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Protective Effects of Vitamin E and Selenium Administration on Small Intestinal Damage Prior to Abdominal Radiation

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ABSTRACT

The purpose of this study was to investigate whether vitamin E and selenium have a protective effect against small intestinal damage induced by radiation. Radiotherapy has a quite important role in cancer therapy. Yet the most important problem is cytotoxicity, which occurs in the applied tissues, depending on the radiation underwent. Pre-treatment with antioxidants has been known to have a useful effect against radiation damage. Wistar albino rats were divided into three groups. Group I: control group, Group II: Only single-dose administration of 1000 cGy radiation, Group III: Vitamin E and selenium were administered, followed by the administration of abdominal radiation. Light and electron microscopic examinations have revealed that administration of radiation caused degenerative changes on small intestinal tissue. Treatment with vitamin E and selenium seemed to reverse these effects. Biochemically, blood glutathione levels in the radiation group were found to decrease. Vitamin E and selenium were found to increase blood glutathione levels. These results indicate that antioxidant treatment prior to irradiation may have protected the small intestine against radiation-induced damage. Dietary vitamin E and selenium have a potentially protective effect on the small intestine of patients subjected to abdominal radiotherapy.

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Authors' Contribution

RY, SB and AK designed the study. OKB and SB executed light and electron microscopic studies. RY performed biochemical analysis. AK was responsible for radiation part of the study. OKB, RY and SB wrote the article.

Key words

Radiation, Selenium, Vitamin E, Oxidative stress, Intestine

INTRODUCTION

In civilized populations, the ever increasing use of radiation in several forms have posed a biological risk to the mankind. In the treatment of cancer, radiation therapy plays an important role. Although they have useful effects in therapy, radiotherapy also causes damage on normal tissues (Pratheeshkumar and Kuttan, 2011; Taunk *et al*., 2015). Exposure to ionizing radiation stimulates the formation of reactive oxygen species (ROS). ROS including superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide further induce oxidative stress (Neoman *et al*., 2002; Chung-Ta *et al*., 2011; Salama, 2011; Kobashigawa *et al*., 2015). Oxidative stress occurs when the imbalance between prooxidant and antioxidant systems has progressed through prooxidants, which causes incapability of antioxidants or increase of free radical formation (Serafini and Del Rio, 2004). These free radicals react with critical cellular components, *i.e.* DNA, RNA, proteins, lipids, and membranes, resulting in cellular dysfunction followed by death (Pereira *et al*., 2010; Droge, 2002). Lipid peroxidation occurring after the effect of free radicals takes place in the cell by self-catalysis and the damage becomes irrepairable (Tomas-Zapico and Coto-Montes, 2006). Although some damages are repaired by intracellular mechanisms, nevertheless lethal damages can occur (Smith and De Cosse, 2006).

Various antioxidants by scavenging ROS and/or blocking peroxidative chain reactions show their protective effect. Endogenous antioxidants are not sufficient to decrease the radiation-induced free radical production. Appropriate antioxidant attempt inhibits or reduces free radical damage and so, it protects against radiation. Antioxidative defence system (AOS) such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) can also be altered by radiation and ROS (Halliwell and Whiteman, 2004; Prasad *et al*., 2005). Antioxidants such as Vit E and selenium (Se) prevent the production of free radicals or suppress the cellular damage (Neoman *et al*.,2002; Chow, 1991; Warren *et al*., 2000;Yanardag and Orak,2001).

Radiation damage is often seen with tissues having a high rate of reproduction. Therefore, the most important side effect in abdominal radiation is the damage to the small intestine (Smith and De Cosse, 1986; Klimberg, 1991). Ionizing radiation on gastrointestinal epithelium might show that this cytotoxic effect is related to oxidative stress (Mutlu-

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Türkoğlu *et al.*, 2000). A therapy was suggested which might prevent complaints due to small intestinal damage caused by radiation (Klimberg *et al.*, 1990).

The aim of this study was to investigate whether the synergistic application of Vit E and Se has a protective effect on small intestinal damage or not.

MATERIALS AND METHODS

Animals

In total, 23 adult male *Wistar albino* rats from Experimental Medical Research Institute of Istanbul University (DETAE) were used. The experiments were reviewed and approved by DETAE. Additionally, the guiding principles for experimental procedures presented in the World Medical Association's Declaration of Helsinki regarding animal experimentation were followed in this study. The animals were fed laboratory pellet chow and water *ad libitum*. Animals were kept in the animal laboratory by maintaining standard conditions of temperature (22ºC to 25ºC) and humidity (50%) with alternating 12 h light/dark cycle.

