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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

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Gamma irradiation/Oral mucosa/Mucositis/Vitamin E/L-carnitine.

The aim of this study was to determine the effects of vitamin E (VE) and L-carnitine (LC) supplementation, separately or in combination, on radiation-induced oral mucositis and myelosuppression. Group 1 received no treatment (control). Group 2 received 15 Gray of ^{60}Co gamma irradiation as a single dose to total cranium (IR). Group 3, 4, and 5 received irradiation plus 40 mg/kg/day VE (IR+VE) or 200 mg/kg/day LC (IR+LC) or in combination (IR+VE+LC) respectively. Clinically and histopathologically, assessments of mucosal reactions were performed by two independent experts in Radiation Oncology and Pathology, respectively. Hematologic analyses and antioxidant enzyme evaluations were also performed. Irradiation significantly increased oral mucositis, and decreased thrombocyte and White Blood Cell counts. A significant increase in malondialdehyde (MDA) levels and decrease in superoxide dismutase (SOD) and catalase (CAT) activities in plasma were found in the IR group. VE and LC administration, separately, plus irradiation significantly delayed the starting day, and reduced the severity of, oral mucositis. This administration also reduced a fall in the numbers of thrombocyte and WBC caused by irradiation, and decreased the MDA level, and increased the activity of SOD and CAT enzymes in the plasma. VE and LC, in combination, plus irradiation did not provide a superior radioprotection against radiation-induced toxicities.

INTRODUCTION

Patients receiving radiotherapy (RT) for head and neck malignancies develop some degree of complications, the severities of which are variable and are influenced by both patient-related and treatment-related factors. These complications may be nausea, vomiting, myelosuppression, weight loss, and mucositis. Mucositis is the major dose limiting side effects in patients receiving RT for head and neck cancer.[1–3)] Mucositis may be intensely painful. It may have substantial effects limiting food intake; and may be a potential portal for infection.[1)] Myelosuppression causes neutropenia and thrombopenia that contribute to morbidity and complications, such as infection and hemorrhage.[4)] Thus, both mucositis and myelosuppression may lead to the delays in administration or to the dosage limitations in anti-neoplastic treatments, to the increased hospitalization stays and the costs, and these complications may have adverse effect on radio-curability of cancer and patient survival.[1,4)]

Unfortunately, at the present time, there is no widely accepted prophylaxis or effective treatment of mucositis that has been of interest to scientists for years. But, previous experimental and clinical studies were carried out by setting off from base on that anti-inflammatory, antioxidant, and cytoprotective drugs were able to reduce injurious effects of irradiation on this toxicity.[1,4–8)] Consequently, evidences have demonstrated the antioxidant, anti-inflammatory and cytoprotective roles of both vitamin E (VE)[9–12)] and L-carnitine (LC).[13–16)]

VE is a free radical scavenger, i.e., a sacrificial molecule with which the peroxy radicals preferentially react, rather than with biological molecules, thus preventing damage to cell structures. It also scavenges $O^{\bullet}_{2}{}^{-}$, OH^{-}, singlet oxygen, lipid peroxyl radicals and other radical species.[11,17)] VE physically stabilizes membrane permeability and fluidity. VE is a potent anti inflammatory agent.[10)] VE may not only protect intact tissues by decreasing apoptosis induced by

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injurious conditions,[18] but also increases apoptosis with a direct selective action on cancer cells.[19,20]

LC was able to scavenge superoxide anion, to inhibit the lipoperoxidation.[14,15] Carnitine can also act as a chelator by decreasing the concentration of cytosolic iron that plays a very important role in free radical chemistry.[21] LC has a capacity to enhance non-enzymatic antioxidants, such as vitamin E.[22]

Studies demonstrated that both VE and LC protected intact tissues against injurious effects of cancer treatments such as radiotherapy or chemotherapy, without an inhibitor effect against their therapeutic effects.[8,10,23–25]

From all these investigations, we have determined that VE and LC have a lot of beneficial effects against cancer treatment-related toxicities. Both VE and LC also have a role as an antioxidant, an anti-inflammatory and a cytoprotective agent. Because LC has a capacity to enhance non-enzymatic antioxidants, such as vitamin E, we considered that the use of LC and VE, in combination, might have additional effects in the prevention of radiation-induced toxicities. Therefore, we have decided to use Vitamin E and L-Carnitine, separately or in combination, in the prevention of radiation-induced mucositis and myelosuppression in a rat model.

MATERIALS AND METHODS

Rats, Drugs and Irradiation

Thirty-five male Sprague-Dawley rats, 8–12 weeks old and weighing 205 ± 25 g at the time of radiation, bred at Atatürk University Medical School, Department of Pharmacology Experimental Animal Laboratory, were used for the experiment. All procedures involving Sprague-Dawley rats were carried out to adhere to principles of the Use of Animals in Research. The rats were quarantined for at least three days before irradiation, housed in seven per group to a cage in a windowless laboratory room with automatic temperature (22 ± 1°C) and lighting controls (12 hr light / 12 hr dark), and fed standard laboratory chow and water ad libitum.

