

## Effect of vitamin E and/or nicotine on the activities of lactate dehydrogenase isoenzyme in serum of gamma irradiated rats

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The present work aims to examine the competence of vitamin E in reducing the changes in lactate dehydrogenase (LDH) isoenzymes activities and the interference of nicotine. Vitamin E and nicotine were administrated to rats via daily i.p injection in concentrations of 10 mg/kg and 80 µg/kg of body weight, respectively, for 12 days before exposure to 7 Gy of whole body gamma irradiation. The activities of isocitrate dehydrogenase, creatinine phosphokinase (CPK) and the concentration of lipid peroxides (thiobarbituric reactive substances; TBARS) were observed. The results obtained revealed abnormal electrophoretic pattern of LDH isoenzyme 1 day after exposure to gamma rays. Three bands only appear on the agarose film comparing with 4 bands in normal control rats. Occasionally, the percentage of activity distribution within bands was differing where the serum LDH total activities displayed significant increase comparing with control rats. Serum isocitrate dehydrogenase and CPK activities were significantly increased from the normal control values. Variation in enzyme activities was associated with significant elevation in the concentration of TBARS in serum. The prolonged administration of vitamin E before radiation exposure reduced the changes occurred in LDH isoenzymes, isocitrate dehydrogenase and CPK activities. Reduction in TBARS concentration was also observed. The administration of nicotine resulted in abnormal pattern of LDH isoenzyme activities comparing with control rats. In rats receiving several doses of nicotine before exposure to gamma irradiation, the changes in measured parameters were more pronounced comparing with irradiated rats. The simultaneous administration of nicotine and vitamin E attenuated the improvement occurred in the case of rats treated with vitamin E before exposure to gamma irradiation. This study demonstrated that vitamin E has an antioxidant and a radioprotective efficacy against changes occurred by radiation exposure and/or nicotine administration in lactate dehydrogenase isoenzymes.

Key words: lactate dehydrogenase isoenzymes, gamma irradiation, vitamin E, nicotine, rats.

Abbreviations: creatinine phosphokinase, CPK; lactate dehydrogenase, LDH; malondialdehyde, MDA; thiobarbituric reactive substances, TBARS.

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## Introduction

Cigarette smoking causes a continuing toll on the public health. Smokers generate a large number of free radicals, which are linked with a number of diseases including coronary heart diseases and chronic obstruction pulmonary diseases (PETTY, 1998). Among the mechanisms hypothesized to contribute to smoking-induced vascular damage is oxidant injury (FREI et al., 1991; LEHR et al., 1993). Highly reactive elements in cigarette smoke facilitate DNA adduct formation (PHILLIPS et al., 1988; GRINDERG-FUNES et al., 1994) and may directly induce platelet activation and vascular dysfunction (SALVEMINI & BOTTING, 1993). Strong increases in many enzymatic activities have been recorded by TUREGANO et al. (2001) as well as by YILDIZ et al. (1999) in lipid peroxides assessed by measurement of changes in malondialdehyde (MDA).

Exposure to ionizing radiation produces also significant alterations in oxidant activity in different tissues. HUI et al. (1996) showed that lipid peroxidation of biological membranes contributes significantly to the development of radiation induced cell injury. YANARDAG et al. (2001) showed also increases in serum values of lactate dehydrogenase (LDH) following gamma irradiation indicating cellular damage.

Cigarette smoking, which is one of the potent oxidant and radiation exposures that may exacerbate its effect, may induce functional and chemical change in living systems. Low dietary intakes of antioxidant vitamins, such as vitamin C and vitamin E, increase the risk of illnesses whereas high dietary intakes seem to be protective (NEUNTEUFL et al., 2000). It has been proven that vitamin E is an effective antioxidant; it converts superoxide radicals and lipid peroxy radicals to less reactive form (VALK & HORNSTRAL, 2000).

However, protection from the impact of smoking and radiation on mammals still awaits further investigation. This work was undertaken to investigate the possible protective role of vitamin E in regulating disturbances in LDH isoenzymes, isocitrate dehydrogenase, and creatine phosphokinase (CPK) activities in irradiated rats subjected to nicotine administration. Changes in the levels of thiobarbituric reactive substances (TBARS) are used as early indicators of peroxidizing effect.