Experimental design and treatment of animals

The animals were randomly selected and divided into three groups: Group I contained control rats, Group II comprised rats which received radiation only, Group III had rats which received 30 mg/kg Vit E along with 0.5 mg/kg Se on the 1st, 3rd, and 5th day, and only Vit E on the 2nd and 4th day by intraperitoneal injection, followed by 1000 cGy single-dose abdominal radiation on the 6th day. Rats of Groups II and III were sacrificed three days after the radiation. However, control group rats were sacrificed on the 9th day of the experiment. After the experiment and 24 h before dissection, access to oral feeding was stopped. They were only allowed to drink water. For anaesthesia, 50 mg/kg ketamin hydrochloride (Ketalar®, Eczacıbaşı) and 10 mg/kg Xylazine HCl (Alfazyne®,The Netherlands) were injected intramuscularly.

Irradiation

The irradiation was done using Chicobalt 75 radiotherapy equipment at the Oncology Institute of Istanbul University. The rats were anesthetized with 40 mg/kg sodium pentothal. The head, thorax, and extremities were protected by lead, allowing exposure to radiation only in the abdomen. Irradiation was accomplished at a dose of 1000 cGy from a ^{60}Co source at a distance of 80 cm at a rate of 96 cGy/min.

Histopathological evaluation

For light microscopic investigations, the small intestinal tissues were fixed in Bouin's fixative, dehydrated with alcohol and then embedded in paraffin. To the cross-sections with 5µm thickness were stained with Masson's trichrome for histopathological scoring and periodic acid Schiff (PAS) reactions (Humanson, 1972). The preparations were investigated using Olympus CX 41 model light microscope and their photographs were taken. Histological damage rates were determined according to the method by Howarth *et al.*(1996) with some modifications.

Transmission electron microscopical (TEM) preparation

Approximately 1 mm^3 tissue blocks from the small intestine were prefixed with 2% glutaraldehyde and post-fixed with 1% osmium tetroxide, dehydrated in a graded series of ethanol, and finally embedded in Epon 812 resin (Fluka, Sigma–Aldrich Chemicals, Steinheim, Switzerland). The ultrathin sections stained with uranyl acetate and lead citrate were examined using a Carl Zeiss EM 9 S-2 microscope.

Biochemical study

Blood samples were taken for biochemical studies. Blood glutathione (GSH) level was determined according to Beutler *et al.* (1963). Serum total protein level was estimated using Lowry's method with bovine serum albumin as standard (Lowry *et al.*, 1951).

Statistical analysis

Graph-Pad Prism 3.0 (GraphPad Software, San Diego, CA, USA) program was used and data were analyzed by using one-way analysis of variance (ANOVA). Differences between groups were determined with the Tukey's multiple comparisons test and data were expressed as mean±standard deviation (SD). Significance level of $p<0.05$ was used.

RESULTS

Histopathological changes

Light microscopic investigations showed that, in radiation-applied group, in comparison to control group (Fig. 1A), disintegration and discontinuity in villous epithelia, irregularity, enlargement, decompression and eudema in villi, decrease in number of crypts, hyperemia and eudema in submucosae, mononuclear cellular infiltration were observed (Fig. 1B). In the experimental group, in comparison to the control individuals, PAS (+) reactive cells fairly decreased, whereas the radiation group administered with Vit E and Se showed, compared to the experimental group, a notable increase (Fig. 1C).

Electron microscopic structure

Electron microscopic findings revealed that, compared to the control group (Fig. 2A), the radiation

