Toxicology and Industrial Health
2015, Vol. 31(12) 1334–1340

DOI: 10.1177/0748233713491811
tih.sagepub.com

The chemoprotective effects of L-carnitine against genotoxicity induced by diazinon in rat blood lymphocyte

Amir Shadboorestan[1], Mohammad Shokrzadeh[1], Nematollah Ahangar[1], Mohammad Abdollahi[2], Mahmood Omidi[1] and Sakine Sadat Hosseini Payam[3]

Abstract

The purpose of this study was to assess the preventive effects of L-carnitine (LC) against DNA damage induced by diazinon (DZN) in rat blood lymphocytes. Animals were concurrently administered intraperitoneally with DZN in proper solvent (20 mg/kg body weight (b.w.)) and LC at three different doses (50, 100, and 150 mg/kg b.w.) for 30 consecutive days. The positive control group received DZN at the same dose without LC. Twenty-four hour after last injection, 0.5 ml blood of each rat was received and cultured in culture medium for 44 h. The lymphocyte cultures were mitogenically stimulated with cytochalasin B for the evaluation of the number of micronuclei (MNs) in cytokinesis-blocked binucleated cells. Incubation of lymphocytes with DZN induced additional genotoxicity and was shown by increase in MNs frequency in rat lymphocytes. LC at all doses had a protective effect and significantly reduced the MNs frequency in cultured lymphocytes ($p < 0.0001$–$p < 0.05$). The maximum effect was observed at 150 mg/kg that reduced the frequency of MN from 12.78 $\pm$ 0.24% for DZN group to 5.61 $\pm$ 0.17%. Our study revealed that LC has a potent antigenotoxic effect against DZN-induced toxicity in rats, which may be due to the scavenging of free radicals and increased antioxidant status. Since LC is a natural compound and is being safe, it is recommended as a daily supplement for body defense against side effects induced by chemical hazardous agents.

Keywords

Chemoprotective, L-carnitine, genotoxicity, diazinon, micronucleus assay, rat blood lymphocyte

Introduction

There are many reports that the hazardous environmental chemical induces genotoxic and carcinogenic effects on mammals. The main mechanism is producing of species-free radical that induces damage to critical macromolecules such as DNA to promote chronic diseases such as cancers. Although human body is equipped with self-defense mechanisms, such as detoxification process through various enzymes, serious exposure to dangerous chemicals can lead to mutagenic and carcinogenic events (Sporn, 1993; Tiwari, 2001).

Diazinon (DZN) is a commonly used organophosphorous (OP) pesticide (diethoxy-[(2-isoprophyl-6-methyl-4-pyrimidinyl) oxy]-thioxophosphorane). It is a synthetic chemical substance with a broad spectrum insecticide activity (Sarabia et al., 2009). Toxic

[1] Pharmaceutical Sciences Research Center, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Islamic Republic of Iran
[2] Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran
[3] Department of Occupational Health, Faculty of Health, North Khorasan University of Medical Sciences, Bojnurd, Islamic Republic of Iran

Corresponding author:
Mohammad Shokrzadeh, Pharmaceutical Sciences Research Center, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, 18 Kilometer of Khazarabad Road, Sari 48175-861, Islamic Republic of Iran.
Email: mslamuk@yahoo.com

effects of DZN are due to the inhibition of acetylcholinesterase activity, an enzyme needed for proper nervous system function. It has been widely used throughout the world with applications in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruits, vegetables, and other food products (El-Shenawy et al., 2010; Grafitt et al., 2002). Some reports have been published with respect to DZN and its effects on hematological and biochemical parameters of rats, rabbits, and mice (El-Shenawy et al., 2009; Kalender et al., 2005, 2006; Quistad et al., 2001; Yehia et al., 2007). Toxicities of OP insecticide DZN cause adverse effects on many organs (Kalender et al., 2005). DZN's mutagenicity studies, its ability to cause genetic damage, showed that DZN in fact can damage DNA in human blood cells, cells from laboratory animals, and bacteria (Grover et al., 2003). DZN exposure was found to increase the occurrence of a type of genetic damage called MNs. MN may be induced by strand breaks in DNA due to oxidative stress (Fenech, 1993). The MN test using peripheral blood cells is used to detect chromosome breaks derived from DNA damage (Igarashi and Shimada, 1997).

