American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 2 (3): 120-132, 2012
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2012.120.132

Protective Effect of L-carnitine Against γ-Rays Irradiation-induced Tissue Damage in Mice

[1,2]Samir A. Elshazly, [1,3]Mohamed M. Ahmed, [4,5]H.E. Hassan and [6,7]Zein S. Ibrahim

[1]Department of Biotechnology, Faculty of Science, Taif University, AL-Hawiah-888, Taif, Saudi Arabia
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Minufiya University, Egypt
[4]Department of Physics, Faculty of Science, Taif University, AL-Hawiah-888, Taif, Saudi Arabia
[5]Cyclotron Facility, Nuclear Research Center, Atomic Energy Authority, Cairo 13759, Egypt
[6]Department of Physiology, Faculty of Medicine, Taif University, AL-Hawiah-888, Taif, Saudi Arabia
[7]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt

Corresponding Author: Samir A. El-Shazly, Department of Biotechnology, Faculty of Science, Taif University, P.O. Box 888, Taif, Kingdom of Saudi Arabia Tel: +966553114940

ABSTRACT

The present study has been conducted to evaluate the radioprotective effect of L-carnitine against γ-irradiation-induced tissue damage in mice. Adult male mice were exposed to 1 Gy γ-irradiation either in the absence and/or presence of L-carnitine at dose of 10 mg kg^{-1} body weight/day over a period of 10 days. Present results revealed that γ-irradiation stimulated TNF-α mRNA expression 12 folds compared to control, meanwhile treatment of L-carnitine for one week before and throughout γ-irradiation exposure period suppressed TNF-α mRNA expression to L-carnitine-induced level. Similar results were obtained for IL-1β and IFN-γ mRNA expression. On histopathological examination, treatment with L-carnitine alone did not induce any detectable pathological lesions. However, γ-irradiation induced a variety of pathological changes in liver, kidney and spleen. In liver, there was a marked dilatation and congestion of hepatic veins, most of hepatocytes showed vacuolation and displayed distinct nuclear changes in response to radiation while L-carnitine pretreatment showed regenerative activity as represented by high frequency of binucleated hepatocytes. In case of kidney, γ-irradiation induced congestion, interstitial haemorrhages and degenerative changes. Renal tissue structures were restored on co-treatment with L-carnitine. Additionally, spleen showed marked congestion and haemorrhages. The marginal zones revealed depletion of their cellular contents, especially from the macrophages and lymphocytes. L-carnitine restored more or less normal organization of spleen in γ-irradiation treated group. These results clarified the immunomodulatory effects of L-carnitine and its radioprotective role against tissue injury induced by γ-irradiation on molecular basis.

Key words: L-carnitine, γ-irradiation, protection, cytokines, mice

INTRODUCTION

The medical applications of radiation technology have been increased rapidly in the last two decades due to development of new diagnostics and therapeutic methods. Radiation treatment increases the annual dose received by both the patients and physicians because of regular exposure to radiation or radionuclides intake. Therefore, studying the biological damage induced by ionizing

radiation is necessary in assessment of maximum absorbed dose during radiotherapy or diagnosis. Moreover, development of protective agents presented new solutions for recovery of undesired tissue damage induced by ionizing radiation.

The ionizing radiation causes damage of the cells directly by ionization of DNA and other cellular targets and indirectly by effect through Reactive Oxygen Species (ROS) (Borek, 2004). Exposure to ionizing radiation produces oxygen-derived free radicals in the tissue environment; these include hydroxyl radicals (the most damaging), superoxide anion radicals and other oxidants such as hydrogen peroxide. About two-thirds of X-ray and gamma-ray damage is caused by indirect action (Konopacka and Rogolinski, 2004). Although radiotherapy is effective in killing tumor cells, ROS produced during radiotherapy threaten the integrity and survival of surrounding normal cells. The increasing use of diagnostic radiology is unquestionably beneficial. However, individual exposure to medical irradiation has grown six folds in the last two decades and appears to be still increasing. It is well established that the effects of irradiation are cumulative and lead to increase the incidence of cancers, cell deaths, genetic damage and numerous forms of body tissue pathology. According to classical radiation biology, irradiation cell killing is based on DNA strand breaking (Hall, 1999). Immunologic mechanisms of cell killing including killing of tumor cells by immune cells and humoral immune cytotoxicity are mediated by cytokines. Furthermore, lymphokines have been shown to mediate molecular pathways common to ionizing irradiation-induced apoptosis (Epperly *et al*., 2001; Epperly *et al*., 2003a). A recent evidence has suggested that mitochondrial membrane permeability and leakage of cytochrome C into the cytoplasm mediate a critical step in the activation of caspase-3 and distal caspase death effectors cascade proteins leading to poly-ADP-ribosyl polymerase activation and DNA fragmentation (Epperly *et al*., 2002). These later steps have been shown to be common in induction of apoptosis by TNF-α binding to its receptor, as well as other cytokine receptor mediated apoptotic mechanisms (Epperly *et al*., 2003a). As the diversity of radiation used in medicine, agriculture, industry, biochemical research and military operations increases, the risk from exposure is to be evaluated. Thus, the protection of individuals against severe damage due to irradiation is an important issue (Epperly *et al*., 2003b; Pearce *et al*., 2001).