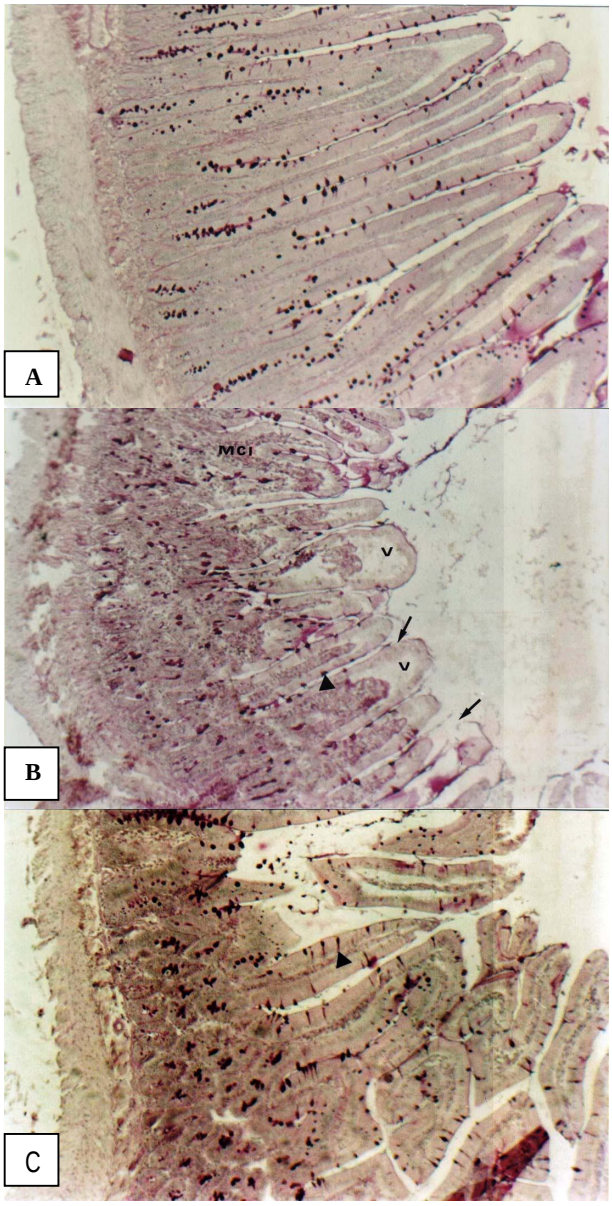

Fig. 1. Light microscopy of histological structure of small intestine of the male *Wistar albino* rats: A, control individual; B, irradiated rats. Disintegration and discontinuity in villi epithelium (→), irregular, wide, compressed villi with eudema (**V**), mononuclear cellular infiltration (MCI), PAS (+) reactive goblet cells' counts are decreasing; C, irradiated rats pretreated with vitamin E + selenium. Decrease of degenerative changes in the small intestinal structure. PAS (+) reactive goblet cells had an increased count compared to the experimental group (➢). PAS. ×430.

only group had swelling in mitochondria, decrease and disorder in the arrangement of cristae, decrease in number of the short and irregular microvilli, invagination in the nuclei, vesiculation in granulated endoplasmic reticulum, a transition from granulated endoplasmic reticulum to smooth endoplasmic reticulum, and enlargement within the intercellular area (Figs. 2B,C).

Although there were individual differences, the group given Vit E and Se and radiation showed less degenerative changes when compared to the radiation only group as evidenced by light and electron microscopic means (Figs. 2D,E).

Histological score of damage

The following light microscopic criteria for each individual were evaluated for small intestinal cross-sections: Disintegration and discontinuity in epithelial areas and villi epithelium, irregularity, enlargement, compression and edema in the villi, decrease in the number of crypts, hyperemia and eudema in submucosae, and mononuclear cellular infiltration. Every histologic criterion was graded according to this scoring scheme: 0 (no damage), 1 (light damage), 2 (medium damage), or 3 (severe damage) (Fig. 3).

According to these histological criteria, the highest score of damage was obtained for the radiation-only group. When compared to the control individuals, there was a statistically significant increase for histological score of damage for radiation-only ($p<0.01$) and Vit E + Se + radiation groups ($p<0.05$). Radiation-receiving individuals administered with vitamin E and selenium had a statistically significant decrease ($p<0.05$) compared to the radiation-only group**.**

Table I.- Blood GSH and serum total protein levels of control and experimental groups.

	Control	Radiation	Radiation+ Vit E., Se	*p*- ANOVA
GSH (mg%)*	35.88±9.3	26.24± 6.70[a]	35.38± 14.43[b]	0.0001
Total protein (g%)*	8.40± 0.71	8.49± 0.97	10.33± 1.72[c]	0.0001

*Mean± SD
[a]*P* <0.0001 compared with the control group.
[b]*P* < 0.001 compared with the radiation group.
[c]*P* <0.0001 compared with the radiation group

Blood GSH and Total Protein Levels

Blood GSH levels in the radiation-only group decreased significantly in comparison with the control group ($p < 0.0001$). Administration of Vit E and Se to the irradiated group significantly increased the blood GSH levels ($p<0.001$). Serum total protein levels were not