Systems that protect the living body from oxidative stress-induced damage include superoxide dismutase, catalase, and glutathione peroxidase enzymes that remove reactive oxygen species (ROS); proteins ferritin and transferrin that mask the heavy metal ions; antioxidant system including vitamins E and C; enzymatic repair systems for damaged DNA; and enzyme systems ubiquitin–proteasome that remove damaged proteins (Kikugawa, 2004; Limon-Pacheco and Gonsebatt, 2009). Antioxidant micronutrients are being widely studied for their alleged beneficial properties in the prevention of human diseases, cancer, arthritis, and cardiovascular diseases (Faure et al., 2007). Much attention of preventive medicine research is focused on natural antioxidants.

L-carnitine (LC) is a vitamin-like substance that is structurally similar to amino acids. Most carnitine is obtained from diet such as mushroom, carrot, bread, rice, and tomato. It can also be synthesized endogenously by skeletal muscle, heart, liver, kidney, and brain from the essential amino acids lysine and methionine (Rebouche and Seim, 1998). LC is an important cofactor in the fatty acid metabolism and is known to have antioxidant properties (Rani and Panneerselvam, 2001). It plays an important role in β-oxidation of long-chain fatty acids in the mitochondria (Brass, 2000). It is known that LC and its derivatives prevent the formation of ROS and protect cells from peroxidative stress (Dokmeci et al., 2006). Some studies indicate a carnitine-dependent reduction in either DNA single-strand breaks in isolated human lymphocytes after *in vitro* treatment with an oxygen radical-generating system (Boerrigter et al., 1993) or DNA cleavage induced by hydrogen peroxide ultraviolet–photolysis (Vanella et al., 2000). Also, LC has DNA repair capability and decreased induction of aberrations in *Ataxia telangiectasia* patients (Berni et al., 2008).

Taken together, LC showed a potent antioxidant activity and free-radical-scavenging properties. This characteristic of LC could introduce a safe and right candidate agent to prevent diseases caused by free radicals. On the other hand, the use of DZN as a pesticide has been increasing in farmer lands and as a result, it can indirectly enter into the body of people through the consumption of crops such as rice. So, in the long-term, DZN can reveal its side effects. One of the most important side effects of DZN is that it can induce genetic damage, chromosomal aberration, cell death, and eventually cancer. Thus, the search to obtain the best and effective chemoprotective agent that protects the body cells from DNA damage induced by DZN with fewer side effects will be a merit. Therefore, the current study was performed to assess the *in vivo* protective effects of LC against genotoxicity induced by DZN in rat peripheral blood lymphocytes using the MN test.

Materials and methods

Animals

Male Wistar albino rats ($n = 40$), aged 5–7 months and weighing 150–200 g, were purchased from the Pasteur Institute of Iran (North Branch, Amol, Iran) and were kept in a good condition at the university animal section and were given standard food pellets and water *ad libitum*. All the animals were maintained under controlled conditions of light (12/24 h) and temperature ($23 \pm 1^{\circ}C$). Their usage and the experimental protocol used in this study were approved by the Ethical Committee of the Medical Sciences, University of Mazandaran, Iran.

Experimental design

In the experiment, a total of 40 rats were used. Animals were concurrently administered by intraperitoneal (i.p.) injection of DZN in a proper solvent (20 mg/kg b.w.) and L-Carnitine at three different doses

(50, 100, and 150 mg/kg body weight (b.w.)) for 30 consecutive days. The positive control group also received DZN at the same dose without LC. The rats were randomly divided into nine groups (groups 1–9), with five animals in each group:

- Group 1: normal control; received normal saline (10 ml/kg b.w.) by i.p. injection for 30 consecutive days.
- Group 2: vehicle control, received soy bean oil (10 ml/kg b.w.) by i.p. injection for 30 consecutive days.
- Group 3: positive control; received DZN (20 mg/kg b.w.) by i.p. injection in soy bean oil (10 ml/kg b.w.) for 30 consecutive days.
- Group 4: treated with LC (50 mg/kg b.w.) in normal saline (10 ml/kg b.w.) by i.p injection for 30 consecutive days.
- Group 5: treated with LC (100 mg/kg b.w.) in normal saline (10 ml/kg b.w.) by i.p. injection for 30 consecutive days.
- Group 6: treated with LC (150 mg/kg b.w.) in normal saline (10 ml/kg b.w.) by i.p. injection for 30 consecutive days.
- Group 7: concomitantly treated with LC (50 mg/kg b.w.) and DZN (20 mg/kg b.w.) by i.p. injection for 30 consecutive days.
- Group 8: concomitantly treated with LC (100 mg/kg b.w.) and DZN (20 mg/kg b.w.) by i.p. injection for 30 consecutive days.
- Group 9: concomitantly treated with LC (150 mg/kg b.w.) and DZN (20 mg/kg b.w.) by i.p. injection for 30 consecutive days.

The dose of 20 mg/kg DZN and the period of treatment were selected on the basis of previous studies (El-Shenawy et al., 2009, 2010; Grafitt et al., 2002; Kalender et al., 2005, 2006; Quistad et al., 2001; Sarabia et al., 2009; Yehia et al., 2007). Also, screening pretests were performed to achieve the best effective dose range of LC. In the current study, the dose range of LC was similar to other studies.

MNs assay

Twenty-four hours after last injection, blood samples (0.5 ml) from each rat were added to 4.5 ml of RPMI 1640 culture medium (Gibco, Karlsruhe, Germany), which contained a mixture of 20% fetal calf serum, 20 µl/ml phytohemagglutinin (Gibco, Paisley, UK), 50 U/ml penicillin, 50 µg/ml streptomycin, and 2 mM glutamine (Sigma, Poole, UK) at final concentration. All cultures were incubated at 37 ± 1°C in a humidified atmosphere of 5% carbon dioxide and 95% air. Cytochalasin B (Final concentration: 6 µl/ml) (Fluka, Buchs, Switzerland) was added after 21 h of culturing. After 44 h of incubation, the cells were collected by centrifugation for 8 min at 1000 r/min, resuspended in 0.075 M cold potassium chloride, and immediately fixed in a fixative solution (methanol: acetic acid, 6:1) three times. The fixed cells were dropped onto clean microscopic slides, air-dried, and stained with Giemsa solution. All slides were evaluated using 40× magnification microscope to determine the frequency of MNs in the cytokinesis-blocked binucleated cell with a well-preserved cytoplasm. A typical image of binucleated cells with and without MNs is shown in Figure 1. The criteria for scoring MNs were the diameter should be between 1/16th and one-third of the main nuclei, nonrefractile, not linked to the main nuclei, and not overlapping with the main nuclei (Hosseinimehr et al., 2009). For each rats, three slides were prepared, and for each slide, a total of 1000 binucleated cells were scored to record the percentage of frequency of MNs.

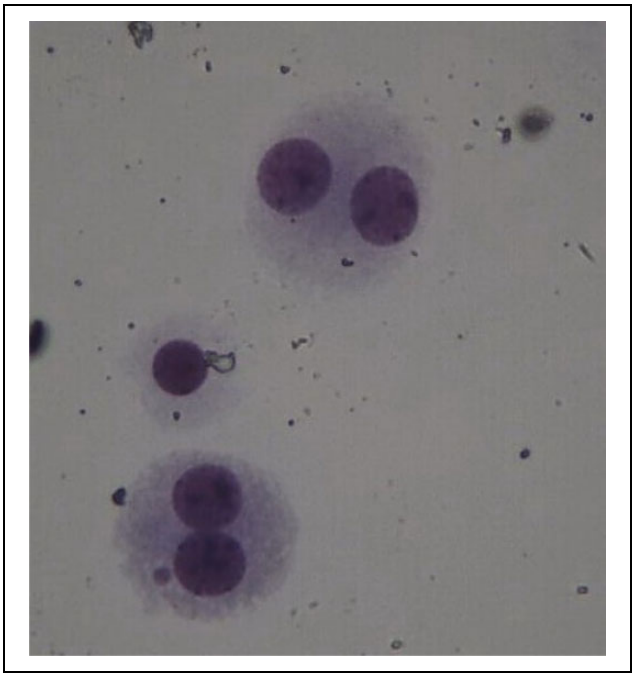

Figure 1. A typical binucleated lymphocyte with micronuclei (down) and without micronuclei (up).