A recent research has supported that a variety of cytokines play important roles during irradiation (Ao *et al*., 2009). The response to ionizing radiation involves a number of mediators including inflammatory cytokines produced by macrophages, epithelial cells and fibroblasts (Chen *et al*., 2002; Chen *et al*., 2005). Rats that received 10 Gy of whole-body gamma irradiation exhibited increased levels of IL-1β, TNF-α and IL-6 mRNA at 3 and 6 h post irradiation (Linard *et al*., 2004). Additionally, doses of irradiation as low as 1 cGy, could elicit an inflammatory response. This low dose is close to that used for many diagnostic procedures (Fujimori *et al*., 2005). It is important to make the connection between pro-inflammatory cytokines and free radicals, since many cytokines can be induced by increased free radical formation and other cytokines can aid in their production through activation of signal transduction pathways. Gamma irradiation of endothelial cells using doses as low as 2 Gy induced secretion of inflammatory cytokines including IL-6 and IL-8 from the cells (Van Der Meeren *et al*., 1997). IL-8 also has been found to be upregulated in normal human fibroblasts after irradiation with α-particles. These investigators also found that IL-8 is directly correlated with increases in ROS and that the levels of ROS were decreased when cells were irradiated in the presence of antioxidants (Narayanan *et al*., 1999). As Irradiation can exert a significant inflammatory response in cells so, it is essential to develop methods to target the radiosensitive tumors and/or to protect the normal tissues.

Antioxidants eliminate the free radicals and neutralize ROS ions before they can do their damage. However, much remains unknown about mechanisms of radio-protection. Existing studies do not provide definitive answers about which are the most effective antioxidants and combinations of antioxidants for human radioprotection. Both mitochondrial membrane stability and antioxidant reserves appear to be important to radioprotection. Natural products such as herbal medicines have only recently begun to receive some attention as possible modifiers of the irradiation response (Kim *et al*., 2003). L-carnitine is a naturally occurring quaternary ammonium compound, which is endogenously synthesized in man and also found in the diet (Goa and Brogden, 1987; Izgut-Uysal *et al*., 2001). It is an essential cofactor of several enzymes necessary for energy metabolism and also acts as a scavenger of oxygen free radicals in mammalian tissues and its role in energy production is essential to maintain membrane structure and cell viability (Izgut-Uysal *et al*., 2001; Famularo *et al*., 1997). L-carnitine was early shown to enrich leukocytes, including peripheral blood mononuclear cells indicating its immunomodulatory effect (Deufel, 1990). Moreover, L-carnitine has been proven for its antioxidant and antiapoptotic effects (Monti *et al*., 1992; Famularo *et al*., 1994). L-carnitine also decreased Irradiation-induced increase of malondialdehyde level and increased the activity of SOD and CAT enzymes in the plasma, which prove its antioxidant properties (Ucuncu *et al*., 2006). The irradiation-induced cell injury and biological roles of L-carnitine motivated us to investigate the cytoprotective effects of L-carnitine in mice exposed to gamma irradiation and possible mechanisms involved in this effect.

MATERIALS AND METHODS

Animals: Twenty four male mice were purchased from experimental animal center, faculty of Pharmacy, King Saud University, KSA. The animals were housed in the animal facility of the faculty of Science, Taif University, KSA, at 22°C and 55% humidity with 12 h light:12 h dark cycle. The mice were fed a standard pellet diet and water *ad libitum*.

Experimental design and animal irradiation: After one week accommodation, the mice were divided into four groups. The first group used as a negative control injected with 50 μL saline. The second group received L-carnitine (Sigma-Aldrich Co., St Louis, MO) at a dose 10 mg kg^{-1} b.wt./day intra-peritoneally . The third group exposed to gamma irradiation at a dose one Gray over 10 days. While the fourth group was pre-treated for one week with intra-peritoneal L-carnitine at a dose 10 mg kg^{-1} b.wt./day then, exposed to gamma irradiation at a dose rate of one Gray over additional 10 days along with L-carnitine treatment.

RNA extraction: At the end of the experiment, mice were anesthetized using diethyl ether. Whole blood was collected from medial cansus of the eye into sterile heparinzed tube for Total RNA extraction. Briefly, 100 μL blood were homogenized in 1 mL QIAzol (QIAGEN Inc., Valencia, CA) then 0.3 mL chloroform was added to the homogenate. The mixtures were then shaken for 30 sec followed by centrifugation at 4°C and 12,500 rpm for 20 min. The supernatant layer were transferred to a new set of tubes and an equal volume of isopropanol was added to the samples, shacked for 15 sec and centrifuged at 4°C and 12500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, dried then, dissolved in diethylpyrocarbonate (DEPC) water. The prepared RNA samples integrity and concentration were checked by electrophoresis and by measuring the optical density respectively. The optical density of all RNA samples was 1.7-1.9 based on the 260/280 ratio.

Table 1: Oligonucleotide primers used for gene expression analysis by RT-PCR

Gene	Accession No.	Primer sequence	Product size (bp)
β-actin	X03672.1	Forward 5´-TTC TTT GCA GCT CCT TCG TTG CCG -3´	457
		Reverse 5´- TGG ATG GCT ACG TAC ATG GCT GGG -3´	
TNF-α	M11731	Forward 5´-TGT CTC AGC CTC TTC TCA TT-3´	429
		Reverse 5´ -CAG AGA GGA GGT TGA CTT TC-3´	
IL-1β	NM_008361	Forward 5´-GTG ACG TTC CCA TTA GAC AA-3´	431
		Reverse 5´-TGT CCT GAC CAC TGT TGT TT-3´	
IFN-γ	FJ617514	Forward 5´-TGC ATC TTG GCT TTG CAG CTC TTC-3´	350
		Reverse 5´-GGG TTG TTG ACC TCA AAC TTG GCA-3´	

cDNA synthesis: For synthesis of cDNA, mixture of 5 μg total RNA and 0.5 ng oligo dT primer in a total volume of 24 μL sterilized DEPC- water was incubated at 70°C for 10 min. Then, the total volume were made up to 40 μL by adding 16 μL of a mixture contains 8 μL (5X) RT-buffer, 2 μL 10 mM dNTPs, 4 μL DEPC water and 200 U Revet Aid Premium reverse transcriptase (Fermentas Canada Inc. Harrington Court, Burlington Ontario). The mixtures were then re-incubated in the PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma) at 30°C for 10 min, at 42°C for 1 h and at 90°C for 10 min then, preserved at 4°C until used.