## Material and methods

Male Swiss Albino rats (100–120 g) obtained from the Egyptian Organization for Biological Products and Vaccines were used as experimental animals. Animals were kept under standard conditions during the experiment period. The rats were fed on pellet concentrated diet containing all the necessary nutritive elements. Liberal water intakes were available.

Whole body gamma irradiation was performed with a Canadian gamma cell-40 ( $^{137}\text{Cs}$ ) at the National Center for Radiation Research and Technology, Cairo, Egypt, at dose rate 0.61 Gy/min. Rats were exposed to 7 Gy delivered as a shot dose.

Animals were divided into 8 groups: (1) Control (C): rats of this group were neither treated nor irradiated. (2) Vitamin E treated rats (E): rats of this group received vitamin E in concentration of 10 mg/kg of body weight/day for 12 successive days. (3) Nicotine treated rats (N): rats of this group received nicotine in concentration of 80  $\mu\text{g}/\text{kg}$  of body weight/day for 12 successive days. (4) Vitamin E and nicotine treated rats (E+N): rats of this group received vitamin E and nicotine in concentrations of 10 mg/kg and 80  $\mu\text{g}/\text{kg}$  of body weight/day, respectively, for 12 successive days. (5) Irradiated rats (R): rats of this group were exposed to 7 Gy, shot dose of whole body gamma irradiation. (6) Vitamin E and irradiated rats (E+R): rats of this group received vitamin E in concentration of 10 mg/kg of body weight/day for 12 successive days before exposure to 7 Gy of gamma irradiation. (7) Nicotine-treated and irradiated rats (N+R): rats of this group received nicotine in concentration of 80  $\mu\text{g}/\text{kg}$  of body weight/day for 12 successive days before exposure to 7 Gy of gamma irradiation. (8) Vitamin E, nicotine-treated and irradiated rats (E+N+R): rats of this group received vitamin E and nicotine in concentrations of 10 mg/kg and 80  $\mu\text{g}/\text{kg}$  of body weight/day, respectively, for 12 successive days before exposure to 7 Gy whole body gamma irradiation.

Vitamin E (Sigma) was dissolved in sesame oil and delivered to rats via i.p injection. Nicotine (Sigma) was dissolved in distilled water and administered to rats via i.p injection.

Six rats from each group were sacrificed 1 day after irradiation time. Blood samples were collected and serum was separated. Total activity of serum LDH was estimated according to the method by KLIN (1972). The activities of LDH isoenzymes in serum were determined after electrophoretic separation on 1% agarose gel and the activity of each isoenzyme fraction were considered as a part of total serum LDH activity (CHAPMAN et al., 1986). Isocitrate dehydrogenase and CPK activities in serum were assayed according to the methods by ELLIS & GOLDBERG (1971) and REC (1977), respectively. The lipid peroxidation products were assayed as TBARS (YOSHIOKA et al., 1979). Student's t-test was applied for the statistical analysis of collected data (BYRKIT, 1980).

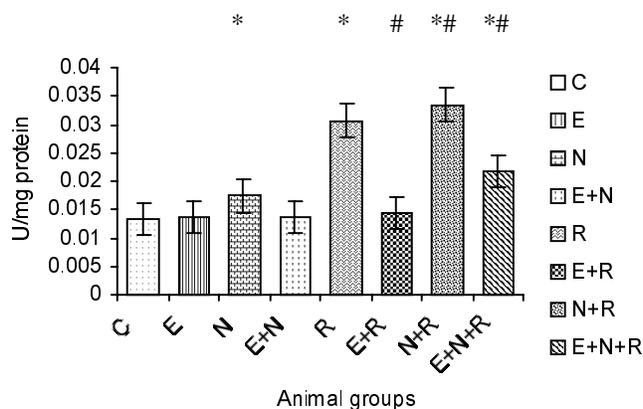


Fig. 1. Changes in serum LDH activities (U/mg protein) in different rat groups. C: control rats, E: vitamin E treated rats, N: nicotine supplemented rats, E+N: vitamin E and nicotine treated rats, R: irradiated rats, E+R: vitamin E supplemented rats before and during irradiation, N+R: nicotine supplemented rats before and during irradiation, N+E+R: nicotine and vitamin E supplemented rats before and during irradiation. Each column represents the mean of 6 records  $\pm$  S.E. \* - significant changes from control rats, # - significant changes from irradiated rats.