Statistical analysis

For each rat, at each group, the incidence of DZN-induced MNs per 3000 binucleated lymphocyte and

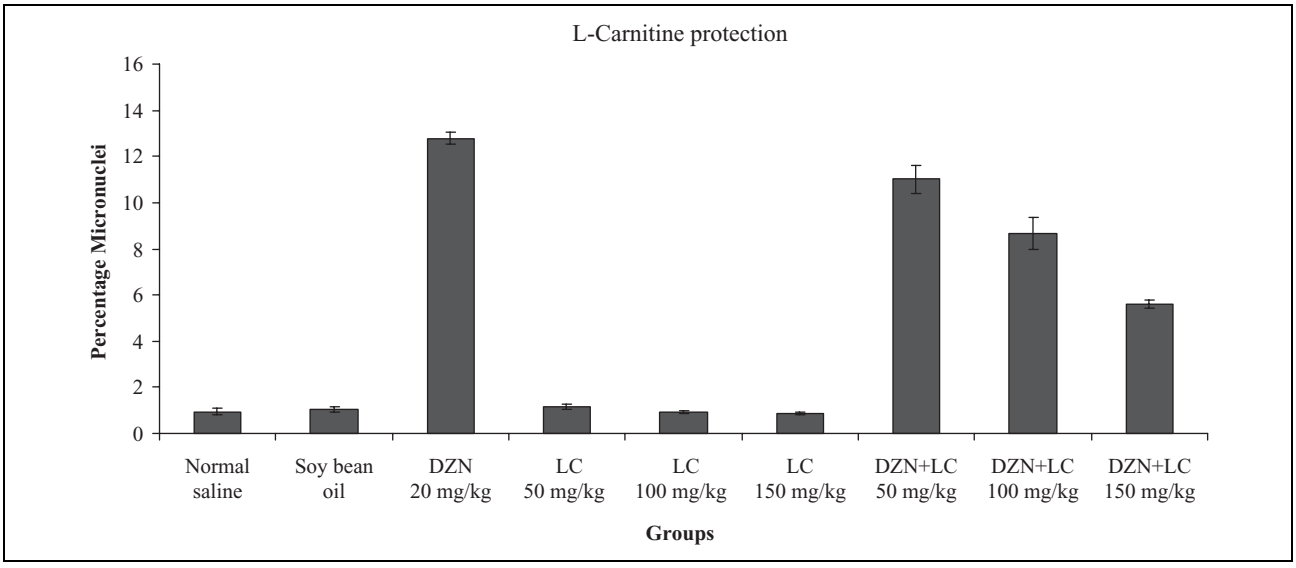

Figure 2. *In vivo* protection by LC at different doses (in milligram per kilogram) against DZN-induced genetic damage in rat blood lymphocyte. The frequency of micronucleus is represented as the percentage of the mean ± SD of five rats. Three slides were prepared for each rat and a total of 3000 binucleated lymphocytes were scored to determine the number of MNs for each rat. $p < 0.0001$: control sample compared with similarly administrated lymphocytes from the blood sample treated with DZN. $p < 0.0001$: DZN sample compared with DZN + LC 100 mg/kg, DZN + LC 150 mg/kg. $p < 0.05$: DZN sample compared with DZN + LC 50 mg/kg. $p < 0.05$: control sample compared with similarly administered lymphocytes from the blood sample treated with LC 50 mg/kg, LC 100 mg/kg, and LC 150 mg/kg. LC: L-carnitine; DZN: diazinon; MN: micronucleus.

the possible reduction by LC was recorded. The data are presented as means ± SD. Data were analyzed with Student's *t* test. A $p < 0.05$ was considered to be significant.