Semi-quantitative PCR: The used primers were designed using oligo-4 computer program and nucleotide sequence published in genebank (Table 1) and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea. PCR was conducted using 1 μL cDNA in a final volume of 25 and 1 μL of 10 μM forward primers, 1 μL of 10 μM reverse primer and 23 μL PCR master mix (Promega Corporation, Madison, WI). PCR was carried out using a PeX 0.5 thermal cycler with the cycle sequence at 94°C for 4 minute one cycle, followed by 25 cycles at 94°C for 1 min, at 54°C for 1 min and 72°C for 1 min extended for 5 min at the last cycle. 20 μL of PCR products were electrophoresed on 1% agarose A (Bio BAsic INC. Konrad Cres, Markham Ontario) gel in TE (Tris-EDTA) buffer at 100 volt for 45 min. The gel was stained in 1% ethidium bromide and washed with distilled water. PCR products were visualized under UV light and photographed. The intensities of the bands were quantified densiometrically using NIH image program (http://rsb.info.nih.gov/nih-image) and Macintosh computer.

Histopathological studies: Tissue samples from liver, spleen and kidney were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin-eosin to study their micro architecture by light microscopy.

Statistical analysis: Results were expressed as Mean±standard error (SE). Statistical analysis was performed using Analysis of Variance (ANOVA) and Fischer's protected least-significant difference test, by StatView program for Macintosh computer with $p<0.05$ regarded as statistically significant.

RESULTS

In the present study, we evaluated the protective effect of L-carnitine on γ-irradiation-induced tissue injury in mice. To do so, mice were treated with/or without γ-irradiation in the absence/or presence of L-carnitine and the proinflammatory cytokines mRNA expression was checked by RT-PCR. Among tested cytokines, TNF-α mRNA expression was significantly upregulated (12 folds)

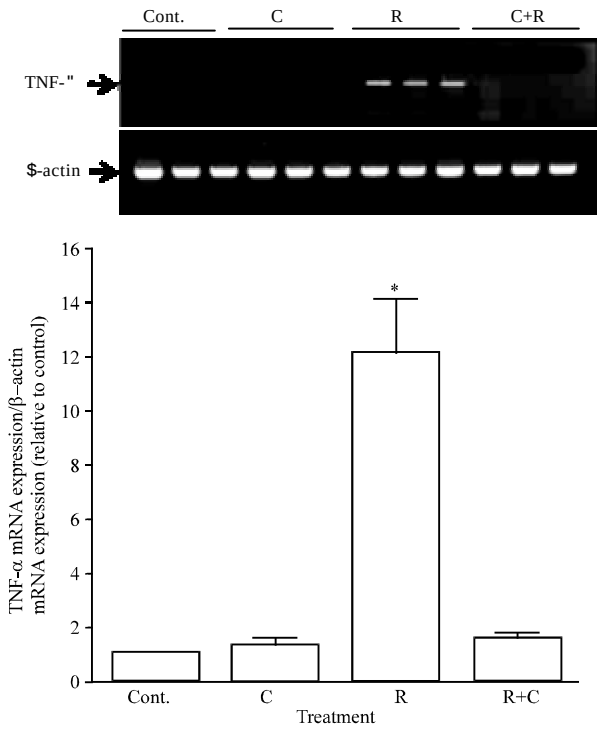

Fig. 1: Effect of L-carnitine on γ-irradiation induced TNF-α mRNA expression in mice. Mice were treated with 1 Gy of γ-irradiation for 10 days either in the absence or presence of 1 week daily pre-treatment of L-carnitine (10 mg kg^{-1}). Total RNA was prepared from the peripheral blood leukocytes and the expression of TNF-α mRNA in leukocytes was analysed by RT-PCR. Cont.: Control group, C: L-carnitine group, R: γ-irradiation group, R+C: γ-irradiation+L-carnitine group. Values are Means±SE of three independent experiments. *p<0.05 vs. radiation group

in response to γ-irradiation. Also L-carnitine inhibited γ-irradiation-induced TNF-α mRNA expression significantly (Fig. 1). We also checked the effect of γ-irradiation and L-carnitine on the mRNA expression of IL-β. As shown in Fig. 2, in case of L-carnitine there was a basal level of induction for IL-β mRNA expression. α-irradiation stimulated (about 6 folds) IL-β mRNA expression significantly and this stimulation was greatly inhibited in the presence of L-Carnitine. Finally, the mRNA expression of IFN-γ in response to γ-irradiation both in the absence or the presence of L-Carnitine was examined. IFN-γ mRNA expression was significantly stimulated in the presence of γ-irradiation (more than 8 folds) and/or L-carnitine treatment alone (Fig. 3). However on co-treatment with γ-irradiation in the presence of L-carnitine, L-carnitine showed a powerful tissue protective effect through significant suppression of this γ-irradiation-induced cytokines induction.

