# Radioprotective activity of L- Carnitine and α -Lipoic acid against whole body γ- irradiation in rats

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**Abstract:** The present study was designed to investigate the radioprotective efficacy of naturally occurring antioxidants, L - carnitine (LC) and α -Lipoic acid (LA) on radiation-induced bone marrow and liver damages in a rat model. The cellular changes were estimated by evaluation the expression of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx), genes using RT-PCR and DNA damage in bone marrow and liver cells. The histopathological and ultra structural changes were also determined. To evaluate the effects of the above antioxidants, adult rats were treated with LC (300 mg/kg b wt) and LA (150 mg/kg b wt) after exposure to whole-body γ-rays (6 Gy) for 10 days, or treated with LC & LA for 7 consecutive days and one hour after the last administration, animals irradiated a single dose of whole-body γ-rays (6 Gy) and received again LC & LA in same dose for 10 days. The obtained data revealed that γ –irradiation significantly decreases the expression of SOD and GPx genes and increases DNA fragmentation in liver cells as well as the incident of micronuclei in bone marrow cells. In addition, different histological and ultra structural alterations in the liver of irradiated animals were recorded. These alterations were varied from hemorrhage, congestion in blood vessels, pyknosis and necrosis as well as complete degenerated area in the liver electron micrographs recorded swollen mitochondria, fragmented endoplasmic reticulum, distorted nuclei and cell membrane. Treatment with LC & LA post-exposure to radiation attenuated most of these changes. Whereas pre- and post- treatment with LC & LA to γ-irradiation normalized the expression of the antioxidant genes enzymes, decreased the DNA fragmentation and micronuclei formation with a normal restoration of histopathologial and ultra structure liver architecture. Thus, our results suggested that pre-treatment with LC & LA offers protection against γ-irradiation induced cellular damage.
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## 1. Introduction

Ionizing radiation (IR) is an important environmental risk factor for various cancers and also a major therapeutic agent for cancer treatment. Radiation toxicity occurs either by direct attack on the genetic material and/or by generating reactive oxygen species (ROS) by radiolysis of water. It also attenuates the endogenous antioxidant enzymes which are considered as first line defense mechanism to maintain redox balance and normal biochemical processes (Parihar *et al.,* 2007). Consequently, the organs become more susceptible to the deleterious effects of ROS that attack various cellular components including DNA, RNA, proteins and membrane lipids, thereby leading to significant cellular damage (Tominaga *et al.,* 2004).

The potential application of radioprotective chemicals in the event of planned exposures or radiation accidents/incidents has been investigated from the beginning of the nuclear era (Weiss and Simic, 1988). It has also been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue. They include sulfhydryl compounds, antioxidants, plant extracts, immunomodulators, and other agents (Nair *et al.,* 2001).

α-Lipoic acid (LA), a naturally occurring sulphydryl compound found in virtually all plants and animal species that functions as a coenzyme in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase mitochondrial reactions, leading to the production of cellular energy. In human body LA

is rapidly reduced to dihydrolipoic acid (DHLA) after intake into the cellular system (Packer *et al.,* 1995). Both, LA and DHLA are effective against conditions in which oxidative stress has a role (Packer *et al.,* 1999). LA, which is a universal antioxidant functions both in aqueous and membrane phases (Kagan *et al.,* 1992). LA has been shown to quench free radicals, prevent singlet oxygen-induced DNA damage, exhibit chelating activity, reduce lipid peroxidation and protein damage through the redox regeneration of other antioxidants such as vitamins C and E, and by increasing intracellular glutathione (Moini *et al.,* 2002; Biewenga *et al.,* 1997). It shows beneficial effects in oxidative stress conditions because of its synergistic action with other antioxidants (Suzuki *et al.,* 1993).

L-carnitine (LC) is a natural compound known as vitamin $B_T$ (γ-trimethyl amino butyrate) widely distributed in the body. It is mainly required in the transport of activated fatty acids from the cytosol into the mitochondrial matrix for subsequent β-oxidation, and in the transfer of the products of peroxisomal β-oxidation to the mitochondria for utilization in energy generation process (Ramsay *et al.,* 2001; Santoro *et al.,* 2005). It has been shown that LC has a scavenger effect on ROS and a stabilizing effect on damaged cell membranes (Fritz and Arrigoni-Martelli, 1993). Carnitine can also act as a chelator by decreasing the concentration of cytosolic iron that plays a very important role in free radical chemistry (Reznick *et al.,* 1992). LC has a capacity to enhance non-enzymatic antioxidants, such as vitamin E (Arockia Rani and Panneerselvam, 2001).

Several studies demonstrated that both LA and LC protected intact tissues against injurious effects of cancer treatments such as radiotherapy or chemotherapy, without an inhibitory effect against their therapeutic effects (Chang *et al.,* 2002; Pisano *et al.,* 2003; Selvakumar *et al.,* 2006; Prahalathan *et al.,* 2006). Previous study has shown that the combined effect of both these drugs improves mitochondrial enzyme activities (Savitha and Panneerselvam, 2006). Muthuswamy *et al.* (2006) found that co-administration of L-carnitine and DL-α-lipoic acid mitigates ROS-induced oxidative damage to macromolecules such as lipids, proteins and DNA in brain of aged rat.

The aim of the present study was to investigate the radioprotective role of L-carnitine and α-Lipoic acid against γ-irradiation-induced liver and bone marrow damage in rat model.

## 2. Materials and methods

### *2.1. Source of chemicals*

L-Carnitine and R-α-lipoic acid were a kind gift from EVA Pharma for pharmaceuticals and medical appliances, Egypt. All other chemicals used were of the highest analytical grade.

### *2.2. Irradiation*

Whole-body Gamma - irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt, using a Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.72 Gy/ min.

### *2.3. Animals*

Female albino rats of Wistar strain weighing 150-200 g was obtained from the animals house Colony, National Research Centre, Dokki, Giza, Egypt. The animals were housed in large spacious cages and given food and water *ad libitum*. The animals were well ventilated with a 12-h light/dark cycle, throughout the experimental period.

### *2.4. Experimental design*

A total of (50) rat was randomly divided into five groups (10 animals per group).

*Group I*: control group, animals were received normal saline (0.5 ml/150 g b wt.) for 10 consecutive days.

*Group II*: LC&LA group, animals were received LC & LA for 10 consecutive days.

*Group III*: IR group, rats received the same dose of normal saline as the first group and on day seven, animals were irradiated with a single dose of whole-body γ-rays (6 Gy) and left for 10 days.

*Group IV*: IR + (LC&LA) group, animals were irradiated with a single dose of whole-body γ-rays then after one hour they administered LC & LA for 10 days.

*Group V*: (LC&LA) + IR group, animals received LC & LA for 7 consecutive days, then after one hour of the last administration, animals irradiated a single dose of whole-body γ-rays (6 Gy) and received again LC & LA in same dose for 10 days.