Results

The data presented in Figure 2 showed a significant difference in the percentage of micronucleated binucleated cells in lymphocytes treated with DZN, compared with those in control cells ($p < 0.0001$). The percentage of MNs in the lymphocytes of rats treated with 20 mg/kg of DZN for 30 consecutive days through i.p. injection was 12.78 ± 0.24%, while the percentage in nontreated control lymphocytes was 0.95 ± 0.12% (Table 1). The frequency of MNs in rats received concomitant treatment with LC at doses of 50, 100, and 150 mg/kg and DZN was 11.02 ± 0.6, 8.66 ± 0.67, and 5.61 ± 0.17%, respectively (Table 1). These results were significantly lower than the rats that received DZN alone ($p < 0.0001$–0.05; Figure 2). The total micronucleated binucleated cell reduction values were 14, 32, and 56% in 50, 100, and 150 mg/kg doses of LC. It was higher at a high dose of 150 mg/kg compared with those at 100 and 50 mg/kg doses that are in agreement with previous studies. LC alone did not cause any genotoxicity even at high dose of 150 mg/kg in rats without DZN ($p > 0.05$) so that LC is completely safe for cells.

Discussion

Our results indicated that LC treatment mitigated the genotoxicity induced by DZN in rat lymphocytes. DZN is one of the most commonly used OP pesticides that is used to control a range of crop pests and also as a veterinary ectoparasiticide in many countries including Iran. It degrades easily, but under condition of low temperature, low moisture, high alkalinity, and lack of suitable microbiological degraders, it may remain biologically active in soil for 6 months or longer (Koprucu et al., 2006). DZN causes changes in liver enzymes and biochemical indices and swelling of mitochondria in hepatocytes (Kalender et al., 2005). It also causes toxic effects on blood cells, spleen, thymus, and lymph nodes of rats (Handy et al., 2002) and on other organisms too (Svoboda et al., 2001). Metabolic processes produce free radicals that are highly reactive and unstable and also can cause oxidative damage to cells and tissues (Kanakis et al., 2009) Increase in the intracellular level of ROS, frequently referred to as oxidative stress,

Table 1. The total number of micronuclei per 1000 binicleated lymphocyte.[a]

Groups	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SD
Treatment						
Control	7.6 ± 2.52	11 ± 3.6	10 ± 2.65	9.67 ± 2.52	9.33 ± 1.52	9.53 ± 1.21
Vehicle	11.66 ± 2.08	10 ± 2.65	11 ± 3.61	9.66 ± 1.15	8.66 ± 1.52	10.19 ± 1.17
DZN (20 mg/kg)	124.66 ± 10.5	125.66 ± 19.3	130 ± 12.49	130 ± 15	128.66 ± 9.6	127.79 ± 2.48
LC (50 mg/kg)	11 ± 2	10.66 ± 3.05	10.33 ± 1.52	13.33 ± 1.52	12 ± 2	11.46 ± 1.21
LC (100 mg/kg)	9.66 ± 1.52	9 ± 3.60	10 ± 5.29	9.66 ± 2.08	8.66 ± 2.08	9.4 ± 0.54
LC (150 mg/kg)	9.66 ± 2.51	9 ± 2	9 ± 3.60	7.66 ± 2.08	8.66 ± 1.52	8.8 ± 0.73
DZN + LC (50 mg/kg)	107.66 ± 3.05	115 ± 14.10	116 ± 12.28	101.33 ± 4.16	111.33 ± 10.96	110.26 ± 5.97
DZN + LC (100 mg/kg)	81.33 ± 4.04	84.66 ± 7.76	84.33 ± 12.01	98.33 ± 15.94	84 ± 6.02	86.6 ± 6.69
DZN + LC (150 mg/kg)	56.66 ± 13.05	54.33 ± 7.76	54.33 ± 10.01	57.33 ± 9.07	58 ± 5	56.13 ± 1.70

LC: L-carnitine; DZN: diazinon; MN: micronucleus.