The rats were randomly divided into five groups. Group 1 received neither VE nor LC or irradiation (control group), but received physiological saline both 0.05-ml intramuscularly (IM) and 0.2-ml intraperitoneally (IP), as placebo, and sham-irradiation. Group 2 received total cranium irradiation of 15 Gy as a single dose plus 0.05-ml IM and 0.2-ml IP physiological saline (IR group). Group 3 received total cranium irradiation plus 40 mg/kg/day VE IM and 0.2-ml physiological saline IP (IR+VE group). Group 4 received total cranium irradiation plus 200 mg/kg/day LC IP and 0.05-ml physiological saline IM (IR+LC group). Group 5 received total cranium irradiation plus 40 mg/kg/day VE IM and 200 mg/kg/day LC IP (RT+VE+LC group). The rats in the IR+VE and IR+LC and IR+VE+LC groups received daily either 40 mg/kg/day (0.05 ml for a day) VE (EVIGEN ampule, Eras llac, lstanbul Turkey) IM or L-Carnitine (CARNITINE ampule, Sigma-tau, Rome) IP or both, respectively; starting 4 hours before irradiation and 10 days after irradiation. 40 mg/kg/day dose of VE was selected to determine its middle dose activity on radioprotection in our study protocol, because the previous studies demonstrated that tocopherol at doses of 5–100 mg/kg/day given intramuscularly or orally (acetate form) 7 days before tumor irradiation significantly enhanced the radiation-induced retardation of growth of a rat sarcoma.[26,27] This dose of VE is a bit more than that of guaranteed dose of VE for 50 kg-human. No reports of L-carnitine toxicity from overdosage exist. In mice, the LD50 is 19.2 g/kg. Studies indicate no mutagenicity. No change in the blood concentration appears to exist in giving a dose greater than 2 g at one time, since the studies indicate saturation at this dose.[28] According to the results of the protection studies such as in the prevention of oxidative stress[29] and chemotherapy-induced neuropathy,[25] LC was selected to determine its middle dose activity in our study protocol, because the doses of LC in these protection studies had been selected in different doses from 100 mg/kg/day to 300 mg/kg/day. In these doses, both VE and LC have been reported to be well-tolerated without toxic effects.

Prior to total cranium radiotherapy, the rats were anesthetized with 50 mg/kg ketamin HCl (Pfizer Ilac, Istanbul, Turkey) and placed on a Plexiglas tray in a prone position. While the rats in the control group received sham-irradiation, the rats in the IR, the IR+VE, the IR+LC and the IR+VE+LC group were irradiated using a cobalt-60 teletherapy unit (Picker, C 9, Maryland, NY, USA) from a source-to-surface distance of 80 cm, by 5×5 cm anterior fields with 15 Gy to the total cranium as a single fraction. The dose was calculated for the central axis at a depth of 1 cm. The dose rate was 0.68 Gy/min. Total cranium irradiation of 15 Gy in a single dose and histopathological examination time of oral mucosa were selected from previous mucositis studies carried out in the rats.[30–32]

Determination of Clinical Findings of Radiation-Induced Mucositis

After irradiation, the mouths of the rats were examined daily for signs of mucositis (erythema) by two independent physicians, experts of Radiation Oncology; the extent of mucositis was scored by being based on the scale proposed by Parkins *et al.*[33] as follows:

Score 0: Normal
Score 0.5: Slightly pink
Score 1: Slightly red
Score 2: Severe reddening
Score 3: Focal desquamation
Score 4: Exudation or crusting covering less than one-half of lip
Score 5: Exudation or crusting covering more than one-half

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of lip

When the erythema on the mouths of the rats progressed score 3, we administered 10 mg/kg/day of morphine (Morfin HCl amp, Biosel İlaç, İstanbul) for pain and distress subcutaneously twice in a day. The erythema scores in the rats of IR, IR+VE, IR+LC, and IR+VE+LC groups were graded to be comparative with those of the control group.

Determination of Histopathological Findings of Radiation-Induced Mucositis

For the histopathologic study, at the endpoint of the study, the rats were anesthetized again with 50 mg/kg ketamin HCl (Pfizer İlaç, İstanbul, Turkey) and specimens of right and left oral regions were obtained from the irradiated field by doing biopsy. The tissue samples were fixed in 10% formalin. After routine processing, the tissues were imbedded in paraffin wax. Four-μm-thick slices were prepared and stained with hematoxylin and eosin for evaluation with light microscopy. Light-microscopic findings were assessed by two independent physicians, experts of Pathology. Damaged areas were evaluated using damage (degeneration and vacuolar alteration of the basal layer, congestion and inflammatory infiltrate in submucosa, and the findings of cell changes in stratified squamous epitelium such as hyperchromasia, pleomorphism, binucleation, and necrosis) in terms of percentages, which were scored on a 5-points ordinal scale as follows; Grade 0 = Normal, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, Grade 4 = marked, and Grade 5 = severe. The semiquantitative scores reflect the population examined as follows; Grade 1 = < 5%, Grade 2 = 6–20%,

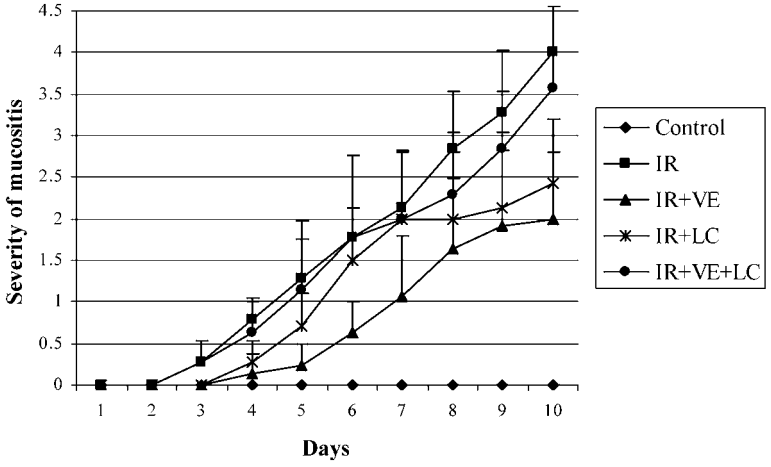


Fig. 1. The time courses of the mean clinical mucositis score after 15 Gy irradiation. Each data point (±SE) represents an average of seven animals. n = 7 rats per group, IR: irradiation group, IR+VE: irradiation plus vitamin E supplementation group, IR+LC: irradiation plus L-carnitine supplementation group, IR+VE+LC: irradiation plus vitamin E and L-carnitine supplementation group, in combination.