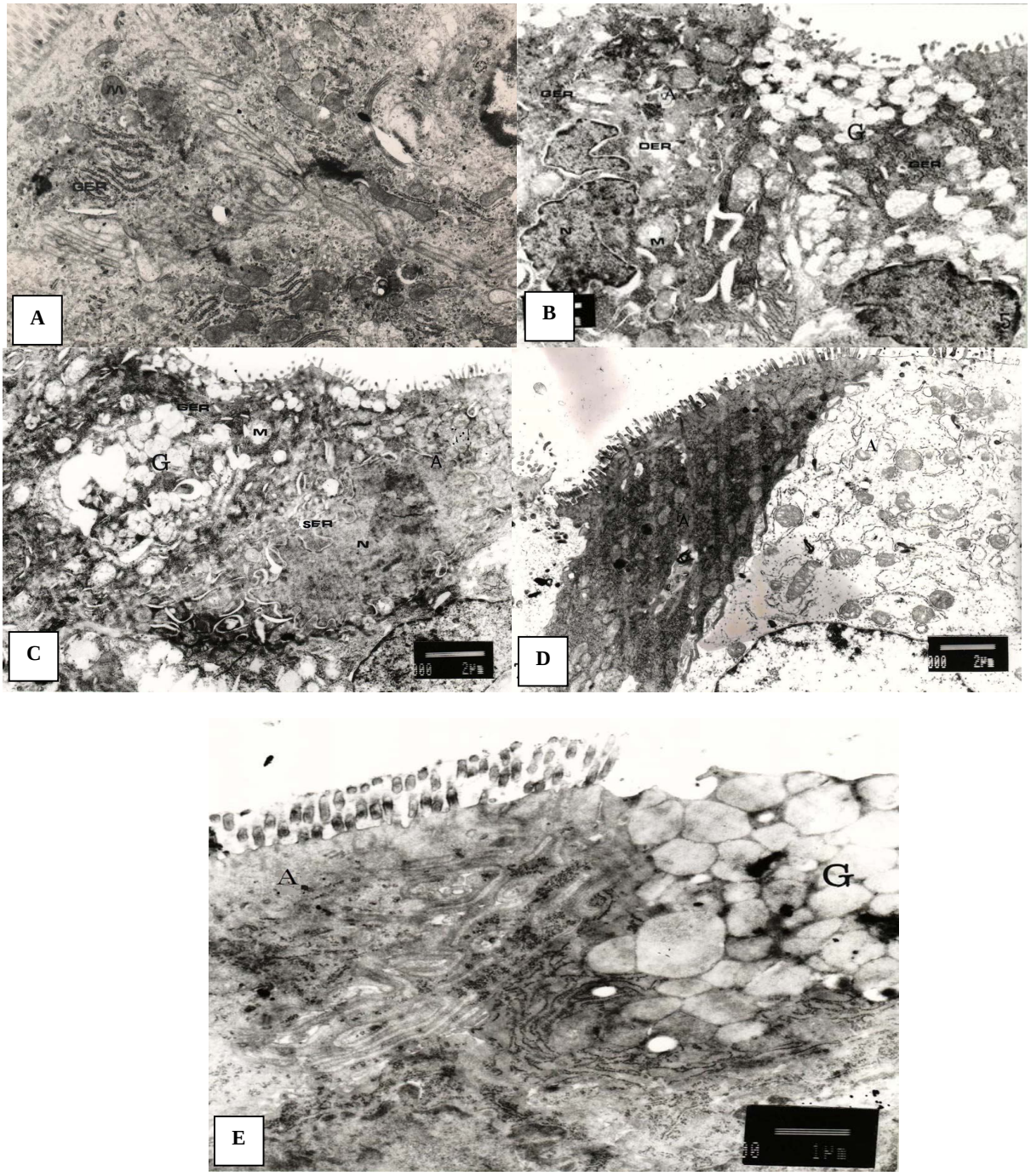

Fig. 2. Electron microscopical structure of small intestine of male Wistar albino rats: A, control individual's absortive (A) and goblet (G) cells, granulated endoplasmic reticulum (GER), mitochondria (M). B, C, intestine of irradiated rats' absorptive (**A**) and goblet (**G**) cells, displaying inflated mitochondria with disordered and decreased crystae (**M**), nucleus having invaginations (**N**), and a transition from granulated endoplasmic reticulum (**GER**) to smooth endoplasmic reticulum (**SER**). D, E, irradiated rats pretreated with vitamin E + selenium displaying less degenerative changes for their absortive (**A**) and goblet (**G**) cells. Bar in Figs. A, C, E, 2 μm; B, D, 1 μm.