[a]For each rat, three slides were prepared and in each slide, 1000 micronucleated binucleated cells were scored. The number of MN was calculated for each rat per 1000 binucleated cells in each treatment group with mean ± SD calculations. At the end, the number of total MN per 1000 binucleated cells is represented as mean ± SD for five animals in each group treatment.

represent a potentially toxic insult, which interact with macromolecules to induce cell membrane dysfunction, lipid peroxidation, and DNA damage (Ahmadi et al., 2008) The results of many studies confirmed that DZN exposure induced DNA damage. Using MN assay, DZN treatment to human blood cells (lymphocytes and erythrocytes) and skin fibroblasts has been shown to results in a significant increase in the number of MN via clastogenic mode of action, inducing single- and double-strand breaks on DNA molecule (Colovic et al., 2010).

In the last years, the micronucleus (MN) assay has been extensively used to assess the cytogenetic damage induced by chemicals and radiation. This assay after the improvements introduced by Fenech and Morley (Fenech and Morley, 1985) appears to be sensitive, simple, and fast enough to detect agents that induce chromosome damage (Fenech, 2000). This method is now one of the best established *in vivo* and *in vitro* cytogenetic assays in the field of genotoxic biomonitoring (Decordier et al., 2009). In cytokinesis-blocked MN assay, cells that have completed one nuclear division are blocked from performing cytokinesis using cytochalasin B and are consequently readily identified by their binucleated appearance. MN are scored in binucleated cells only, which enables reliable comparison of chromosome damage between cell populations that may differ in their cell division (Fenech, 2000).

DZN is capable of inducing chromosomal aberrations such as MN. In the present study, DNA assay damage was evaluated by MN test. Administration of DZN resulted in DNA damage and induced MN in rats' blood lymphocyte. It is evident that exposure to DZN resulted in DNA damage as compared to control, and administration of LC for 30 consecutive days reduced this DNA damage in rats. LC reduced DZN-induced MN as a DNA damage index. These results suggest that LC has a protective effect on DZN-induced genotoxicity.

The observed protective effect of LC may be due to the direct protection against DNA damage by scavenging free radicals generated by DZN before they induce damage on the genetic material, that is, the extent of primary damage in cellular DNA may be significantly reduced (Kocer et al.,2007; Sundaram and Panneerselvam, 2006) Second, LC may indirectly alter the final level of DNA damage by enhancing the activity of DNA repairing enzyme, poly(ADP-ribose) polymerase, a nuclear protein that is intimately linked with the occurrence of DNA strand breaks and also other related repair mechanisms (Boerrigter et al., 1993; Szabodos et al., 1999), so that the damaged DNA is repaired more rapidly in DZN cells cotreated with LC. Other studies have also observed that the LC maintains thiol-containing compound and improves the glutathione redox status, which could protect DNA repairing enzymes, thereby preventing DNA strand breaks (Savitha and Panneerselvam, 2006). LC 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 against DNA damage induced by DZN.

Figure 2 shows that LC had a protective effect against DZN-induced DNA damage at all three doses used in this study. Based on the results, it is clear that DZN might be inducing DNA damage by increased generation of reactive free radicals that are scavenged by LC. Administration of DZN in combination with LC showed significant decrease in the percentage of MN as compared to DZN-treated rats. It is evident that LC might be reducing the MN formation by scavenging the DNA damaging free radicals generated following DZN exposure. Another possible mechanism of LC might involve selective removal of cells with DNA damage by apoptosis. These results demonstrate that administration of LC along with DZN decreased the DNA damage and thus protected the cells against genotoxic effect of DZN.

Conclusion

Our findings demonstrate that DZN is genotoxic as assessed by MN assays and had adverse effects on peripheral blood lymphocytes. LC, on the other hand, was observed to repair the genotoxicity and reduced the frequency of MN induced by DZN. It can be concluded that LC, as an antioxidant, has protective effect against DZN adverse effects by inactivating (scavenging) free radicals generated following DZN exposure, and daily supplement of LC might be beneficial to pesticide-exposed populations.