The histological sections of livers from mice treated with L-carnitine showed almost normal histological structure compared to control (Fig. 4a, b). Liver from mice exposed to a total dose of 1Gy of gamma irradiation over a period of 10 days showed marked dilatation and congestion of hepatic veins. Most of hepatocytes were vacuolated and displayed distinct nuclear changes. Some nuclei were irregular; others were pyknotic while other some appeared surrounded by large clear area

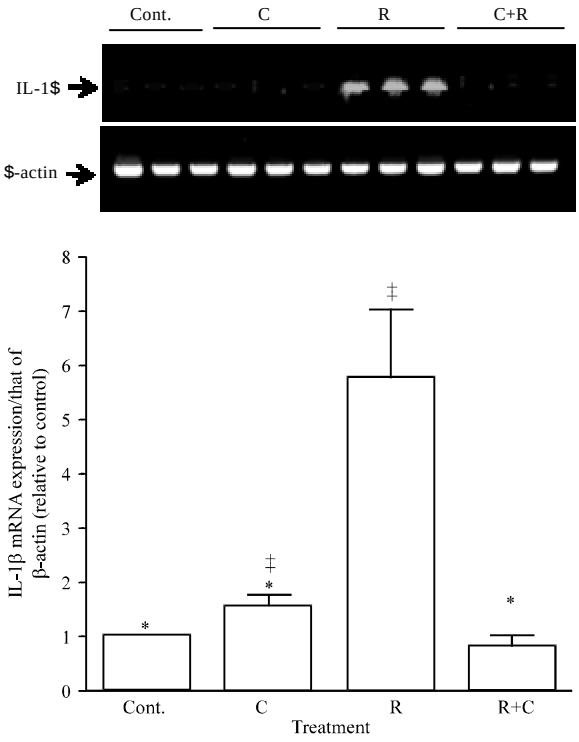

Fig. 2: Effect of L-carnitine on γ-irradiation induced IL-1β mRNA expression in mice. Mice were treated with 1Gy of γ-irradiation for 10 days either in the absence or presence of 1 week daily pre-treatment of L-carnitine (10 mg kg^{-1}). Total RNA was prepared from the peripheral blood leukocytes and the expression of IL-1β mRNA in leukocytes was analyzed by RT-PCR. Cont.: Control group, C: L-carnitine group, R: γ-irradiation group, R+C: γ-irradiation+ L-carnitine group. Values are Means±SE of three independent experiments. *p<0.05 vs. radiation group, ‡p<0.05 vs. control group

(Fig. 4c). Numerous vacuolated and hypertrophied hepatocytes were seen obstructing the sinusoidal spaces that showed Kupffer cell hyperplasia. Small haemorrhagic areas with mononuclear cells infiltration were also seen, especially in portal tracts. In hepatic sections from irradiated mice pretreated with L-carnitine showed regenerative activity as represented by high frequency of binucleated hepatocytes (Fig. 4d).

Histopathological examination of kidney from L-carnitine treated group showed no pathological changes as compared with control group (Fig. 5a, b) while those from gamma rays irradiated group, revealed congestion and interstitial haemorrhages. The renal cortex showed glomerular and tubular degenerative changes including glomerular hypercellularity, obliterating capsular space. Additionally, tubular changes ranged from cloudy swelling to necrosis with disruption of the tubular basement membrane (Fig. 5c). Many inflammatory cells were seen in the interstitium. Interestingly, L-carnitine showed a protective effect on the kidney tissue that exhibited by more or less normal structure in irradiated group receiving L-carnitine (Fig. 5d).

The histological sections of spleen from L-carnitine treated group showed no pathological changes as compared with control group (Fig. 6a, b) while those from gamma rays irradiated group, showed marked dilatation and congestion of splenic blood vessels with prominent

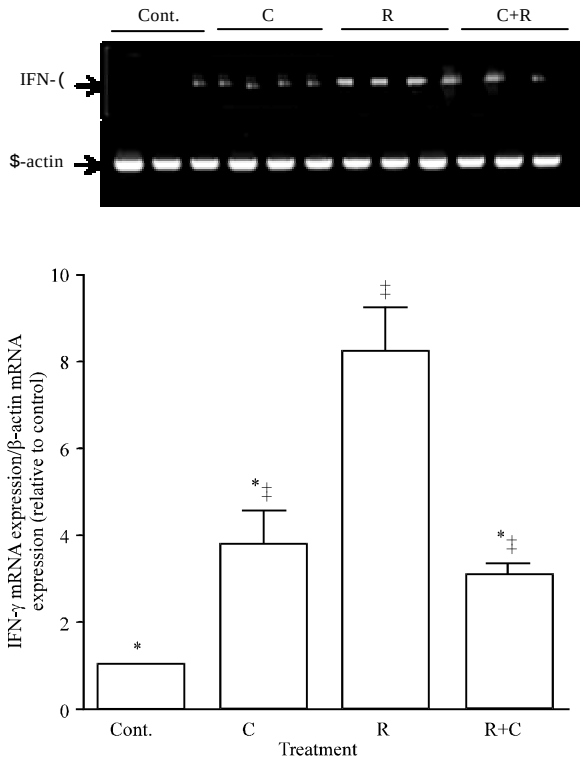

Fig. 3: Effect of leptin on γ-irradiation induced IFN-γ mRNA expression in mice. Mice were treated with 1Gy of γ-irradiation for 10 days either in the absence or presence of 1 week daily pre-treatment of L-carnitine (10 mg kg^{-1}). Total RNA was prepared from the peripheral blood leukocytes and the expression of IFN-γ mRNA in leukocytes was analyzed by RT-PCR. Cont.: Control group, C: L-carnitine group, R: γ-irradiation group, R+C: γ-irradiation+ L-carnitine group. Values are Means±SE of three independent experiments. *p<0.05 vs. radiation group, ‡p<0.05 vs. control group

haemorrhages. Thickening of follicular arterioles and periarteriolar haemorrhages were frequently seen in splenic follicles within the white pulp. The marginal zones revealed depletion of its cellular content, especially from the macrophages and lymphocytes (Fig. 6c). L-carnitine almost restored the normal organization of spleen (Fig. 6d) in irradiated group.