Table 1. Histopathological values in all groups (mean ± SD)

Groups	Histopathological values		
	Degeneration and vacuolar alteration of the basal layer	Congestion and inflammatory infiltrate in submucosa	Findings of cell changes in stratified squamous epitelium such as hyperchromasia, pleomorphism, binucleation, and necrosis
Control	$0.71 \pm 0.48^{b,c,d,e}$	$0.42 \pm 0.53^{b,d,e}$	$0.71 \pm 0.48^{b,c,d,e}$
IR	$3.71 \pm 0.48^{a,c,d}$	$3.00 \pm 0{,}00^{a,c,d}$	$3.71 \pm 0.48^{a,c,d}$
IR+VE	$2.14 \pm 0.69^{a,b,e}$	$0.57 \pm 0.53^{b,d,e}$	$2.00 \pm 0.57^{a,b,e}$
IR+LC	$2.42 \pm 0.53^{a,b,e}$	$1.71 \pm 1.11^{a,b,c,e}$	$2.57 \pm 0.53^{a,b,e}$
IR+VE+LC	$3.14 \pm 0.37^{a,c,d}$	$3.28 \pm 0.48^{a,c,d}$	$3.14 \pm 0.69^{a,c,d}$

n = 7 rats per group, IR: irradiation, IR+VE: irradiation plus vitamin E supplementation, IR+LC: irradiation plus L-carnitine supplementation, IR+VE+LC: irradiation plus vitamin E and L-carnitine supplementation, in combination. [a] $p < 0.05$ vs control group, [b] $p < 0.05$ vs irradiation group, [c] $p < 0.05$ vs IR+VE group, [d] $p < 0.05$ vs IR+LC group, [e] $p < 0.05$ vs IR+VE+LC group.

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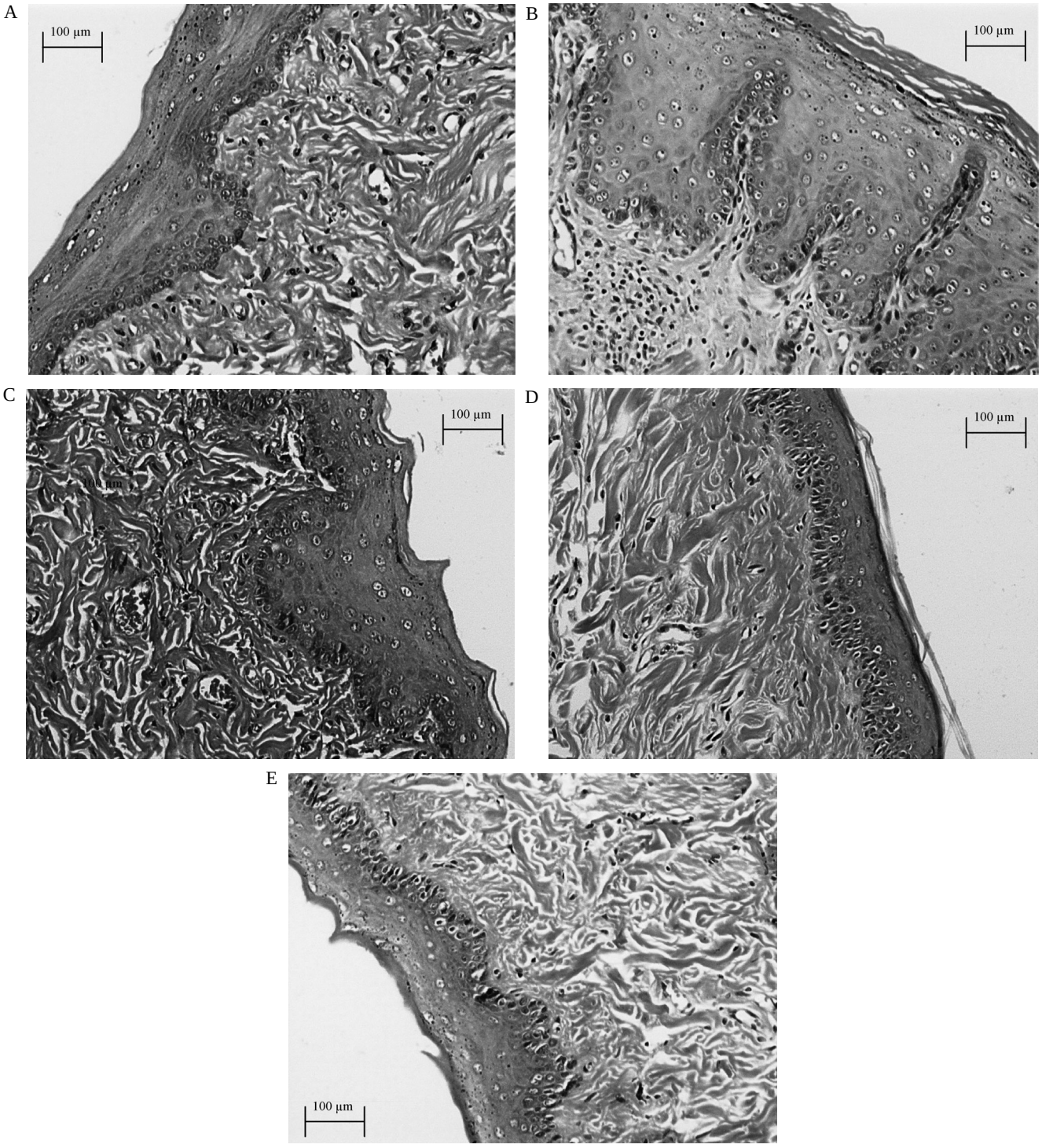