Conflict of interest

The authors declared no conflicts of interest.

Funding

This study was approved and supported by a grant from the Mazandaran University of Medical Sciences, Sari, Iran.

References

Ahmadi A, Hosseinimehr SJ, Naghshvar F, Hajir E, and Ghahremani M (2008) Chemoprotective effects of hesperidin against genotoxicity induced by cyclophosphamide in mice bone marrow cells. *Archives of Pharmacal Research* 31: 794–797.

Berni A, Meschini R, Filippi S, Palitti F, Arnicisb A, and Chessab L (2008) L-carnitme enhances resistance to oxidative stress by reducing DNA damage in *Ataxia telangiectasia* cells. *Mutation Research* 650: 165–174.

Boerrigter ME, Franceschi C, Arrigoni-Martelli E, Wei JY, and Vijg J (1993) The effect of L-carnitine and acetyl-L-carnitine on the disappearance of DNA single-strand breaks in human peripheral blood lymphocytes. *Carcinogenesis* 14: 2131–2136.

Brass EM (2000) Supplemental carnitine and exercise. *The American Journal of Clinical Nutrition* 72: 618–623.

Colovic M, Krstic D, Petrovic S, Leskovac A, Joksić G, Savić J, et al. (2010) Toxic effects of diazinon and its photodegradation products. *Toxicol Letteres* 193: 9–18.

Decordier I, Papine A, Plas G, Roesems S, Vande Loock K, Moreno-Palomo J, et al. (2009) Automated image analysis of cytokinesis-blocked micronuclei: an adapted protocol and a validated scoring procedure for biomonitoring. *Mutagenesis* 24: 85–93.

Dokmeci D, Akpolat M, Aydogu N, Uzal C, Doganay L, and Turan FN (2006) The protective effect of L-carnitine on ionizing radiation induced free oxygen radicals. *Scandinavian Journal of Laboratory Animal Science* 33: 75–83.

El-Shenawy NS, Al-Eisa RA, El-Salmy F, and Salah O (2009) Prophylactic effect of vitamin E against hepatotoxicity, nephrotoxicity, haematological induces and histopathology induced by diazinon insecticide in mice. *Current Zoology* 55: 219–226.

El-Shenawy NS, El-Salmy F, Al-Eisa RA, and El-Ahmary B (2010) Amelioratory effect of vitamin E on organophosphorus insecticide diazinon-induced oxidative stress in mice liver. *Pesticide Biochemistry and Physiology* 96: 101–107.

Faure P, Baeclay D, Joyeux-Faure M, and Halim S (2007) Comparison of the effects of zinc alone and zinc associated with selenium and vitamin E on insuline sensitivity and oxidative stress in high-fructose-fed rats. *Journal of Trace Elements in Medicine and Biology* 21: 113–119.

Fenech J, Morley AA (1985) Measurement of micronuclei in lymphocytes. *Mutation Research* 147: 29–36.

Fenech M (1993) The cytokinesis blocks micronucleus technique: a detailed description on the method and its application to genotoxicity studies in human population. *Mutation Research* 285: 35–44.

Fenech M (2000) The *in vitro* micronucleus technique. *Mutation Research* 455: 81–95.

Grafitt SJ, Jones K, Mason HJ, and Cocker J (2002) Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. *Toxicology Letters* 134: 105–113.

Grover P, Danadevi K, Mahboob M, Rozati R, Saleha-Banu B, and Rahman MF (2003) Evaluation of genetic damage in workers employed in pesticide production utilizing the comet assay. *Mutagenesis* 18: 201–205.

Handy RD, Abd-El Samei HA, Bayomy MFF, Mahran AM, Abdeen AM, and El-Elaimy EA (2002) Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse. *Toxicology* 172: 13–34.

Hosseinimehr SJ, Ahmadi A, Beiki D, Habibi E, and Mahmoudzadeh A (2009) Protective effects of hesperidin against genotoxicity induced by ^{99m}Tc-MIBI in human cultured lymphocyte cells. *Nuclear Medicine and Biology* 36: 863–867.