L-Carnitine (300 mg/ (kg b wt) day) (Kumaran *et al.,* 2005) was dissolved in 0.89% physiological saline and R -α- lipoic acid (150 mg/ (kg b wt) day)

(Savitha *et al.,* 2005) was dissolved in 0.5% of KOH in physiological saline and administered orally. Animals were killed by cervical decapitation and a part of liver was immediately frozen and stored at - 80°C for RNA extraction and DNA fragmentation analysis. Other part of liver was collected for the histopathological and ultra structural examination.

### *2.5. Molecular genetics assays*

#### *2.5.1. RNA isolation and Reverse transcription (RT)*

Total RNA was isolated from liver tissues using Trizol reagent (Invitrogen, Paisley, UK). RNA samples were subjected to DNase1 treatment to remove genomic DNA contamination in the presence of RNase inhibitor. The purity and integrity of the total RNA was determined by spectrophotometry and agarose gel electrophoresis (Sambrook *et al.,* 1989). The first-strand cDNA was prepared from the 5 µg of total RNA using Fermentas kits (Sigma, St. Louis, MO) as per the manufacturer's instructions. The RT-program used was: 60 min at 42$^0$C (cDNA synthesis); 5 min at 94$^0$C (denaturation). Afterwards the reaction tubes containing RT preparations were ash-cooled in an ice chamber until used for DNA amplification through polymerase chain reaction (PCR) (Brun *et al.,* 2006).

#### *2.5.2. Polymerase chain reaction (PCR)*

The first-strand cDNA from different rat samples was used as the template for amplification by the PCR with the following pairs of specific primers (from5′to3′):

Cu-ZnSOD forward: GCAGAAGGCAAGCGGTGAAC,
Cu-ZnSOD reverse: TAGCAGGACAGCAGATGAGT,
GPx forward: CTCTCCGCGGTGGCACAGT,
GPx reverse: CCACCACCGGGTCGGACATAC,

that are taken from the literature (Limaye *et al.,* 2003). β-actin, a house-keeping gene, was used for normalizing mRNA levels of the target genes. The PCR cycling parameters were one cycle of 94 $^0$C for 5min, 35 cycles of 94 $^0$C for 30 s, 60 $^0$C (Cu-Zn SOD and GPx gene) for 30 s, 70 $^0$C for 40 s, and 72 $^0$C for 5 min. The PCR products were electrophoresed onto ethidium bromide stained a 2.0% agarose gels. The ethidium bromide-stained gel bands were scanned and the signal intensities were quantified by the computerized Gel-Pro program.

### *2.6. Quantification of fragmented DNA by diphenylamine (DPA)*

To measure hepatic DNA fragmentation by spectrophotometry, a portion of the liver was homogenized in hypotonic lysis buffer (0.2% Triton X-100, 10 mM Tris, 1 mM EDTA, pH 8.0) and centrifuged for 15 min at 10,000 rpm to separate intact chromatin in the pellet from fragmented/damaged DNA in the supernatant. Pellets were resuspended in 0.5 N perchloric acid and 5.5 N perchloric acid was added to supernatant fractions to final concentration of 0.5 N. Samples were heated at 90 $^0$C for 15 min and centrifuged at 10,000 rpm for 10 min to remove proteins. Supernatant fractions were reacted with diphenylamine (DPA) reagent [0.088 M DPA, 98% (v/v) glacial acetic acid, 1.5% (v/v) conc. $H_2SO_4$ and 0.5% of 1.6% acetaldehyde solution] and the samples were kept at room temperature for 20 h Paradones *et al.* (1993). Absorbance was measured at 600 nm using a UV-double beam spectrophotometer (Shimadzu 160A).The percentage of DNA fragmentation was taken as the ratio of DNA in the supernatant to total amount of DNA in pellet and supernatant.

### *2.7. Agarose gel electrophoresis for DNA fragmentation*

Agarose gel electrophoresis was carried out for the analysis of DNA fragmentation by the method of Yokozawa and Dong (2001). The DNA samples (1 µg) were electrophoresed on 1.4% agarose gel using TBE buffer at 40 V for 5 h. Then the gel was stained with ethidium bromide and viewed under UV transilluminator and photographed.

### *2.8. Micronucleus assay*

Immediately after the animals were sacrificed, bone marrow was collected from each animal for the micronucleus assay as described by Schmid (1975). In brief, the femurs were dissected and washed with 1ml of fetal calf serum, smeared on clean and dry slide, fixed with absolute methanol for 10 min and stained with 5% (v/v) Giemsa stain diluted in phosphate buffer. One thousand polychromatic erythrocytes (PCEs) were analyzed per animal to ascertain the frequency of micronuclei and the micronucleated cells in the bone marrow of each rat in the different treatment groups. The ratio of PCEs to normochromatic erythrocyte (NCEs) was calculated for the determination of the cytotoxicity in bone marrow.

### *2.9. Histopathological study*

After animals dissections, liver were removed immediately and fixed in 10% normal saline and neutral buffered formalin for 7 days, then the tissues were washed and dehydrated in ascending grades of ethyl alcohol cleared in benzene and impregnated in paraffin for 1.5 h in the oven at 55$^0$C. Serial section, 5 µm were cut and stained with

Haematoxylin & Eosin (H&E) as described by Bancrofet and Stevens (1977).

### *2.10. Electron Microscopic Study*

For electron microscopic examination, portion of liver were cut into small pieces, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) and post in 1% osmium tetroxide in 0.3 M cacodylate buffer. The specimens were then dehydrated and embedded in Epson 812 (Hayat, 1973). Ultrathin sections were cut and stained according to Reynold (1963). Sections were examined with Joel 100.cx a transmission electron microscope at NCRRT.

### *2.11. Statistical analysis*

Data were analyzed using One-way analysis of variance (ANOVA) using the SPSS 11 program, followed by Post-Hoc test for multiple comparisons. The data were expressed as means ± standard error of the mean. Differences were considered significant at $P<0.05$.

## 3. Results

### *3.1. Evaluation of gene expression*

Bands produced from amplifying cDNA of GPx, SOD and the house keeping gene β-actin as a control were analyzed and the results of gene expression was based on quantifying the signal intensities in each band. Results were expressed as the ratio between maximum optical density (max OD) for each band of the target amplification product and the corresponding max OD of β-actin. Expression of GPx and SOD mRNA in liver of the different groups of rats are summarized in Figs. (1 and 2). The results show that there was a significant decrease in the expression level of the examined genes in the γ-irradiated group of rats as compared to the other groups. However, treatment with LC & LA resulted in a significant increase in the expression level of GPx and SOD mRNA ($P < 0.05$) versus the control group. As well as treatment of LC & LA prior to γ-irradiation exposure significantly ($P < 0.05$) up-regulated the expression of both genes. Moreover, pre- and post- treatment with LC & LA to γ-irradiation normalized the expression levels of these genes relative to the control level.