Fig. 2. The histopathological images of all groups in the present study. **A**: Normal histopathological image of the mucosa in the control group. Stratified squamous epitelium, submucosa and basal layer were intact; **B**: Radiation damage of the mucosa in the irradiation group. Irradiation group clearly reflected degeneration and vacuolar alteration of the basal layer, congestion and inflammatory infiltrate in submucosa, and the findings of cell changes in stratified squamous epitelium such as hyperchromasia, pleomorphism, binucleation, and necrosis. **C**: The group, received irradiation plus either 40 mg/kg/day Vitamin E (IR+VE group) intramuscularly, reflected a radioprotection against radiation-induced mucosal damage in terms of degeneration and vacuolar alteration of the basal layer, congestion and inflammatory infiltrate in submucosa, and the findings of cell changes in stratified squamous epitelium such as hyperchromasia, pleomorphism, binucleation, and necrosis. **D**: The group, received irradiation plus 200 mg/kg/day L-Carnitine intraperitoneally, (IR+LC group) reflected a radioprotection against radiation-induced mucosal damage similar to those of 40 mg/kg/day Vitamin E (IR+VE group). **E**: In contrary to our hypothesis, the use of use of VE and LC, in combination, plus irradiation interestingly did not exhibit an increased radioprotective effect. This combination did not protect the mucosa from radiation induced-mucositis. (H&E × 40).

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Grade 3 = 21–50%, Grade 4 = 51–75%, and Grade 5 = 76–100%. This method is modified to assess the acute oral mucosal reactions from the light microscopic changes for radiation-induced skin damage proposed by Ertekin *et al*.[34)]

Blood examinations

At the end of the 10th day, rats were anesthetized with 50 mg/kg ketamin HCl (Parke-Devis, Turkey) and then, total four-milliliter blood samples, two-milliliter for the counting blood cells and two-milliliter for the measuring antioxidant enzymes status in the plasma, were obtained by intracardiac puncture. The blood samples were collected in a vial containing 0.5 µl of 0.5 M EDTA and were coded.

Blood cells were counted with an automatic counter (GEN-8 Hematology Analyser). Total white blood cell (WBC), red blood cell (RBC), thrombocyte count, hemoglobin (Hb) and Hemotocrit (Htc) levels were determined in all groups.

For the measuring antioxidant enzymes status in the plasma, two-milliliter blood samples were centrifuged at 2000 g for 15 min, and plasma was removed with a Pasteur pipette. The analyses of MDA level and the activity of SOD, and CAT enzymes were carried out in plasma.

MDA method, an important indicator of oxidant stress, was based on the spectrophotometric absorbance measurement of the pink colored product of the thiobarbituric acid-reactive substances complex.[35)] Results were expressed as nmol/mg Hb.

SOD activity was detected by the nitroblue tetrazolium (NBT) reduction by $O^{\bullet}_{2}{}^{-}$ generated by the xanthine/xanthine oxidase system.[36)] A SOD unit is defined as the enzyme amount causing 50% inhibition in the NBTH2 reduction rate. SOD activity was also expressed as U/mg Hb.

CAT activity was measured in sera at according to the Aebi method.[37)] A unit of CAT activity is defined as the amount of enzyme that degrades 1 µmol H_2O_2 per min. CAT

Table 2. White Blood Cell (WBC), Red Blood Cell (RBC) and Thrombocyte (Plt) Counts and Hemoglobin (Hb) and Hematocrit (Htc) Levels in All Groups (mean ± SD).

Groups	WBC (10^3)	RBC (10^9)	Plt (10^3)	Hb	Htc
Control	$7.74 \pm 2.15^{b,d}$	8.22 ± 0.57	$887 \pm 120^{b,c}$	15.1 ± 0.8^{d}	42.1 ± 2.8
IR	$1.75 \pm 4.15^{a,c,d,e}$	7.60 ± 0.18^{c}	$142 \pm 107^{a,c,d,e}$	14.1 ± 0.3^{c}	40.3 ± 1.7^{c}
IR+VE	6.30 ± 2.25^{b}	$8.57 \pm 0.44^{b,d}$	$738.5 \pm 110^{a,b,e}$	$15.4 \pm 0.7^{b,d}$	44.4 ± 2.0^{b}
IR+LC	$5.18 \pm 1.70^{a,b}$	7.87 ± 1.06^{c}	914.5 ± 196^{b}	$13.6 \pm 2.0^{a,c}$	41.3 ± 4.5
IR+VE+LC	5.44 ± 2.86^{b}	8.09 ± 0.54	$939.5 \pm 140^{b,c}$	14.7 ± 1.0	42.5 ± 1.7

n = 7 rats per group, IR: irradiation, IR+VE: irradiation plus vitamin E supplementation, IR+LC: irradiation plus L-carnitine supplementation, IR+VE+LC: irradiation plus vitamin E and L-carnitine supplementation, in combination. [a] $p < 0.05$ vs control group, [b] $p < 0.05$ vs irradiation group, [c] $p < 0.05$ vs IR+VE group, [d] $p < 0.05$ vs IR+LC group, [e] $p < 0.05$ vs IR+VE+LC group.