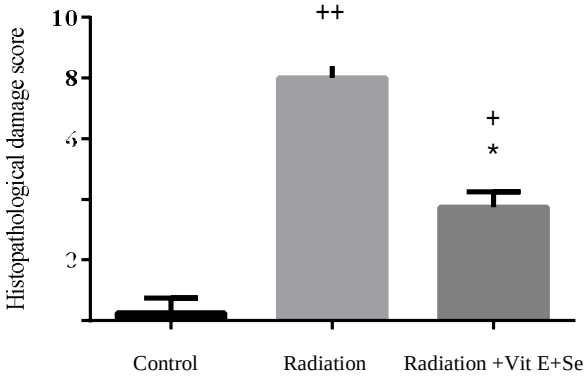

Fig. 3. Histopathological damage score of small intestine for all groups. The data were given as mean±SE for each group. Control Group: 0.25±0.5, Radiation Group: 8±0.81, Vitamin E+Selenium+Radiation Group: 3.75±0.5, +p<0.05, ++p<0.01 compared to control group. *p<0.05 compared to radiation group.

changed in radiation-only group as compared to the control group. Administration of Vit E and Se to the radiation group increased the serum total protein levels in a significant manner when compared to radiation-only and control group ($p < 0.0001$) (Table I).

DISCUSSION

Radiotherapy is an important treatmentoption in the cancer; but it has serious side effects on the normal tissues. Therefore, the effectiveness oftreatmentis limited (Grdina et al., 2002). Radiation causes changes in the structure and function of cellular components in different tissue and organs, resulting in the damage of the tissues and cell death (Akpolat *et al*., 2009). Radiation damage is mostly observed in regenerative tissues such as the gastrointestinal (GI) tract. Radiation readily damages the regenerating epithelial cells in the mucosal layer of the intestinal lumen. Digestive, absorptive, and barrier functions of the small intestine are impaired, therefore radiation contributes to sugar malabsorption and diarrhea (Smith and De Cosse, 1986; Klimberg, 1991). Radiation generates free radicals and ROS, thus it indirectly causes to damage of DNA and other biomolecules, which lead to cell death and mutations (Somosy *et al*.,2000; Akpolat *et al*., 2009; Chung-Ta *et al*., 2011). Enzymes such as SOD, GPx, and CAT protect the cells from oxidative damage of radiation (Pratheeshkumar and Kuttan, 2010; Roche *et al*., 2011).

Experimental studies have determined that the damages occurring are mostly of free radical nature and they can be prevented, if not fully, by antioxidative substances. Antioxidative substance-employing support is thought to have both increased the response of radiation to tumoral areas, and increased the toxic effects on the normal cells, thereby increasing the therapeutic yield (Mutlu-Türkoğlu *et al*.,2000; Neoman *et al*., 2002; Dokmeci *et al*., 2006).For this purpose, in our study, in order to prevent or decrease the damage posed on the small intestinal tissue by radiotherapy, the effects of Vit E and Se, both of which are known to have administered prior to abdominal radiation and possess antioxidant properties, were investigated by histopathological and biochemical means. Somosy *et al*. (2000) have shown that ionizing radiation causes some morphological changes in the gastrointestinal system. To grade these morphological changes helps determine the degree of the radiation damage. In order to evaluate the early morphological parameters, the criteria used are not only the changes in the shape of the cell, but also the changes in the intercellular connections, and changes in the cellular surface morphology, in addition to morphological changes like the determination of cellular death and degree of fibrosis.

Radiation alters the expression of antioxidant enzymes in intestinal cells in the crypt region and thus the symptoms of malabsorption such as ulceration, infection, and perforation can be encountered. A mixture of antioxidant vitamins of A, C, and E protect the small intestine from radiation damage (Harapanhalli *et al*., 1994).

In our study, we observed the following light microscopic criteria: Disintegration and discontinuity in villous epithelium, irregularity, enlargement, compression, and edema in villi, decrease in the number of crypts, hyperemia and edema in sub-mucosae, mononuclear cellular infiltration, and PAS (+) reactive cellular counts. In addition, the following electronic microscopic criteria were also observed: Inflation in mitochondria, disintegration and decrease in the order of and a generic decrease in the number of crystae, short and irregular microvilli, invagination in the nuclei, vesiculation in the granular endoplasmic reticulum, transition from granular endoplasmic reticulum to a smooth one, and inflations within the intercellular areas. Then we considered these findings to better observe the radiation-only and radiation + Vit E + Se groups' individuals in terms of their small intestine-based histological damages and/or recovery.