### *3.2. Analysis of DNA Fragmentation*

Results of DNA fragmentation are shown in Table (1). γ-irradiated animals produced 33.01 % fragmented DNA in hepatic tissue, whereas control group shows a negligible 6.5 % fragmented DNA, which are associated significantly ($P < 0.05$). Supplementation with LC & LA significantly brought down the levels of DNA damage that reached 22.41 % for post – treated group, and 13.33% in pre- and post-treated group. In addition, the pattern of DNA fragmentation elicited by γ-irradiation showed the characteristic DNA ladder which is significantly restored in the groups treated with LC & LA (Fig.3).

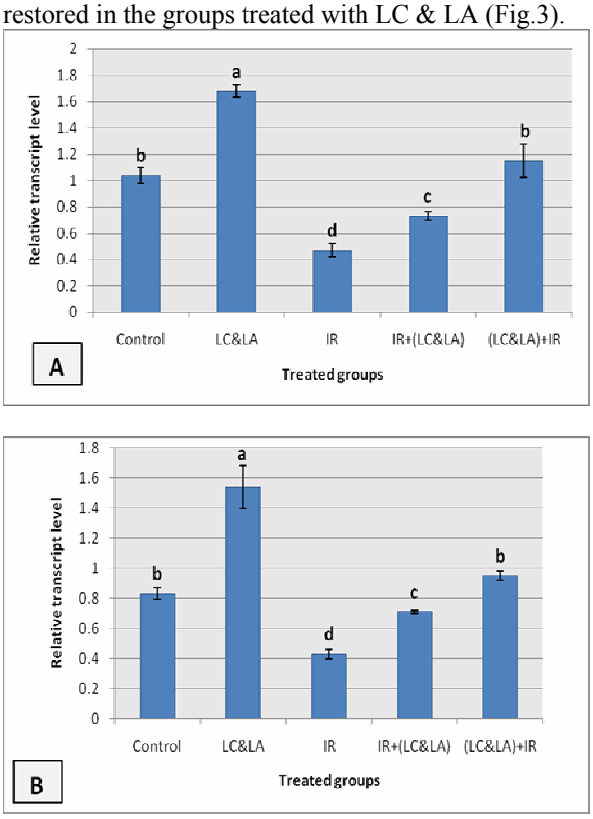


**Fig. 1.** RNA expression of SOD (A) and GPx (B), in the liver of control and treated rats. The results depicted are normalized to levels of β-actin gene. Data are mean ± S.E. of ratios of intensity for each gene divided by that for β-actin.

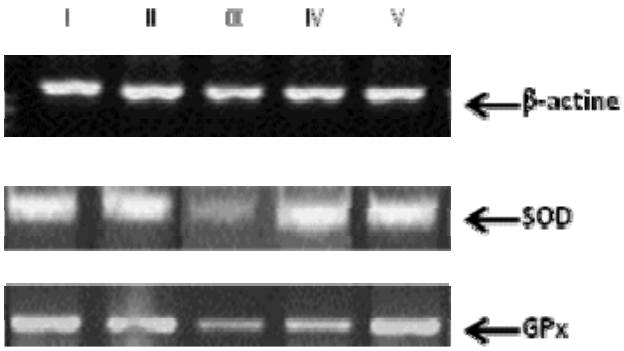


**Fig.2.** Effects of LC & LA on transcript product of hepatic antioxidant genes in γ-irradiated rats. Agarose gel electrophoresis of SOD, GPx and β-actin RT-PCR products of different groups. Group I: Control, Group II: LC&LA, Group III: IR, Group IV: IR + (LC&LA) and Group V: (LC&LA) + IR.

**Table 1.** Effects of LC & LA on the percentage of DNA fragmentation in liver of rats exposed to γ- irradiation.

| Treated Groups | Percent DNA fragmentation Mean ±S.E. | Change % |
|---|---|---|
| Control | 6.5±0.64[d] | - |
| LC&LA | 6.0±0.40[d] | 0.5 |
| IR | 33.01±3.04[a] | 26.5 |
| IR+(LC&LA) | 22.41±2.22[b] | 15.91 |
| (LC&LA)+IR | 13.33±1.30[c] | 6.83 |

Within each column, means superscript with different letter are significantly different ($P < 0.05$).

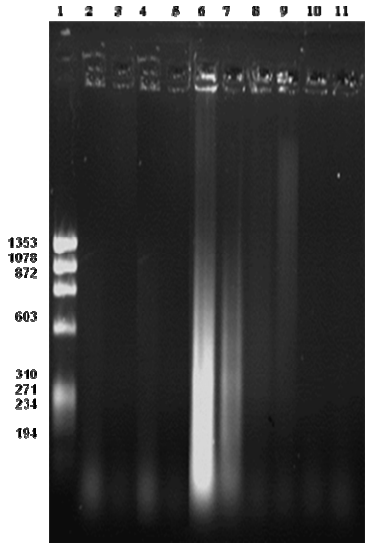


**Fig.3.** Agarose gel electrophoretic pattern of DNA isolated from liver tissue of control and treated rats. Lane 1: phi x marker; Lane 2-3: control group; Lane 4-5: LC&LA group; Lane 6-7: IR group; Lane 8-9: IR + (LC&LA) group; Lane 10-11: (LC&LA) + IR group.

### *3.3. Effects of LC and LA on γ-irradiation–induced micronuclei formation*

The cytogenetic damage induced by γ-irradiation as well as the antimutagenic effects of treatment with LC and LA were investigated in bone marrow of female rats utilizing micronucleus assay. Table (2) showed that the frequencies of micronucleated polychromatic erythrocytes (Mn-PCEs) as resulted from administration of LC & LA was within the accepted spontaneous range for control. Exposure to γ-irradiation induced a significant increase in the frequencies of Mn-PCEs (26.6 ± 1.44) as compared to the control group (4.2 ± 0.58). Pre - and post- treatments with LC & LA to γ-irradiation was found to decrease the Mn-PCEs (7.0 ± 0.45) significantly when compared to the post – treated group (12.4 ± 0.81). Moreover, the inhibition percentages reached (63.4%) in post-treated group meanwhile, a maximum inhibition (87.5%) was shown in pre- and post- treated group (Fig. 4 A and B). In spite of this reduction, the frequencies of Mn-PCEs were significantly higher than the control group. Our results clearly indicated that animals exposed to γ-irradiation showed severe bone marrow cytoxicity as indicated by the reduction in PCEs percentage and the PCEs/NCEs ratio as compared to the control group. The treatment with LC & LA resulted in an increase in PCEs percentage and the PCEs/NCEs ratio when compared with γ-irradiated rats.