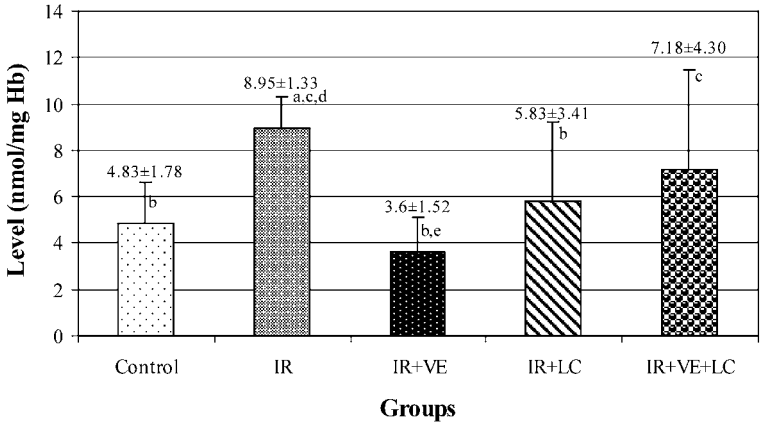


Fig. 3. The level of malondialdehyde (MDA) in the rat plasma. Each data point (±SD) represents an average of seven animals. n = 7 rats per group, IR: irradiation, IR+VE: irradiation plus vitamin E supplementation, IR+LC: irradiation plus L-carnitine supplementation, IR+VE+LC: irradiation plus vitamin E and L-carnitine supplementation, in combination. [a] $p < 0.05$ vs control group, [b] $p < 0.05$ vs irradiation group, [c] $p < 0.05$ vs IR+VE group, [d] $p < 0.05$ vs IR+LC group, [e] $p < 0.05$ vs IR+VE+LC group.

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result was expressed as IU/ mg Hb.

Biochemical measurements were carried out at room temperature using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

Statistics

In the study, we planned to determine the effects of vitamin E and L-carnitine, separately or in combination, on radiation-induced oral mucositis and myelosuppression. After necessary data had been collected, statistical analyses were made by using SPSS 11.0 packet programme (Statistical Package for Social Science; Windows version 11.0). The results were given as mean ± standard deviation. In histopathological and clinical grading, hematological and biochemical data, the potential difference among groups was evaluated using Anova test, and statistical significance of differences between the groups was tested with the Mann Whitney-U test. $p < 0.05$ was accepted as statistically significant.

RESULTS

The drugs were well-tolerated without toxic effects. There was significant reduction between the IR (174 ± 16) and the control groups (205 ± 2 5) in the measurement of body weight carried out in every three days ($p < 0.05$). The dif-

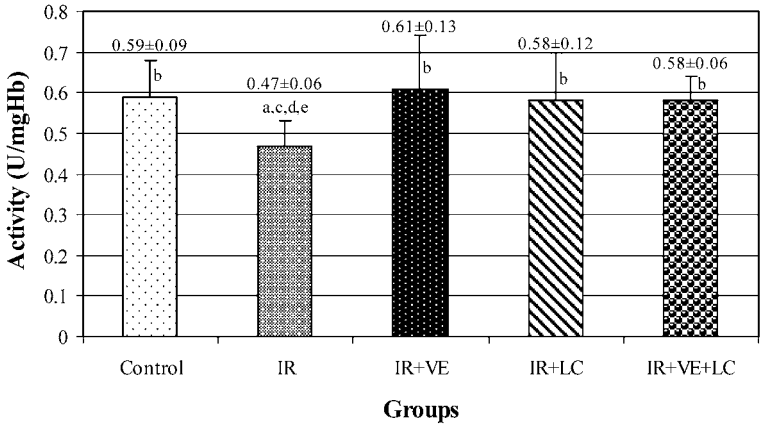


Fig. 4. The activity of superoxide dismutase (SOD) enzyme in the rat plasma. Each data point (±SD) represents an average of seven animals. n = 7 rats per group, IR: irradiation, IR+VE: irradiation plus vitamin E supplementation, IR+LC: irradiation plus L-carnitine supplementation, IR+VE+LC: irradiation plus vitamin E and L-carnitine supplementation, in combination. [a] $p < 0.05$ vs control group, [b] $p < 0.05$ vs irradiation group, [c] $p < 0.05$ vs IR+VE group, [d] $p < 0.05$ vs IR+LC group, [e] $p < 0.05$ vs IR+VE+LC group.

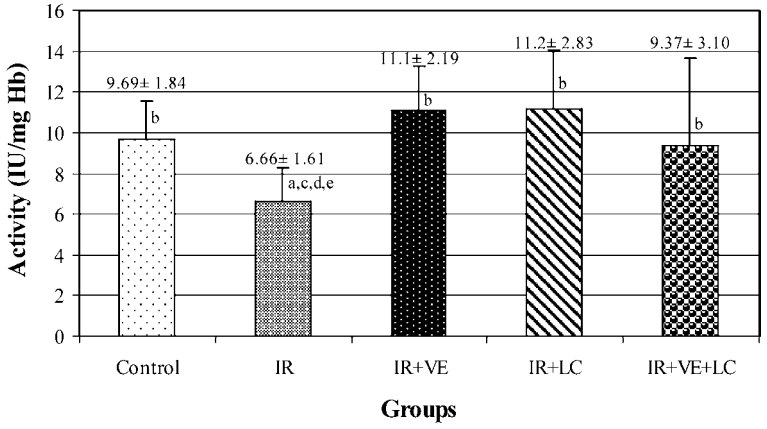


Fig. 5. The activity of catalase (CAT) enzyme in the rat plasma. Each data point (±SD) represents an average of seven animals. n = 7 rats per group, IR: irradiation, IR+VE: irradiation plus vitamin E supplementation, IR+LC: irradiation plus L-carnitine supplementation, IR+VE+LC: irradiation plus vitamin E and L-carnitine supplementation, in combination. [a] $p < 0.05$ vs control group, [b] $p < 0.05$ vs irradiation group, [c] $p < 0.05$ vs IR+VE group, [d] $p < 0.05$ vs IR+LC group, [e] $p < 0.05$ vs IR+VE+LC group.