DISCUSSION

Immune cell functions are greatly affected by the antioxidant/oxidant balance which is necessary to maintain redox homeostasis especially during oxidative stress conditions (Thangasamy *et al.*, 2009). Exposure to ionizing radiation produces reactive oxygen species like hydroxyl radicals, superoxide anion radicals and other oxidants such as hydrogen peroxide which cause antioxidant/oxidant imbalance. About two-thirds of X-ray and gamma-ray damage is caused by indirect action of ROS (Konopacka and Rogolinski, 2004). Previous studies proved that a variety of cytokines play important roles during irradiation (Ao *et al.*, 2009). The response to ionizing radiation involves a number of mediators including inflammatory cytokines produced by macrophages, epithelial cells and fibroblasts (Chen *et al.*, 2002; Chen *et al.*, 2005). Rats that

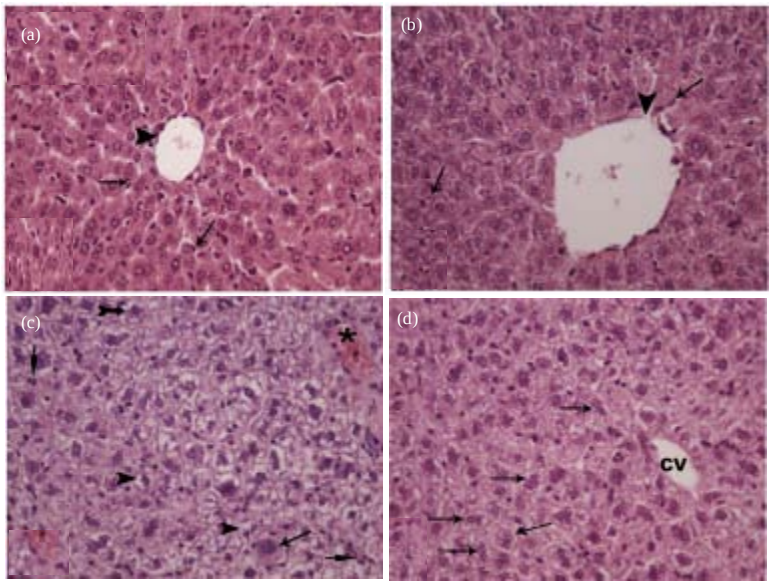

Fig. 4(a-d): a and b: Photomicrographs of hepati tissue from contro and L-carnitine treated groups, respectively showing central veins (arrowheads) and Kupffer cells (arrows). c: Photomicrograph of hepatic tissue from gamma rays irradiated group displaying hepatocytes with vacuolated cytoplasm, hypertrophy (arrow) and nuclear changes including irregular (arrowheads) and pyknotic (forked-tailed arrow) forms, Kupffer cells hyperplasia (longhead arrows) and venous congestion (asterisk). d: Photomicrograph of hepatic tissue from L-carnitine pretreated, gamma rays irradiated group showing central vein (CV) and regenerative activity as represented by high frequency of binucleated hepatocytes (arrows). Original magnification: X400 (a-d)

received 10 Gy of whole-body gamma irradiation exhibited increased levels of IL-1β, TNF-α and IL-6 mRNA at 3 and 6 h post irradiation (Linard *et al*., 2004). Additionally, doses of irradiation as low as 1 cGy, could elicit an inflammatory response. This low dose is close to that used for many diagnostic procedures (Fujimori *et al*., 2005) and therefore, we used this dose in the present study.

In order to protect normal tissues from potential irradiation damage, it would be important to identify biological or chemical agents which, when given before exposure to radiation could protect normal tissues. Most of these agents are antioxidants in nature. These antioxidants include vitamin C, vitamin E, selenium, L-carnosine and L-carnitine (Zhou *et al*., 2003).

In the present study, we evaluated the effect of L-carnitine as a natural antioxidant in protection against gamma irradiation-induced tissue injury in mice. As expected gamma irradiation upregulated the mRNA expression of proinflammatory mediators; TNF-α, IL-1β and IFN-γ (Fig. 1-3). The extent of increase in the proinflammatory cytokines was more than 12 folds increase in TNF-α, about 6 folds increase in case of IL-1β and more than 8 folds increase in IFN-γ. Higher TNF-α levels are generally disadvantageous and capable of producing tissue injures as it is stated previously that TNF-α induces apoptosis through activation of caspase-3 and distal caspase effectors cascade protein leading to poly-ADP-ribosyle polymerase activation and DNA fragmentation