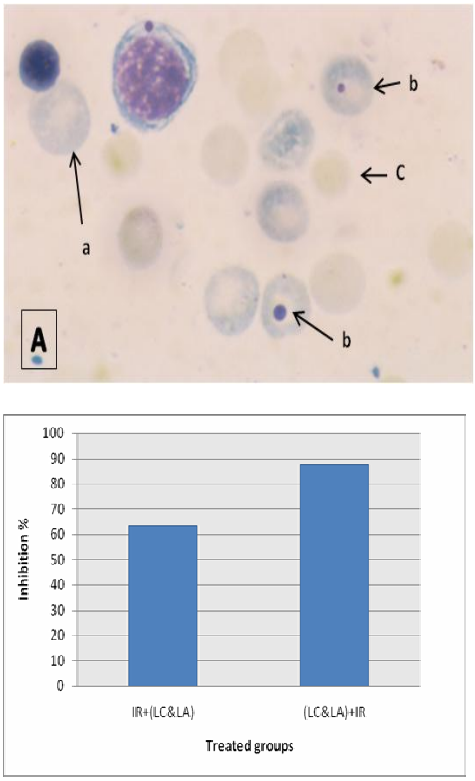


**Fig.4.** **(A)** Showing (a) polychromatic erythrocyte (PCE), (b) micronucleated polychromatic erythrocyte (Mn- PCE), and (c) normo chromatic erythrocyte (NCE). **(B)** Histogram showing inhibition of micronuclei incidence – induced by γ-irradiated by LC&LA.

**Table 2.** Effects of LC&LA on the frequency of Mn-PCEs and PCEs in the bone marrow of rats exposed to γ- irradiation.

| Treated Groups | No of Mn-PCEs/5000PCEs (Mean ± S.E.) | PCEs % | NCEs % | PCEs/NCEs ratio (Mean ± S.E.) |
|---|---|---|---|---|
| Control | 21<br>4.2 ± 0.58[d] | 51 | 49.0 | 1.04 ± 0.05[b] |
| LC&LA | 11<br>2.20 ± 0.37[e] | 59.8 | 40.2 | 1.49 ± 0.08[a] |
| IR | 133<br>26.6 ± 1.44[a] | 39.6 | 60.4 | 0.65 ± 0.03[e] |
| IR+(LC&LA) | 62<br>12.4 ± 0.81[b] | 45.6 | 54.4 | 0.84 ± 0.02[d] |
| (LC&LA)+IR | 35<br>7.0 ± 0.45[c] | 48.8 | 51.6 | 0.94 ± 0.02[C] |

Within each column, means superscript with different letter are significantly different ($P < 0.05$).

### *3.4. Histopathological evaluation*

Examination of H & E stained liver sections obtained from control rats showed a normal lobular architecture with central veins and radiating hepatic cords are separated by narrow blood sinusoids lined with two types of cells, endothelial (with thin, small, rod-like nucleus) and van-kupffer cells (containing oval dense nuclei), the hepatocytes are large spherical cells with spherical nuclei and prominent deeply stained nucleoli (Fig.5 A). The liver sections of rats treated with LC and LP illustrating the hepatic architecture shows no abnormalities. The hepatocytes reveal the same appearance as the normal ones. Also, the hepatic, sinusoids and kupffer cells show no abnormalities (Fig.5 B). However, liver sections of γ-irradiated rats showed radiolesions in the form of dilatation and congestion in blood vessels and appearance of inflammatory cells. The hepatocytes showed vacuolated cytoplasm, dilated sinusoidal spaces, large number of binucleated hepatocytes cells and necrotic cells along with many pyknotic nuclei (Fig. 5 C, D and E). In Fig. (5 F, G and H) liver of rat in group post- treated with LC&LA showed hepatocytes were found in focal hepatic hemorrhage and binucleated hepatocytes wider sinusoidal space, vacuolar degeneration of some hepatocytes, dilated blood vessel were noticed. Nevertheless, other hepatocytes were abnormal with either pyknotic or karyolitic nuclei with noticeable increase of endothelial and of kupffer cells within the tissue. Whereas photomicrograph of liver in pre- and post-treated with LC&LA to γ-irradiation showed lesser damage when compare to post-treated one. Liver sections of these rats almost have normal hepatic architecture as well as mild cytoplasmic vacuolation, binucleated cells were evident in photomicrograph, hemorrhage disappeared, slight dilated blood vessels and dilated blood sinusoids were noticed (Fig. 5 I).

### *3.5. Ultra structural evaluation*

The ultra structural of a normal hepatocyte obtained from control and LC&LA is presented in Fig. (6 A and B). Electron micrographs showed large rounded euchromatic nuclei with prominent nucleoli. The cellular organelles were uniformly distributed throughout the cytoplasm, where numerous mitochondria, cisternae of rough endoplasmic reticulum and electron dense cytoplasm. Electron micrographs showed ultra structural changes in different hepatocyte components after exposure to γ-irradiated revealed focal degeneration of cytoplasmic matrix accompanied by ill defined cytoplasmic organelles, degeneration hepatocytes with lysis cytoplasm matrix, degeneration nuclei with wrinkled nuclear membrane (Fig. 6 C and E). Swelling mitochondria with rupture of its cristae. Also, fragmentation of the rough endoplasmic reticulum (Fig. 6 D). In addition, the blood sinusoids exhibited prominent dilatation with abnormal lymphocytes and also contain numerous R. B. Cs. (Fig. 6 C, E and F). Also, dilated bile canal with abnormal microvillus (Fig. 6 F). Mitosis of the nuclei appears in Fig. (6 A).