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ference between the IR+VE+LC (179 ± 20) and the control (205 ± 25) groups were also statistically significant ($p < 0.05$).

Clinically, the signs of radiation-induced mucositis (erythema) in the mouths of rats that received radiation only and those treated with vitamin E or L-carnitine, separate or in combination, are shown in Fig. 1. While the finding of mucositis began in four of seven rats in the IR and IR+VE+LC groups on the 3rd day of postirradiation, it began in three of seven rats in the IR+LC group and two of seven rats in the IR+VE group on the 4th day of postirradiation. Mucositis scores on the 10th day of postirradiation were at mean figure of 4.00 ± 0.81 in the IR group; 3.57 ± 0.97 in the IR+VE+LC group;2.42 ± 0.78 in the IR+LC group; 2.00 ± 0.81 in the IR+VE group. There was statistically significant difference between the starting days of mucositis in the IR+LC and IR+VE groups when compared with those of the IR group ($p < 0.05$), but not between the IR+VE+LC and the IR groups ($p > 0.05$). While statistically significant difference between the IR and the IR+VE+LC groups, when com-

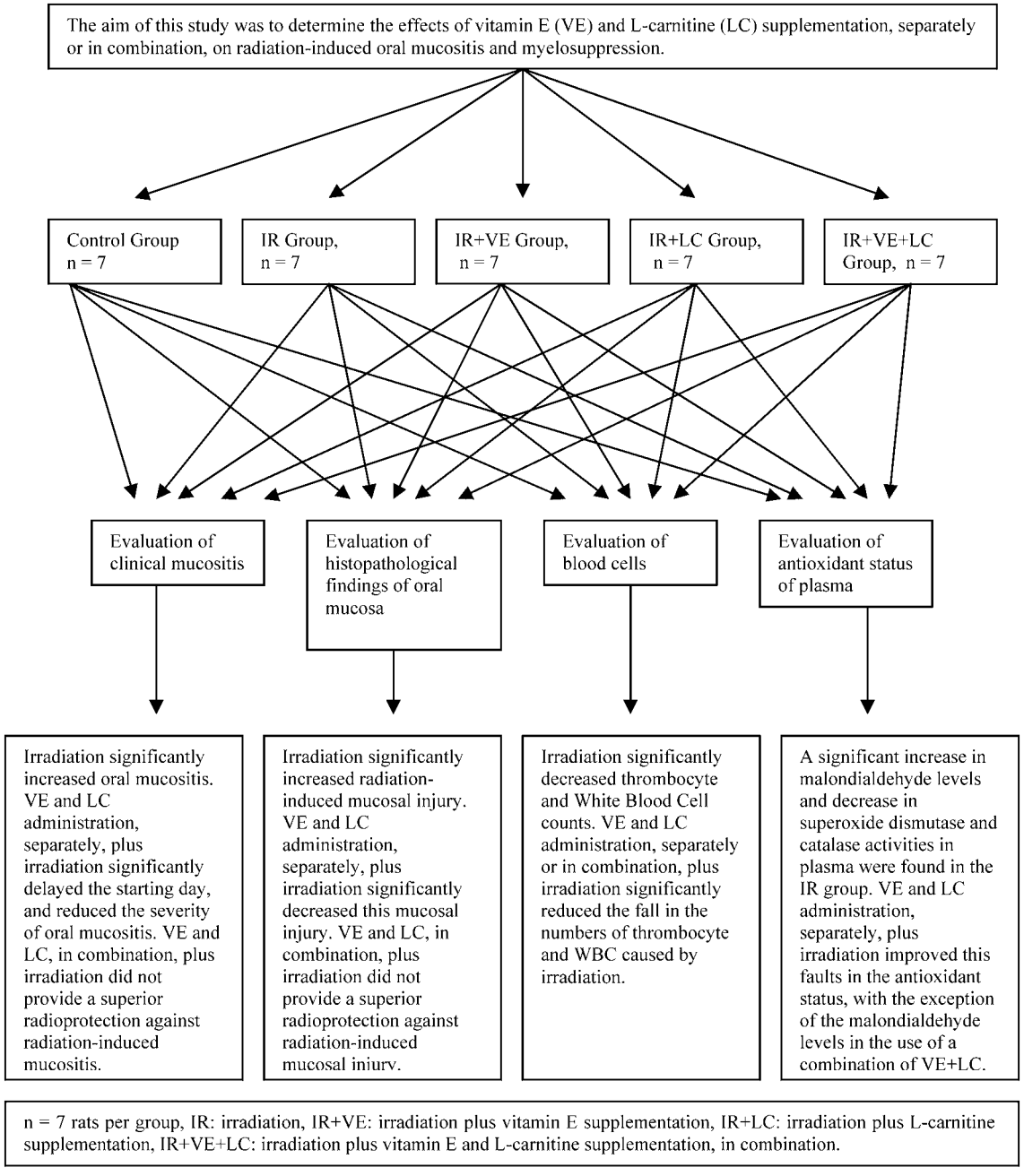


Fig. 6. The experimental flow chart summarize the experimental design and results of the study