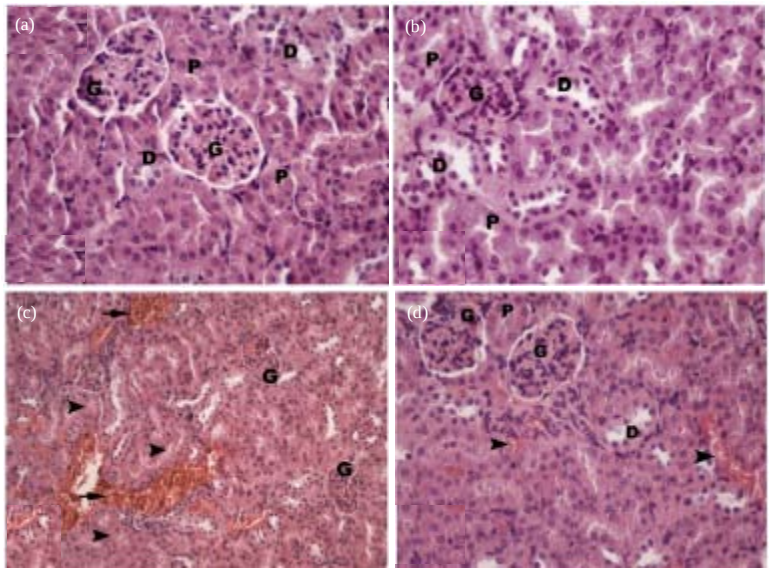

Fig. 5(a-d): a and b: Photomicrographs of renal tissues from control and L-carnitine treated groups respectively showing glomeruli (G), proximal (P) and (D) convoluted tubules. c: Photomicrograph of renal cortex from gamma irradiated group presenting glomerular hypercellularity (G), congestion and interstitial haemorrhages (longhead arrows) and cloudy swelling in proximal tubules (arrowheads). d: Photomicrograph of renal tissue from L-carnitine pretreated, gamma irradiated group showing reduction of congestion and interstitial haemorrhages (arrowheads) and more or less normal structures represented by glomeruli (G), proximal (P) and distal (D) convoluted tubules. Original magnification: X400 (a, b and d), X200(C)

(Epperly *et al.*, 2002; Thangasamy *et al.*, 2009). For, IFN-γ it is suggested to play a central role in Con A-hepatitis by activating Fas-induced apoptosis of liver cells (Tagawa *et al.*, 1997). Both IL-1β and IFN-γ, activate signaling pathways involved in cell apoptosis (Collier *et al.*, 2011). These obtained results at molecular level were consistent with those of histopathological examination (Fig. 4). Cytokines are signaling molecules that are key mediators of inflammations and immune response. These signaling molecules have a wide variety of cellular functions and are stimulated when tissue homeostasis is altered. Among these cytokines, IL-1, IL-6, TNF-α are inflammatory while IL-4, IL-10, IL-13 are anti-inflammatory. Depending on the balance of cytokines, their collective effect can be either pro-or anti-inflammatory. Upon binding to their membrane receptor, cytokines activate signal transduction pathways that lead to apoptosis, cell proliferation, angiogenesis and cellar senescence (Schetter *et al.*, 2010). Th precursor (THP) cells can differentiate into Th1, Th2, or Th0 cells. Th0 cells can differentiate to Th1 and Th2 subpopulations depending primarily on the cytokines provided exogenously or from dendritic cells. Th1 cells are involved in cellular immunity, whereas Th 2 cells are mainly associated with humeral immunity. Th1 cytokines include interferon (IFN)-γ, IL-12 and TNF-α. They can activate macrophages to produce reactive oxygen intermediates and NO, stimulate their phagocytic functions and enhance their antigen presenting capacity by upregulating Major Histocompatibility Complex (MHC) class molecules

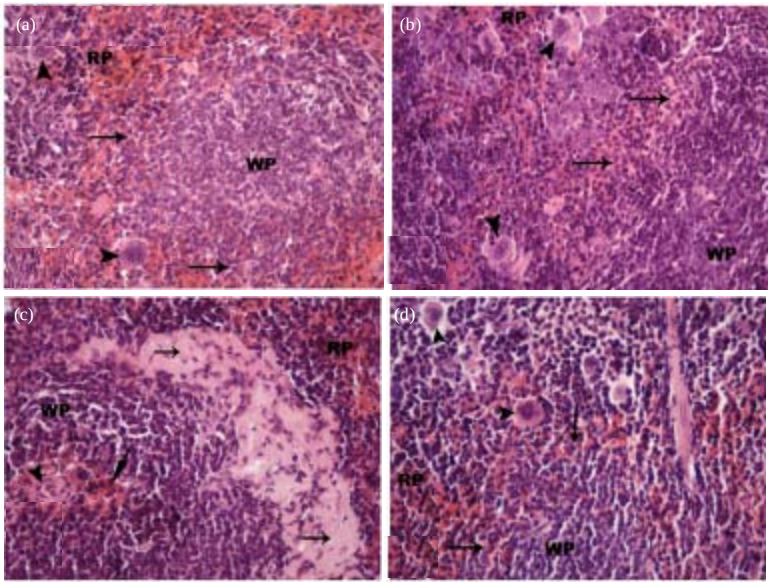

Fig. 6(a-d): a and b: Photomicrographs of splenic tissues from control and L-carnitine treated groups, respectively displaying red (RP) and white (WP) pulps and macrophages (arrowheads) within the marginal zone (arrows). c: Photomicrograph of splenic tissue from gamma rays irradiated group presenting thickening of follicular arteriole (arrowhead) and periarteriolar haemorrhage (longhead arrow) in splenic follicles and depletion of lymphocytes and macrophages from the marginal zone (arrows) at the interface between the white (WP) and red (RP) pulps. d: Photomicrograph of splenic tissue from L-carnitine pre-treated, gamma rays irradiated group showing the normal histology of the spleen and reappearance of lymphocytes and macrophages (arrowheads) in the marginal zone (arrows) at the interface between the white (WP) and red (RP) pulps. Original magnification: X400 (a-d)