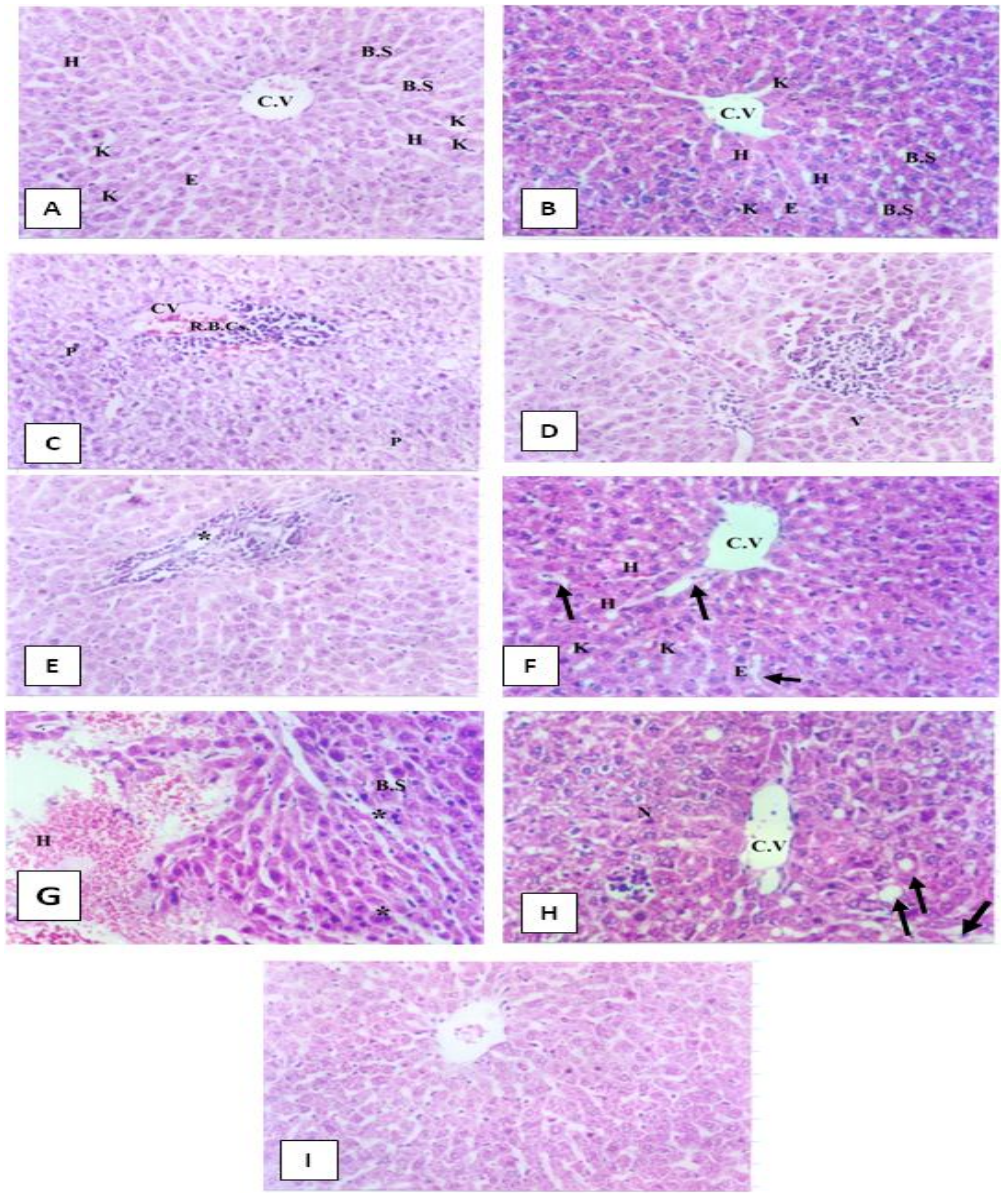


**Fig. 5. (A) (Group I-control)** Showing normal histological structure of rat liver; hepatocytes (H.), central vein (CV), blood sinusoid (BS), endothelial cell (E) and Kupffer cell (K). **(B) (GroupII-LC&LA)** Showing normal architecture of hepatic cells; the hepatocytes (H). The blood sinusoids (BS) are lined by a layer of endothelial cells (E) as well as Kupffer cell (K). **(C) (GroupIII-IR)** Showing central vein (CV) appears congested with blood cells (RBCs), the central vein (CV); is located at the center of the hepatic lobules. phyknosis in the hepatocytes nuclei (P) and vacuolated hepatocytes cytoplasm, cellular edema and binucleated hepatocytes, necrotic cells portal infiltration with leucocytes. **(D) (GroupIII-IR)** Showing focal area of hepatic necrosis associated with leucocytic cells infiltration and vacuolated hepatocytes cytoplasm (V) binucleated hepatocytes, dilated sinusoidal spaces. **(E) (GroupIII-IR)** Showing portal infiltration with leucocyte (*) **(F) (Group IV- IR+(LC&LA))** Showing dilated blood sinusoids (thick arrow), dilated central vein (CV) normal arrangement of the hepatic cords with invasion of endothelial cells (E) and kupffer cells (K)and haemorrhage (H). **(G) (Group IV- IR+(LC&LA))** Showing focal hepatic hemorrhage (H) and binucleated hepatocytes (*) wider sinusoidal space (B.S). **(H) (Group IV-IR+(LC&LA))** Showing vacuolar degeneration (arrow) of some hepatocytes, dilated central vein (CV), dilated blood sinusoids (thick arrows) and necrosis (N). **(I) (Group V-(LC&LA)+IR)** Showing the arranged hepatic architecture, dissociation of giant hepatocytes with parenchymatous islands. (H & E stain, X -200).

However, electron micrograph of liver from post–treated group revealed that LC & LA minimized most the injuries noticed in the hepatocytes of those rats exposed to γ-irradiated. Improvement in the nucleus and its nuclear membrane and chromatin, normal mitochondria, regeneration in the endoplasmic reticulum mean while, mild irregularity in nuclear membrane and fragmented endoplasmic reticulum were still found in different hepatocytes (Fig. 7 A, B and C). Ultra structurally, the hepatocyte sections of pre- and post-treated group (Figs. 7 D, E, F and G) revealed normal appearance of the liver cell membrane. The hepatocytes showing complete regeneration in the nucleus, nuclear membrane regular appear. Regeneration in the endoplasmic reticulum, the bile canal demonstrated in normal shape normal mitochondria in between rough endoplasmic reticulum.