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pared with those of the control group, started on the 3rd day of postirradiation ($p < 0.05$), this significant difference appeared the 5th day in the IR+LC group ($p < 0.05$) and the 7th day in the IR+VE group ($p < 0.05$). In the analysis for mucositis scores carried out among the groups, in the IR+LC and IR+VE groups, there was significant decrease in severity of mucositis, compared with the IR and the IR+VE+LC groups, and the significance continued until the 10th day of postirradiation ($p < 0.05$). However, there was no statistically significant difference in the severity and the starting days of mucositis between the IR+LC and the IR+VE groups ($p > 0.05$). There was no statistically significant difference for these parameters between the IR and the IR+VE+LC groups ($p > 0.05$) (Fig. 1), either.

In the histopathologic examination of oral mucosa in the rats after 10 days, there was statistically significant difference between the control and the IR groups in degeneration and vacuolar alteration of the basal layer, congestion and inflammatory infiltration in submucosa, and the findings of cell changes in stratified squamous epithelium such as hyperchromasia, pleomorphism, binucleation, and necrosis ($p < 0.05$). There was statistically significant difference in all of investigated histopathological parameters between the control and the other groups ($p < 0.05$), with the exception of congestion and inflammatory infiltration in submucosa in the IR+VE group ($p > 0.05$). A statistically significant difference in all of these parameters was also observed in the IR+VE and IR+LC groups when compared with that of the IR group ($p < 0.05$), but not between the IR+VE+LC and the IR group ($p > 0.05$). While there was statistically significant difference in congestion and inflammatory infiltration in submucosa ($p < 0.05$), there was no statistically significant difference in the other histopathological parameters between the IR+VE and the IR+LC groups ($P > 0.05$). In the IR+VE and IR+LC groups, a significant protection was observed when compared with the IR+VE+LC group ($p < 0.05$; $p < 0.05$, respectively) (Table 1; Fig. 2).

The results of the hematological parameters such as total white blood cell (WBC), red blood cell (RBC), thrombocyte count, hemoglobin (Hb) and hemotocrit (Htc) levels are presented in Table 2. Total cranium irradiation by 15 Gy of gamma irradiation as a single dose significantly decreased WBC and thrombocyte count ($p < 0.05$), and did not cause any changes in RBC counts, Hb and Htc levels ($p > 0.05$), when compared with the other groups. In the IR+LC group, WBC counts were significantly lower than in the control group, and Hb levels were statistically higher both in the control and in the IR+VE groups, when compared with the IR+LC group. There was statistically significant difference for RBC counts between the IR+VE and the IR+LC groups. In the IR+VE group, RBC counts and Htc levels were significantly higher than those of the IR group.

The level of MDA and the activity of SOD, CAT in the rat plasma is all presented in Figs. 3, 4, and 5. Total cranium irradiation by 15 Gy of gamma irradiation as a single dose significantly increased the MDA level, and decreased the activity of SOD, CAT enzymes in the plasma ($p < 0.05$) when compared with the control group. VE and LC supplementation, separately, along with total cranium irradiation significantly decreased the MDA level and increased the activity of SOD, CAT enzymes in the plasma ($p < 0.05$). Interestingly, although the supplementation of these two drugs, in combination, did not reduce the MDA level ($p > 0.05$), the combination increased the activity of SOD, CAT enzymes in the plasma ($p < 0.05$).

DISCUSSION

Mucositis may have substantial effects limiting food and water intake, and it may cause cachexia by losing body weight.[1)] The previous studies carried out on the rats demonstrated that, clinically, the finding of mucositis began in rats in the IR group on the 3rd day of postirradiation, and mucositis scores rapidly progressed on the 5th day of postirradiation and increased to peak levels on the 10th day of postirradiation after total cranium irradiation by 15 Gy as a single dose.[30,31)] In our study, the finding of mucositis began in four of seven rats in the IR group on the 3rd day of postirradiation, and mucositis scores progressed to the peak levels in the rats in the radiation group until on the 10th day of postirradiation.

Hemopoietic tissue, mainly bone marrow and lymphoid tissues are highly radiosensitive. The most marked effects are on the parent (stem) cells of the leukocytes, lymphocytes, and platelets. Red cells are much less radiosensitive, as their life cycle is much longer.[38)] Nagler *et al*.[39)] delineated systemic side effects in the short-term period post-IR in an animal model after head and neck irradiation with 15 Gy as a single dose. They reported that the severe cachectic and immunocompromised status occurred to be the severe short-term systemic effects of head and neck IR in the rat. They recommended that the examined animals need nutritional and immunological support during this period. In our study, there was significant reduction between the IR and the control groups in the measurement of body weight carried out in three days, and total cranium irradiation by 15 Gy of gamma irradiation as a single dose significantly decreased WBC and thrombocyte count.

Radiation is known as a producer of reactive oxygen species (ROS). When water, which constitutes around 80% of the cell, is exposed to ionizing radiation, decomposition occurs through which a variety of reactive oxygen species, such as the superoxide radical ($O^{\bullet}{}_2^-$), the hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) are generated. These ROS formed in cells contribute to radiation injury in cells. Although all respiring cells are equipped with protective enzymes such as SOD and CAT or GSH-Px, increased oxidative stress in cells stemming from ionizing radiation may

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overwhelm the protective systems, leading to cell injury.[40] One of the indices of oxidative damage is the malondialdehyde (MDA) formation as an end product of lipid peroxidation.[41,42] In our study, total cranium irradiation by 15 Gy of gamma irradiation as a single dose significantly increased the MDA level as an end product of lipid peroxidation and decreased the activity of SOD, CAT enzymes in the plasma by emphasizing the increased generation of oxidative stress.