(Zhang *et al*., 2011). There is a correlation between ROS production and pro-inflammatory cytokines expression as oxygen species contribute to the production pro-inflammatory cytokine. Free radical scavenger may be a useful treatment and prevention of the systemic inflammatory response occurs in shock states (Tamion *et al*., 2000). In response to a stimulus, phagocytic cells release ROS and non-phagocytic cells are stimulated to produce ROS by pro-inflammatory cytokines (Hussain *et al*., 2003). Proper regulation of ROS is vital for an efficient immune response and for limiting tissue damage. In our results, L-carnitine showed an immunostimulatory effect as indicated by the upregulation of mRNA expression of TNF-α, IL-1β and IFN-γ (Fig. 1-3). In L-carnitine group, meanwhile the degree upregulation did not reach to the extent of tissue injury as L-carnitine group showed more or less normal tissues architectures in liver (Fig. 4b), kidney (Fig. 4b) and spleen (Fig. 6b), on histopathological examination. This immunostimulatory effect of L-Carnitine on the tested cytokines is in accordance to the results of previous study (Dionyssopoulou *et al*., 2005). On co-treatment L-carnitine and γ irradiation, L-carnitine exhibited a powerful down regulation of mRNA expression of all tested cytokines. The histopathological findings are in accordance with results of cytokine expressions. This suppressive effect of

L-carnitine on the γ- irradiation-induced cytokine expression may be explained by the previously proven antioxidant effect of L-carnitine (Zhou *et al*., 2003) as removing of the free radicals reduce the inflammatory stress. These results are in agreement with the previous study reported that the protective role of L-carnitine against ROS-induced damage could be attributed to its antioxidant role (Thangasamy *et al*., 2009). This is also supported by a study of Hao *et al*. (2009) where they observed that 12 week long treatment of diabetic rat with a combination of mitochondrial targeting nutrients like L-carnitine enhanced immune functions, inhibited oxidative damage and apoptosis processes. In accordance with these results, Famularo *et al*. (2004) reported that L-carnitine down regulates proinflammatory cytokines including IL-1 and TNF-α (Famularo *et al*., 2004). Moreover, this effect of L-carnitine on cytokine expression in the presence of γ-irradiation may be explained by the ability of L-carnitine to mimic the suppressive effect of glucocorticoides on proinflammatory cytokines expression through their action on glucocorticoides receptor-α (GR-α) (Seike *et al*., 2009).

CONCLUSION

Present results clarified the immunomodulatory effects of L-carnitine and also its radioprotective role against tissue injury induced by γ-irradiation on molecular basis. Moreover, the results suggest that pharmacological doses of L-carnitine might be used as a radioprotective agent for people receiving radiotherapy and those working at the field of radiodiagnostics.

ACKNOWLEDGMENT

This work has been done under the financial support of Taif University of Kingdom of Saudi Arabia (Project number1/432/1349).

The authors are thankful to Dr. Mohamed Alkafafy; Associate Prof. at the Faculty of Science, Taif University, KSA for his valuable help to achieve the histopathological examination in this study.

REFERENCES

Ao, X., L. Zhao, M.A. Davis, D.M. Lubman, T.S. Lawrence and F.M. Kong, 2009. Radiation produces differential changes in cytokine profiles in radiation lung fibrosis sensitive and resistant mice. J. Hematol. Oncol., 2: 6-6.

Borek, C., 2004. Antioxidants and radiation therapy. J. Nutr., 134: 3207S-3209S.

Chen, Y., J. Williams, I. Ding, E. Hernady and W. Liu *et al*., 2002. Radiation pneumonitis and early circulatory cytokine markers. Semin Radiat Oncol., 12: 26-33.

Chen, Y., O. Hyrien, J. Williams, P. Okunieff, T. Smudzin and P. Rubin, 2005. Interleukin (IL)-1A and IL-6: Applications to the predictive diagnostic testing of radiation pneumonitis. Int. J. Radiat. Oncol. Biol. Phys., 62: 260-266.

Collier, J.J., S.J. Burke, M.E. Eisenhauer, D. Lu, R.C. Sapp , C.J. Frydman and S.R. Campagna, 2011. Pancreatic β-cell death in response to pro-inflammatory cytokines is distinct from genuine apoptosis. PLoS One, 6: e22485-e22485.

Deufel, T., 1990. Determination of L-carnitine in biological fluids and tissues. J. Clin. Chem. Clin. Biochem., 28: 307-311.

Dionyssopoulou, E., S. Vassiliadis, A. Evangeliou, E.E. Koumantakis and I. Athanassakis, 2005. Constitutive or induced elevated levels of L-carnitine correlate with the cytokine and cellular profile of endometriosis. J. Reprod. Immunol., 65: 159-170.

Epperly, M.W., J.A. Gretton, S.J. DeFilippi, C.A. Sikora, D. Liggitt, G. Koe and J.S. Greenberger, 2001. Modulation of radiation-induced cytokine elevation associated with esophagitis and esophageal stricture by manganese superoxide dismutase-plasmid/liposome (SOD-PL) gene therapy. Radiat. Res., 155: 2-14.

Epperly, M.W., C. Sikora, S. DeFilippi, J. Gretton, Q. Zhan, D.W. Kufe and J.S. Greenberger, 2002. MnSOD inhibits iradiation-induced apoptosis by stabilization of the mitochondrial membrane against the effects of SAP kinase p38 and Jnk1 translocation. Radiat. Res., 157: 568-577.

Epperly, M.W., J.E. Gretton, M. Bernarding, S. Nie, B. Rasul and J.S. Greenberger, 2003a. Mitochondrial localization of copper/zinc superoxide dismutase (Cu/ZnSOD) confers radioprotective functions *in vitro* and *in vivo*. Radiat. Res., 160: 568-578.

Epperly, M.W., M. Bernarding, J. Gretton, M. Jefferson, S. Nie and J.S. Greenberger, 2003b. Overexpression of the transgene for manganese Superoxide dismutase (MnSOD) in 32D cl 3 cells prevents apoptosis induction by TNF-α , IL-3 withdrawal and ionizing iradiation. Exp. Hematol., 31: 465-474.