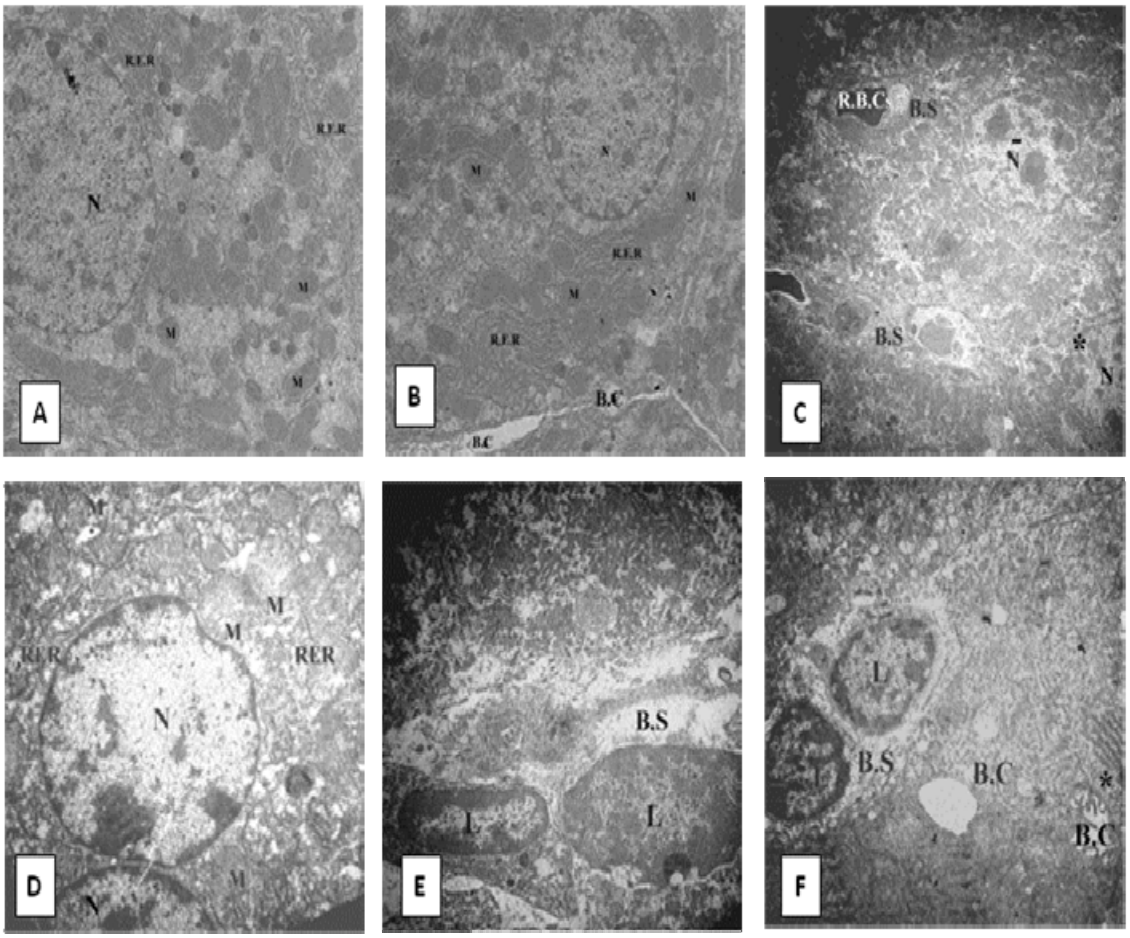


**Fig. 6.** Electron micrograph of hepatocyte section of albino rat **(A) (Group I-control)** Showing normal nucleus (N), normal variable sized mitochondria (M), and rough endoplasmic reticulum R. E. R. (X-8000). **(B) (GroupII-LC&LA)** Showing nearly normal ultrastructure, normal nucleus (N), normal mitochondria (M) R.E.R and normal bile duct (BC) and its canal (BC) (X-8000). **(C) (GroupIII-IR)** Showing degeneration hepatocytes with lysis cytoplasm matrix, degenerated nuclei (N) with wrinkled nuclear membrane (*) and ill defined cytoplasmic organelles, another showing mitosis (N), dilated B.S which contain, numerous R.B.Cs. (X-4000). **(D) (GroupIII-IR)** Showing irregularity in nuclear membrane (arrow), swollen mitochondria and rupture in its cristae (M), fragmentation of R.E.R. and binucleated (N) cells. (X -12000). **(E)(GroupIII-IR)** Showing degeneration hepatocytes with lyses and ill defined cytoplasmic organelles and dilated blood sinusoids (B.S) with abnormal lymphocytes (L) (X-100.000). **(F) (GroupIII-IR)** Showing: dilated bile canal (B.C.) with abnormal microvilli (*), also dilated blood sinusoids (B.S) with abnormal lymphocytes (L) (X -10.000).