Currently, there are increasing evidences, from human and experimental studies, suggesting that both VE [8,10,23] and LC [16,25,43] as an antioxidant, anti-inflammatory and a cytoprotective agent should be a beneficial agent in the protection against cancer treatment-related normal tissue injury.

Ferreira *et al*.[8] reported that VE decreased the incidence of symptomatic oral radio-induced mucositis in patients with cancer of the oropharynx and oral cavity. Mutlu-Turkoglu *et al*.[44] found that VE was effective in the prevention of radiation induced-intestinal injury in rats by ameliorating disturbances in prooxidant-antioxidant balance. Yoshimura *et al*.[45] determined that VE prevented the increase in oxidative damage to lipids and DNA in liver of Osteogenic Disorder Shionogi (ODS) rats given total body X-ray irradiation. Chan *et al*.[46] showed that, for patients with cerebral radionecrosis, alpha-tocopherol had the potential to be a complementary intervention for patients with cognitive dysfunction due to temporal lobe radionecrosis. Shaheen *et al*.[47] reported that administration of vitamin E preceding gamma-radiation exposure gave a significant radioprotection to the hematological parameters. In a previous study, Karslioglu *et al*.[10] demonstrated that VE had a protective effect on radiation-induced cataract by decreasing oxidative stress. Kumar *et al*.[23] reported that the use of alpha-tocopherol during radiation therapy might have improved the efficacy of radiation therapy by enhancing tumor response and decreasing some of the toxicity towards normal cells. In contrast, according to a current study, Biarati *et al*.[48] reported that alpha-tocopherol supplementation after treatment with radiation therapy produced unexpected adverse effects on the occurrence of second primary cancers and on cancer-free survival. In the present study, VE clinically proved to be a radioprotectant agent by delaying the starting day and reducing the severity of radiotherapy-induced oral mucositis. Histopathological evaluation also supported to be a reduction in the severity of radiotherapy-induced oral mucositis. At the same time, VE significantly reduced the fall in the numbers of thrombocyte and WBC caused by irradiation. VE administration plus irradiation significantly decreased the MDA level and increased the activity of SOD and CAT enzymes in the plasma, which might have indicated its antioxidant properties.

In the previous studies, it had been reported that LC was effective in preventing the kidney,[49] the retina,[50] the spinal-cord,[51] and the heart[52] from post ischemic reperfusion-induced oxidative damage. Recent *in vivo* and *in vitro* studies have demonstrated that LC has the potential ability to protect the myocardium against adriamycin-induced cardiotoxicity,[43,53] the kidney and small intestine against cisplatin-induced injury[16] and the sciatic nerves against cisplatin or paclitaxel induced-neuropathy[25] without showing any interference with the antitumor activity of the drugs. LC has been recently shown to act as an important anti-apoptotic mediator.[54,55] It has been shown that magnetic field (MF) decreased splenocyte viability, white blood cell count, as well as mitogens-induced lymphocyte proliferation, and L-carnitine could ameliorate the adverse effects of MF on the vast majority of the immune parameters tested, suggesting a possible immunoprotective role of L-carnitine.[56] There is no data about the use of LC in the prevention of radiation induced-surrounding normal tissues injury in literature. But, the agents which are similar to biologic activities as an antioxidant, anti-inflammatory, anti-apoptotic and a cytoprotective are used in prevention or treatment of radiation induced-surrounding normal tissues injury. These are some of other reasons why we used LC as a potent radioprotector agent in the present study. In the present study, LC clinically proved to be a radioprotectant agent by delaying the starting day and reducing the severity of radiotherapy-induced oral mucositis. Histopathological evaluation also supported to be a reduction in the severity of radiotherapy-induced oral mucositis. LC significantly reduced the fall in the numbers of thrombocyte and WBC caused by irradiation. LC administration plus irradiation significantly decreased the MDA level and increased the activity of SOD and CAT enzymes in the plasma, which might have indicated its antioxidant properties.

In this study, we hypothesized that the use of VE and LC, in combination, plus irradiation might have had additional antioxidant effects, because recent evidences indicated that treatment with LC enhanced the status of VE and suppressed lipid peroxidation, but, did not have direct effect on VE.[22] Dietary LC enhanced the rates and amounts of lymphatic absorption of VE and fat in ovariectomized rats.[57] Dhitavat *et al*.[58] demonstrated that, against oxidative stress in Alzheimer's disease, administration of a combination of vitamin E (which prevents de novo membrane oxidative damage), folate (which maintains levels of the endogenous antioxidant glutathione), and acetyl-L-carnitine (which prevents Abeta-induced mitochondrial damage and ATP depletion) provided superior protection that derived from each agent alone. At present, no information exists on the mechanism underlying the effect of LC supplementation on the nutritional status and metabolism of VE, especially irradiated animals. These observations invite a discussion on the need for maintenance the use a combination of LC and VE supplementation.

In conclusion, the data in our study supported the antioxidant and radioprotective roles of vitamin E and L-carnitine. However, the use of VE and LC, in combination, plus irradiation interestingly did not exhibit a superior radioprotective effect.

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It would be worthwhile studying the effect of vitamin E and L-carnitine supplements, separately or in combination, in radiation-treated cancer patients in a randomised clinical study with a greater number of patients, in the hope of reducing radiation-induced toxicity.

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