبد بيروكسيداز و الهيدروجين بير أوكسيد. أشعة اكس أيضا تسبب نقص في عدد الحيوانات المنوية و نسبة الحيوانات المنوية الحية و نسبة الحركة فيها مع إحداث زيادة في نسبة التشوهات بها. و قد وجد أن تجريع إل-كارنيتين مع أشعة اكس أدى إلى تحسن جميع القياسات السابقة وعلى ذلك ، فان إل-كارنيتين يمكن استخدامه كمادة واقية من الآثار السمية لأشعة اكس وذلك بالاعتماد على نشاطه كمضاد للأكسدة.