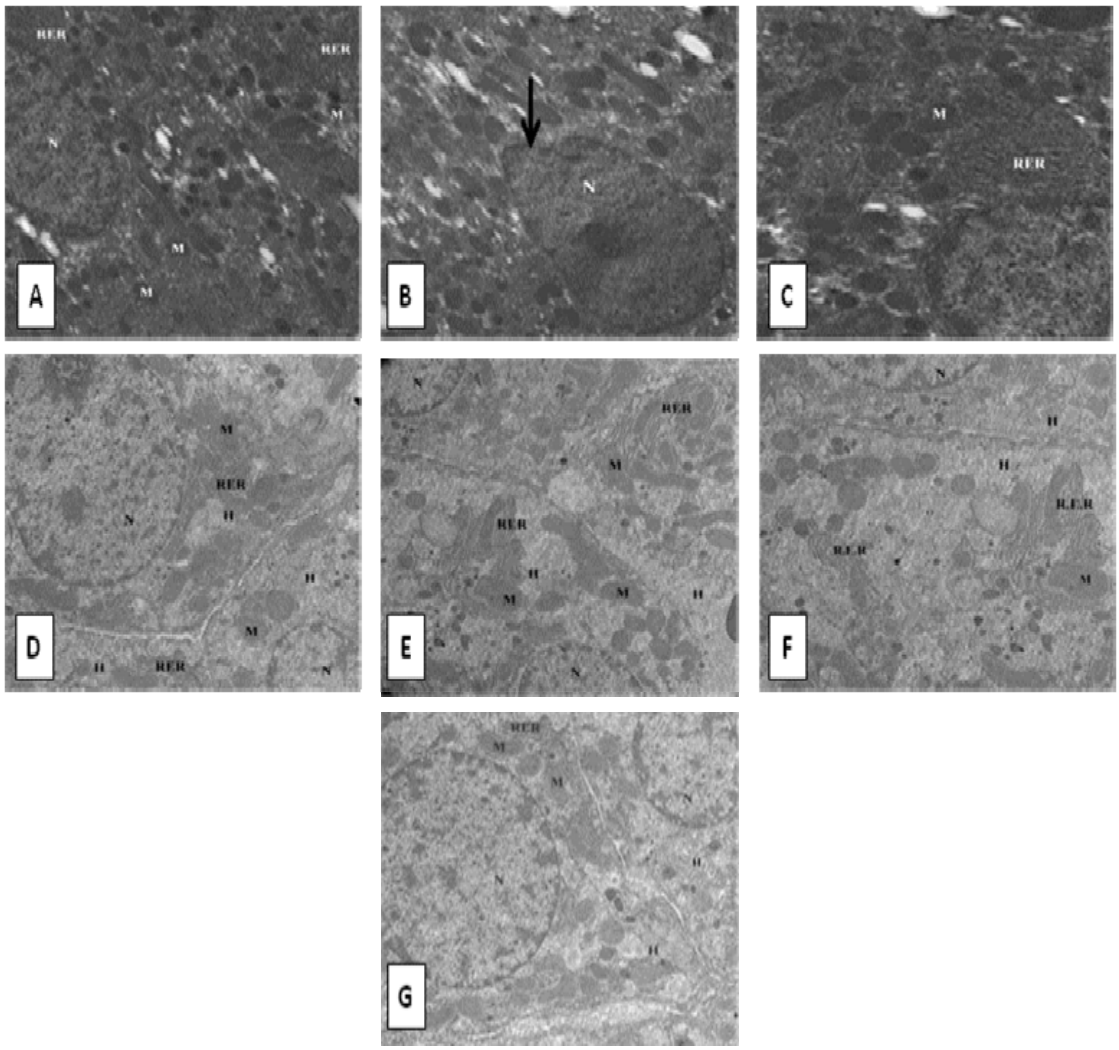


**Fig. 7.** Electron micrograph of hepatocyte section of albino rat: **(A) (Group IV- IR+(LC&LA))** Showing regeneration in the hepatocyte, notice: improvement in the nucleus (N), nuclear membrane, regeneration in the rough endoplasmic reticulum (RER), improvement of mitochondria (M) (X-8000). **(B) (Group IV- IR+ (LC&LA))** Showing regeneration in the hepatocyte, improvement in nucleus (N), nuclear chromatin and neucleolus and irregularity in nuclear membrane (arrow) (X 8000). **(C) (Group IV-IR+ (LC&LA))** Showing regeneration in the hepatocyte, improvement in the nucleus and its chromatin, regular nuclear membrane, regeneration in the rough endoplasmic reticulum (RER) and improvement of mitochondria (X -13300). **(D) (Group V-(LC&LA)+IR)** Showing complete regeneration in the hepatocyte with nucleus (N), regular nuclear membrane, regeneration in the rough endoplasmic reticulum (R.E.R) normal mitochondria in between R.E.R, and an intact cell membrane between 3 hepatocytes (H) (X-8000). **(E) (Group V-(LC&LA)+IR)** Showing bile canal can be demonstrated B. C. Showing complete regeneration in the hepatocyte with nucleus (N), regular nuclear membrane, regeneration in the rough endoplasmic reticulum (R.E.R) normal mitochondria in between R.E.R, and an intact cell membrane between 2 hepatocyte (H) (X -6400).**(F) (Group V-(LC&LA)+IR)** Showing complete regeneration in the hepatocyte with nucleus (N), regular nuclear membrane, regeneration in the rough endoplasmic reticulum (R.E.R) normal mitochondria in between R.E.R, and an intact cell membrane between 2 hepatocytes (H) (X -8080). **(G) (Group V-(LC&LA)+IR)** Showing complete regeneration in the hepatocyte with nucleus (N), regular nuclear membrane, regeneration in the rough endoplasmic reticulum (R.E.R) normal mitochondria (M) in between R.E.R, and an intact cell membrane between 2 hepatocytes (H) (X- 8080).

**4. Discussion**

The deleterious effects of ionizing radiation in the biological systems are mainly mediated through the generation of ROS, a process called oxidative stress, in a variety of cells as a result of water radiolysis (Kamat *et al.,* 2000). To control the flux of ROS, aerobic cells have developed their own defense system, the antioxidant system, which includes the superoxide dismutase (SOD), the first line of defense against oxygen-derived free radicals, catalysis the dismutation of superoxide anion into $H_2O_2$. Glutathione peroxidase (GPx) reduces lipidic or nonlipidic hydroperoxides as well as $H_2O_2$ (Taysi *et al.,* 2002). Antioxidant enzymes are regulated by multiple factors. The oxidative status of the cell is the primary factor regulating gene expression and activity of these enzymes (Rodriguez *et al.,* 2004). Both, endogenous (Nicotera *et al.,* 1989) and exogenous (Yoo *et al.,* 1999) agents act as oxidants and alter cellular oxidative equilibrium and, consequently, antioxidant enzyme gene expression.

In the present study, the expression of GPx and SOD was down-regulatated at the liver of γ-irradiated animals. It is reported that translocation of redox-sensitive transcription factors into the nucleus is influenced by oxidative stress (Sen and Packer, 1996). Besides, elevation of oxidative stress is known to cause destabilization of mRNAs (Mayo *et al.,* 2002). Therefore, the reduction in the mRNA of SOD and GPx in γ-irradiated rats could be either due to the oxidation of transcription factors or due to the decrease in the half lives of mRNAs. In agreement with our results, Mansour *et al.* (2008) recorded a significant decrease in the activities of SOD and GPx in hepatic tissues after whole body γ-irradiation. Ushakova *et al.* (1999) reported that radiation inhibited the expression of SOD gene in splenocytes from control mice.

The down-regulated mRNA of the hepatic GPx and SOD in γ-irradiated rats is alleviated by antioxidant treatments, suggesting the transcriptional control by LC & LA. In additions pre- and post-treatments with LC & LA significantly increased the levels of mRNA expression of hepatic antioxidant enzymes in irradiated rats than the post-treated group. These results are in agreement with that found by Miguel-Carrasco *et al.* (2010) who reported that L-carnitine treatment was able to reverse the down-regulation of cardiac GPx and SOD mRNA expression observed in hypertensive rats, increasing values to the levels observed in control, normotensive animals. Mansour (2006) reported that LC could increase the endogenous antioxidant defense mechanism in rats and thereby protect the animals from radiation-induced organ toxicity. Kocer *et al.* (2007) found that LC protected the lenses of rats against the damage produced by γ- irradiation by increasing the activity of the SOD enzyme and by scavenging free radicals generated by ionizing radiation. In another study, Davis *et al.* (2009) found that LA treatment restored antioxidant levels, reduced cell injury and protected cells against irradiation-induced cytotoxicity. Yang *et al.* (2008) determined that LA ingestion up-regulated the expression of genes related to free-radical scavenger enzymes of mice fed with high fat diet. LA also enhances the decrease of SOD activity and this may be related to its direct scavenger effect (Sundaram *et al*., 2006).

Numerous studies have demonstrated that exposure of mammalian cells to ionizing radiation induces several types of damage to DNA when the capacity of enzymatic and nonenzymatic antioxidant system is inadequate (Ames *et al.,* 1993). They induce a variety of lesions in DNA, including double and single-strand breaks, base and sugar damage, as well as DNA–DNA and DNA–protein cross-links (Barker *et al.,* 2005). Our results clearly showed that γ-irradiation, induces DNA damage in rat liver that was detected by the elevation the percentage of DNA fragmentation and the visualization of DNA ladders upon gel electrophoresis in treated animals. In addition, an increase of micronuclei in bone marrow cells was demonstrated in γ-irradiated rat. The induction of micronuclei was accompanied by cytotoxicity that evidenced through the significant reduction in the number of PCE/1000 cells. This reduction could be a consequence of a direct cytotoxicity or due to the micronuclei formation itself or to heavy DNA damages leading to cell death or apoptosis (Zorgui *et al.,* 2009). An increase in the DNA damage after γ-irradiation has been observed in different studies (Hosseinimehr *et al.,* 2003; Mansour *et al.,* 2008). It has long been known that the damaging effects of ionizing radiation on cellular DNA are brought about by both direct and indirect mechanisms. Direct action produces disruption of chemical bonds in the molecular structure of DNA, while indirect effects result from highly reactive free radicals such as •OH and •H produced during the radiolysis of water, and their subsequent interaction with cellular DNA (Pons and Sullivan, 1994).