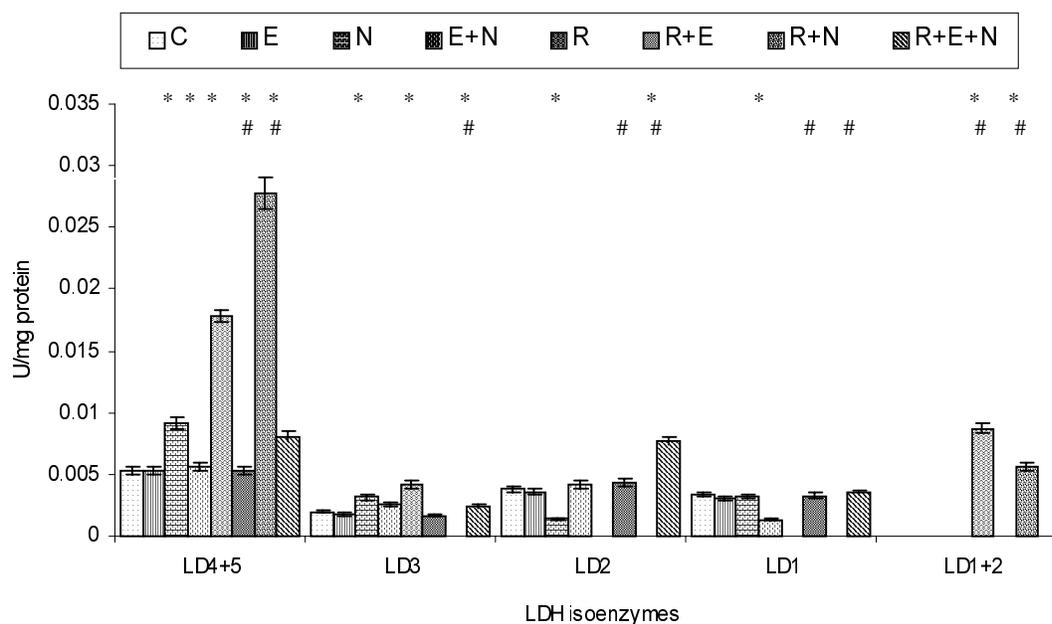


Fig. 2. Changes in LDH isoenzymes activities (U/mg protein) in different rat groups. The symbols are explained in the legend to Figure 1.

## Results

The changes in serum LDH activities in different groups of rats are shown in Figure 1. The activity of LDH in N, R, N+R and N+E+R rat groups increased significantly from control values. The changes are less manifested in the groups E+R and N+E+R as compared to group of irradiated rats (R).

Figure 2 exhibits the changes in electrophoretic pattern of LDH isoenzymes in different rat groups. Administration of nicotine and/or expo-

sure to whole body gamma irradiation significantly altered the distribution of serum LDH isoenzyme fractions; expressed in unit activities/g protein as a portion of total serum LDH activity. The supplementation of vitamin E before and during exposure to gamma irradiation reduced significantly the alteration of LDH isoenzymes patterns. Moreover, vitamin E improves the electrophoretic distribution of LDH isoenzymes in rats supplemented with nicotine.

The changes in the activities of serum isocitrate dehydrogenase, CPK and concentration of

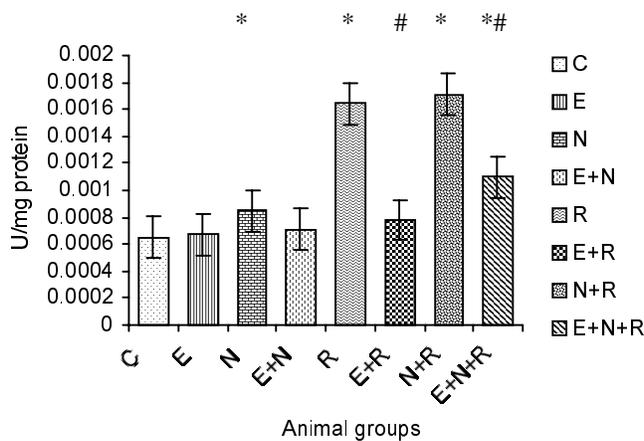


Fig. 3. Changes in serum isocitrate dehydrogenase activities (U/mg protein) in different rat groups. The symbols are explained in the legend to Figure 1.