Famularo, G., C. De Simone, S. Tzantzoglou and V. Trinchieri, 1994. Aopotosis, anti-apoptotic compounds and TNF-alpha release. Immunol. Today, 15: 495-496.

Famularo, G., C. de Simone, V. Trinchieri and L. Mosca, 1997. Carnitine Deficiency: Primary and Secondary Syndromes. In: Carnitine Today, De Simone, C. and G. Famularo (Eds.). Landes Bioscience, Austin, TX., USA., pp: 119-161.

Famularo, G., C. De Simone, V. Trinchieri and L. Mosca, 2004. Carnitines and its congeners: a metabolic pathway to the regulation of immune response and inflammation. Ann. N. Y. Acad. Sci., 1033: 132-138.

Fujimori, A., R. Okayasu, H. Ishihara, S. Yoshida and K. Eguchi-Kasai *et al*., 2005. Extremely low dose ionizing radiation up-regulates CXC chemokines in normal human fibroblasts. Cancer Res., 65: 10159-10163.

Goa, K.L. and R.N. Brogden, 1987. L-Carnitine. Drugs, 34: 1-24.

Hall, E.J.B., 1999. Radiation Biology for Radiobiologist. 4th Edn., Lippincott Inc., Philadelphia, PA., USA.

Hao, J., W. Shen, C. Tian, Z. Liu and J. Ren *et al*., 2009. Mitochondrial nutrients improve immune dysfunction in the type 2 diabetic Goto-Kakizaki rats. J. Cell Mol. Med., 13: 701-711.

Hussain, S.P., L.J. Hofseth and C.C. Harris, 2003. Radical causes of cancer. Nat. Rev. Cancer, 3: 276-285.

Izgut-Uysal, V.N., A. Agac and N. Derin, 2001. Effect of carnitine on stress-induced lipid peroxidation in rat gastric mucosa. J. Gastroenterol., 36: 231-236.

Kim, S.H., S.R. Kim, H.J. Lee, H. Oh and S.Y. Ryu *et al*., 2003. Apoptosis in growing hair follicles following gamma irradiation and application for the evaluation of radioprotective agents. In Vivo, 17: 211-214.

Konopacka, M. and J. Rogolinski, 2004. Thiamine prevents X-ray induction of genetic changes in human lymphocytes in vitro. Acta Biochem. Pol., 51: 839-843.

Linard, C., C. Marquette, J. Mathieu, A. Pennequin, D. Clarencon and D. Mathe, 2004. Acute induction of inflammatory cytokine expression after gamma-irradiation in the rat: effect of an NF-kappa B inhibitor. Int. J. Radiat. Oncol. Biol. Phys., 58: 427-434.

Monti, D., L. Troiano and F. Trpea, 1992. Apoptosis-programmed cell death: A role in the ageing process. Am. J. Clin. Nutr., 55: 1208S-1214S.

Narayanan, P.K., K.E. LaRue, E.H. Goodwin and B.E. Lehnert, 1999. Alpha particles induce the production of interleukin-8 by human cells. Radiat. Res., 152: 57-63.

Pearce, L.L., M.W. Epperly, J.S. Greenberger, B. Pitt and J. Peterson, 2001. Identification of respiratory complexes I and III as mitochondrial sites of damage following exposure to ionizing radiation and nitric oxide. Nitric Oxide Biol. Chem., 5: 128-136.

Schetter, A.J., N.H.H. Heegaard and C.C. Harris, 2010. Inflammation and cancer: Interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis, 31: 37-49.

Seike, M., A. Goto, T. Okano, E.D. Bowman and A.J. Schetter *et al.*, 2009. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proc. Natl. Acad. Sci. USA, 106: 12085-12090.

Tagawa, Y., K. Sekikawa and Y. Iwakura, 1997. Suppression of concanavalin A-induced hepatitis in IFN-γ(-/-) mice but not in TNF-α(-/-) mice: Role for IFN-γ in activating apoptosis of hepatocytes. J. Immunol., 159: 1418-1428.

Tamion, F., V. Richard, G. Bonmarchand, J. Leroy and M. Hiron *et al.*, 2000. Reduced synthesis of inflammatory cytokines by a free radical scavenger after hemorrhagic shock in rats. Crit. Care Med., 28: 2522-2527.

Thangasamy, T., P. Jeyakumar, S. Sittadjody, A.G. Joyee and P. Chinnakannu, 2009. L-carnitine mediates protection against DNA damage in lymphocytes of aged rats. Biogerontology, 10: 163-172.

Ucuncu, H., M.V. Ertekin, O. Yoruk, O. Sezen and A. Ozkan *et al.*, 2006. Vitamin E and L-carnitine, separately or in combination, in the prevention of radiation-induced oral mucositis and myelosuppression: A controlled study in a rat model. J. Radiat. Res., 47: 91-102.

Van Der Meeren, A., J.M. Bertho, M. Vandamme and M.H. Gaugler, 1997. Ionizing radiation enhances IL-6 and IL-8 production by human endothelial cells. Mediators Inflamm., 6: 185-193.

Zhang, H., L.M. Wu and J. Wu, 2011. Cross-talk between apolipoprotein E and cytokines. Mediators Inflamm., Vol. 2011, 10.1155/2011/949072

Zhou, N., H. Xiao, T.K. Li, A. Nur-E-Kamal and L.F. Liu, 2003. DNA damage-mediated apoptosis induced by selenium compounds. J. Biol. Chem., 278: 29532-29537.