Igarashi M, Shimada H (1997) An improved method for the mouse liver micronucleus test. *Mutation Research* 391: 49–55.

Kalender S, Ogutcu A, Uzunhisarcikli M, Açikgoz F, Durak D, Ulusoy Y, et al. (2005) Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicology* 211: 197–206.

Kalender Y, Uzunhisarcikli M, Ogutcu A, Acikgoz F, and Kalender S (2006) Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: the protective role of vitamin E. *Environmental Toxicology and Pharmacology* 22: 46–51.

Kanakis CD, Tarantilis PA, Pappas C, Bariyanga J, Tajmir-Riahi HA, and Polissiou MG (2009) An overview of structural features of DNA and RNA complexes with saffron compounds: models and antioxidant activity. *Journal of Photochemistry and Photobiology B: Biology* 95: 204–212.

Kikugawa K (2004) Strategy in a living body to protect against oxidative stress-induced damage. *Journal of Health Science* 50: 443–455.

Kocer I, Taysi S, Ertekin MV, Karslioglu I, Gepdiremen A, Sezen O, et al. (2007) The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: a rat model. *Graefe's Archive for Clinical and Experimental Ophthalmology* 245: 588–597.

Koprucu SS, Koprucu K, Ural MS, Ispir N, and Pala M (2006) Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (Silurus glanis L.). *Pesticide Biochemistry and Physiology* 86: 99–105.

Limon-Pacheco J, Gonsebatt ME (2009) The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. *Mutation Research* 674: 137–147.

Quistad GB, Sparksand SE, and Casida JE (2001) Fatty acid amide hydrolase inhibition by neruotoxic organophosphorus pesticides. *Toxicology and Applied Pharmacology* 173: 48–55.

Rani PJ, Panneerselvam C (2001) Carnitine as a free radical scavenger in aging. *Experimental Gerontology* 36: 1713–1726.

Rebouche CJ, Seim H (1998) Carnitine metabolism and its regulation in microorganisms and mammals. *Annual Review of Nutrition* 18: 39–61.

Sarabia L, Maurer I, and Bustos-Obregón E (2009) Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on the mouse testis. *Ecotoxicology and Environmental Safety* 72: 663–668.

Savitha S, Panneerselvam C (2006) L-Carnitine and α-lipoic acid improve age associated decline in mitochondrial respiratory chain activity in rat heart muscle. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 61: 650–659.

Savitha S, Panneerselvam C (2007) Mitigation of age-dependent oxidative damage to DNA in rat heart by carnitine and lipoic acid. *Mechanisms of Ageing and Development* 128(2): 206–212.

Sporn MB (1993) Chemoprevention of cancer. *Lancet* 342: 1211–1213.

Sundaram K, Panneerselvam KS (2006) Oxidative stress and DNA single strand breaks in skeletal muscle of aged rats: role of carnitine and lipoic acid. *Biogerontology* 7: 111–118.

Svoboda M, Luskova V, Drastichova J, and Zlabek V (2001) The effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L.). *Acta Veterinaria Brno* 70: 457–465.

Szabodos E, Fischer GM, Gallyas F, Kispal Jr, and Sumegi B (1999) Enhancement of ADP-ribosylation and its diminution by lipoate after ischemia-reperfusion in perfused rat heart. *Free Radical Biology and Medicine* 27: 1103–1113.

Tiwari AK (2001) Imbalance in antioxidant defence and human diseases: multiple approach of natural antioxidants therapy. *Current Science* 81: 1179–1187.

Vanella A, Russo A, Acquaviva R, Campisi A, Di Giacomo C, Sorrenti V, et al. (2000) L-propionyl-carnitine as superoxide scavenger, antioxidant, and cleavage protector. *Cell Biology and Toxicology* 16: 99–104.

Yehia MA, El-Banna SG, and Okab AB (2007) Diazinon toxicity affects histophysiological and biochemical parameters in rabbits. *Experimental and Toxicologic Pathology* 59: 215–225.