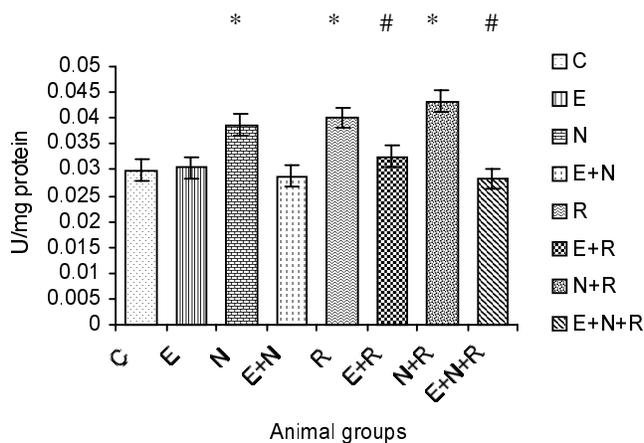


Fig. 4. Changes in serum CPK activities (U/mg protein) in different rat groups. The symbols are explained in the legend to Figure 1.

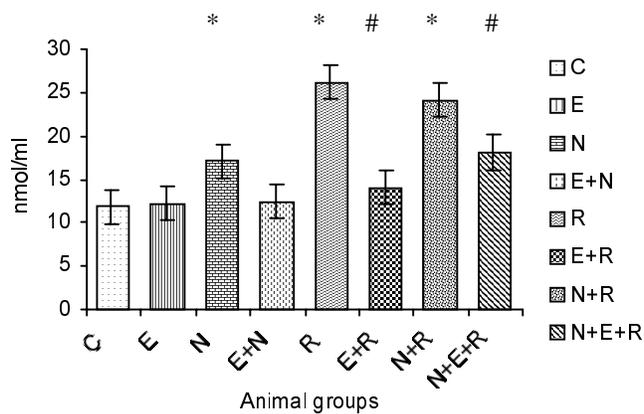


Fig. 5. Changes in serum TBARS concentrations (nmol/mL) in different rat groups. The symbols are explained in the legend to Figure 1.

TBARS are displayed in Figures 3-5. Whole body gamma irradiation significantly increases the activities of isocitrate dehydrogenase and CPK and concentration of TBARS. Also nicotine increased

significantly the activities of serum isocitrate dehydrogenase, CPK and concentration of TBARS. The administration of vitamin E before and during exposure to gamma irradiation reduces the sever-

ity of changes comparing with irradiated rats. The concurrent administration of vitamin E and nicotine improves the changes that occur in nicotine-supplemented group. However, the simultaneous supplementation of vitamin E and nicotine before and during irradiation could interfere with vitamin E competence referring to these parameters (Figs 3-5).

## Discussion

Nicotine affects a variety of cellular processes ranging from induction of gene expression to secretion of hormones and modulation of enzymatic activity. The objective of this study was to characterize the toxicity of nicotine in modifying the pattern of LDH isoenzymes in serum of irradiated rats and the potential protective role of  $\alpha$ -tocopherol. The activities of isocitrate dehydrogenase and CPK were also estimated. The acceleration in lipid peroxidation was measured as MDA content.

Results showed that treatment with nicotine and/or radiation caused a significant increase in all tested enzymes accompanied by significant increase in lipid peroxidation.

Separation of isoenzymes of LDH by serum electrophoresis provides a more information diagnostic tool than single enzyme; this is due to the variation in the isoenzyme composition of different tissues. This has been reported by USHER et al. (1974) showing the increase in concentration of the more anodic isoenzymes (LDH-1 and LDH-2) in myocardial infarction and the increase in the cathodic isoenzymes (LDH-5) in diseases of the liver.

CHAILO & PROTAS (1992) showed that irradiation causes differently directed changes of ratio of the isoenzymes at different times after exposure. The isoenzyme spectra of LDH and malate dehydrogenase were normalized on day 30 after irradiation. THYAGARAJAN et al. (1975) reported that irradiation causes a marked increase in LDH activity and a decrease in H-LDH/M-LDH rat serum and tissues. The increase in M-LDH isoenzymes biosynthesis indicates that an adaptive mechanism is operative in the irradiated rats, whereby in order to augment anaerobic glycolysis synthesis of M-LDH is stimulated.