Treatment with LC & LA in combination with γ-irradiation was markedly efficient and significantly inhibited the micronucleated PCE in bone marrow cells that reached 63.4% in post-treated and 87.5% in pre- and post- treated groups. Moreover, LC & LA treatment decreases the percentage of DNA fragmentation induced in liver cells as evidenced by the reduction of DNA fragments in agarose gel electrophoresis.

The observed protective effect of LC & LA maybe due to the direct protection against DNA damage by scavenging free radicals generated by ionizing radiation before they induce damage to the genetic material, i.e. the extent of primary damage in cellular DNA may be significantly reduced (Sundaram *et al.,* 2006; Kocer *et al.,* 2007). Secondly, LC & LA may indirectly alter the final level of DNA damage by enhancing the activity of DNA repairing enzyme, poly (ADP-ribosyl) polymerase, a nuclear protein that is intimately linked with the occurrence of DNA strand breaks and also other related repair mechanisms (Boerighter *et al.,* 1993; Szabodos *et al.,* 1999), so that the damage DNA is repaired more rapidly in irradiated cells pre-treated with LC & LA. Other studies have also observed that the LC & LA maintains thiol-containing compound and improves the glutathione redox status, which could protect DNA repairing enzymes, thereby preventing DNA strand break (Savitha and Panneerselvam, 2006). LC & LA treatment was also shown to decrease the levels of oxidative stress mediated DNA damage during aging probably by decreasing the oxidant production and by improving the antioxidant status in aged rats (Savitha and Panneerselvam, 2007). These results are in complete agreement with our reports on the protective effect of the LC & LA against DNA damage induced by ionizing radiation.

The histopathological architecture of the liver in γ-irradiated groups showed radiolesions in various form in liver that included congestion and dilatation in blood vessels, haemorrhage, vacuolation, lymphocyte infiltrations and necrobiotic changes in the hepatocytes. Concomitantly, the ultra structural examination for the same group showed different changes in the hepatocytes of treated rats. These changes were represented as swelling and ruptured mitochondria, fragmentation of the endoplasmic reticulum and nuclear damage with wrinkled nuclear membrane. Aforesaid alterations in the liver tissue showed similarity and conforms those recorded by several authors, with various experimental animals exposed to radiation (Nasr *et al.,* 1993; Zavodnik *et al.,* 2003; Mansour *et al.,* 2008). These histopathological and ultra structural changes in rats liver treated with irradiation were attributed to the direct harmful effect of irradiation on the biological system or indirect effect of free radicals liberated in the body after irradiation (Hagn, 1989).

In the current study, LC & LA were histopathologically and ultra structurally proven to be radioprotective agents by reducing the severity of radiation-induced liver damage. Most of the histopathological lesions observed in γ-irradiated group were disappeared to a large extent; where the normal architecture of the liver was restored in the pre- and post-treated group. whereas, slight dilated blood vessels and blood sinusoids were still found, in photomicrograph of liver sections in post- treated group, hepatocytes were found in focal hepatic hemorrhage and binucleated hepatocytes wider sinusoidal space, vacuolar degeneration of some hepatocytes, dilated blood vessel were noticed. Also, other hepatocyts were abnormal with either pyknotic or karyolitic nuclei. Meanwhile, the results of electron microscopy showed that post - treatment with LC & LA minimized most of the injuries noticed in the hepatocytes of rats exposed to γ-irradiation. While, a complete regeneration in the nucleus, nuclear membrane, endoplasmic reticulum and mitochondrial shape were detected in rats treated with LC & LA prior and post exposure to γ-irradiation.

In the previous studies, Altas *et al.* (2006) found that LC was able to ameliorate radiation-induced cochlear histopathologic damage in guinea pigs. Also, Sezen *et al.* (2008) histopathologically demonstrated that LC has a radioprotective feature by reducing the severity of radiation-induced brain damage. Ucuncu *et al.* (2006) reported that LC had radioprotective properties by delaying the starting day and reducing the severity of radiation-induced oral mucositis. L-carnitine is an endogenous mitochondrial membrane compound (Ueda *et al.,* 2002), these data support the idea that L-carnitine-mediated cytoprotection is due, in part, to inhibition of the mitochondrial apoptotic pathway. Moreover, LC has been shown to act as an important anti-apoptotic mediator (Moretti *et al.,* 2002). Ishii *et al.* (2000) reported that acetyl carnitine has shown to attenuate DNA fragmentation and nuclear condensation in cultured neurons promoting neuronal survival. Similarly, Sayed-Ahmed *et al.* (2004) reported that acetyl carnitine has a protective effect against Bleomycin-induced oxidative stress and energy depletion in lung tissues in rats, through its free radical scavenging properties with the consequent improvement in mitochondrial function and ATP production. Recently, Dadhania *et al.* (2010) have reported that LA pretreatment ameliorates MTX-induced intestinal toxicity in rat as evident from the protection against oxidative stress, decrease in DNA damage and protection of cellular morphology. Similarly, LA pretreatment affected cell death by decreasing the number of apoptotic and necrotic cells induced in cyclophosphamide treated rat (Selvakumar *et al.,* 2006). It has also been demonstrated that LA and DHLA both inhibit the apoptosis of rat thymocytes after exposure to either methylprednisolone or etoposide and this inhibition

was manifested at an early stage in the apoptosis as cell shrinkage, chromatin fragmentation (Bustamante *et al.,* 1995).

In the present study, we chose to co-supplement α-lipoic acid, an antioxidant, with L-carnitine to decrease the damaging effects in rat exposed to irradiation. Previous studies have shown an improvement in mitochondrial functions upon supplementation with these two mitochondrial metabolites (Hagen *et al.,* 2002). Moreover, Savitha and Panneerselvam (2006) have also shown that this feeding regimen improves the activities of mitochondrial enzymes and respiration, thereby improving the ATP levels during aging. Alie *et al*. (2008) demonstrated that feeding LA and acetyl carnitine ameliorated age-associated mitochondrial ultrastructural decay.

Treatment with LC & LA could protect against radiation damage by up-regulation the gene expression of the antioxidant enzymes (GPx and SOD), that is in turn may lead to inhibition of DNA fragmentation in liver cells, micronuclei incidence in PCE (bone marrow cells), and normal restoration of histopathologial and ultra structure liver architecture. It is interesting that being injected prior to irradiation, LC and LA could prevent the development of oxidative attack initiated by irradiation suggesting that LC and LA exhibit an antioxidant activity. Thus we can concluded that LC & LA may protect against the damage produced by radiation by preventing oxidative stress by scavenging ROS directly and increasing those of free-radical scavenger enzymes genes expression indirectly.

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