The high serum levels of LDH, CPK and isocitrate dehydrogenase induced by irradiation were reported by MAC WILLIAM & BHAKTHAN (1976) and BASU et al. (1992), who showed that ionizing radiation instigates the alterations in dynamic permeability of membranes allowing leakage of bi-

ologically active material out of the injured cell. The high activity of LDH demonstrated that cellular membrane integrity was disturbed, as shown by WATANABE et al. (1995) and YILDIZ et al. (1998, 1999) following nicotine administration. It appears that chronic nicotine treatment affects cardiac function (HU et al., 2002) by modulating the expression of genes involved in energy metabolism and signal transduction. TUREGANO et al. (2001) demonstrated that chronic as well as acute administration of nicotine produced strong increases in different dehydrogenase activity. Moreover, MALL et al. (1985) concluded that smoking may aggravate the course of acute ischemic heart disease with high CPK values.

It was argued that the oxidant/antioxidant imbalance due to oxidative stress is the main cause of the excessive formation of peroxides. The significant acceleration in lipid peroxidation measured as MDA content is attributed to peroxidation of the membrane unsaturated fatty acids due to free radicals propagation concomitant with the inhibition in biooxidase activities (ZHENG et al., 1996). The generation of reactive oxygen species following treatment with nicotine was assessed by measurement of high levels of MDA (YILDIZ et al., 1998, 1999). YAMAGUCHI et al. (2001) and HELEN et al. (2000) showed an increase on lipid peroxides levels after the nicotine treatment with a corresponding decrease in vitamin E level in plasma and liver.

Antioxidant vitamins may themselves be susceptible to deterioration during inflammation and their levels are consumed in the process of defense against excessive free radical production (GOODE et al., 1995). The potential antioxidant protective role of vitamin E has been investigated. In the present study, vitamin E pretreatment prior to irradiation may have some beneficial effects against nicotine and/or irradiation induced injury.  $\alpha$ -Tocopherol is the most effective chain breaking lipid soluble antioxidant; it protects various fatty acids from being peroxidized (VALK & HORNSTRA, 2000) via free radical scavenging mechanism (CHOW, 1991).

The relevance of interaction of vitamins (E, C, and A) with other dietary factors, such as alcohol and tobacco, are topics of current research (BOLLOSHWEILER et al., 2002). Vitamin E supplementation resulted in preventive oxidative damage by lowering MDA and LDH (GIAKOUSTIDIS et al., 2002; ILAVAZHAGAN et al., 2002).

Because exposure to ionizing radiation induces free radical species, the effective antioxidant vitamin E is used as potential radio-protector. To

test their hypothesis KUMAR et al. (2002) studied vitamin E for its radio-protective efficacy and observed that vitamin E at a dose of 400 IU/kg acts as a good radio-protector against lethal doses of Co<sup>60</sup> radiation, being found more efficacious when given subcutaneously than orally. YANARDAG et al. (2001) showed also the protective effect of  $\alpha$ -tocopherol acetate against radiation damage by decreasing many enzyme activities including LDH.

SRIDHARAN & SHYAMALADEVI (2002) demonstrated that gamma irradiation caused an increase in lipid peroxides accompanied by a reduction in plasma antioxidant vitamins E, C and A. They also attributed that the increase of LDH and CPK are due to the excessive production of free radicals and lipid peroxides that might have caused leakage of cytosolic enzymes and to membrane cell damage which are susceptible for radiation damage. PRZYBYSZEWSKI et al. (1994) reported that the application of vitamin E diminished the MDA and the activity of LDH and CPK in serum of gamma irradiated rats. Changes in LDH isoenzyme patterns or CPK activity were found ameliorated by vitamin E (KRISTINA et al. 2001). ITHAYARASI & SHYAMADA (1998) and ELDALY (1998) concluded that rats with high enzyme activities pretreated with vitamin E exhibited a significant decrease in isocitrate dehydrogenase activity at near normal values.

We can therefore conclude that vitamin E may be a promising compound; it reversed many enzymes and MDA changes induced by radiation exposure and/or nicotine administration. The free scavenging potential of vitamin E that inhibits lipid peroxidation may be the mechanism underlying the protective effect established in our experiments. We thus suggest that the supplementation of vitamin E exerted a beneficial effect on induced oxidative damage in the biological system.

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