Kanter and Akpolat (2008) have shown that in Wistar albinorats treated with 15 Gy radiation, a notable disorder in jejunal mucosae was found. The observation of shortening and merging in the villi due to the loss of

cells and highly atrofic appearance of tunica mucosae is followed by another observation that villi epithelial cells have rubbed off or from place to place, lost completely; mononuclear cellular infiltration as well as heavy capillary congestion and dilatation were observed in lamina propria. Kanter and Akpolat (2008) reported that Lieberkühn gland counts have diminished, as well as losses in gland cells and especially total losses in the criptae. In another publication, picnosis and chromatin disintegration have progressed after radiotherapy, cellular losses were evidenced, and atrophy, villi atrophy, and flattening of mucosae were reported (Berthrong and Fajardo, 1981). When radiation was applied at a high dose or multiple times with gradually increasing doses, inflammation, atrophy in the mucosae, edema in the sub-mucosae, hyperemia, plasmocide in lamina proprea, and leucocyte infiltration with polymorph nuclei, and in the cells that have not been differentiated, inhibition of mitosis were reported (Donald *et al.*, 1995). In a study which investigated the protective effect of vitamin C in small intestine as an antioxidant against gamma radiation, showed that gamma radiation caused injuries of the intestinal mucosae in rats. Electron microscopic evaluations have revealed appendages within the intercellular area, irregular structure in the microvilli, mitochondrial damages, and dilatation in the cysternae of endoplasmic reticulum. In groups administered vitamin C, it has shown that injuries to the intestinal mucosae have diminished, and a protective effect was furnished over the goblet cells in the ileum (Kanter and Akpolat, 2008). Another study with mice has shown that vitamin C administration for two days before 8 Gy radiation significantly increased the survival rate against the number of radiation deaths (Sarmaan and Kesavan, 1993).

Saada *et al*. (2010) showed that lycopene - a carotenoid - which has a structure similar to that of the well-known antioxidant beta-carotene, preserves the morphology of the small intestine against radiation-induced oxidative stress . It is has been shown that the numbers of small intestinal crypt cells, mucosal height, and goblet cell numbers were protected against radiation by vitamin E (Felemovicius *et al*.,1995). They have shown that Vit C and E decreased bleeding and diarrhea in patients with chronic radiation proctitis caused by pelvic irradiation (Kennedy *et al.*, 2001).

In our present study, we observed the following light microscopic findings of the small intestinal cross-sections of the irradiated individuals: In villi epithelia, disintegration and discontinuity, disorder, enlargement, compression, and edema in villi epithelia, decreased number of crypts, hyperemia and eudema in the submucosae, mononuclear cellular infiltration. Electronic microscopic findings, on the other hand, have revealed the following: swelling of the mitochondria, decrease and disorder in the arrangement of cristae, decrease in the number of the short and irregular microvilli , invagination in the nucleus, vesiculation in the endoplasmic reticulum, a transition from the granular endoplasmic reticulum to smooth one, and inflation within the intercellular area. In contrast to the radiation-only group, we have observed a notable decrease in the radiation + Vit E + Se group, in terms of their small intestinal tissues and according to the injury terms defined

As is known, a positive PAS reaction is an indicator for the presence of tissue-based carbohydrates (Bancroft and Stevens, 1982). The changes in the structures and amounts of PAS-positive substances may alter the intensity of the color produced (Dahlquist *et al*., 1965). The reason for a decrease in the PAS (+) reaction might be due to the decrease in building blocks constituting the material, which forms the mucus in goblet cells and the decrease in cellular covering, or the disintegration of their chemical structure. They have also reported that ionizing radiation has caused the diminishing or total disappearance of glycocalyx structure in the epithelial cells of the small intestine and has increased the activity of lined side disaccharides (Mc Ardle *et al*.,1986).

In the current study, our group has observed, that the radiation-only group has yielded a notable decrease in terms of PAS (+) goblet cell count and the color intensity in the reaction, whereas, there was a significant term of increase of the same criteria for radiation+ Vit E + Se group.

Small intestine is one of the most radio-sensitive organs in the mammalian body. High dose radiation exposure causes violent intestinal damage, and intestinal injury has been shown to play a basis role in patient survival (Monti*et al*., 2005). Radiation causes mucosal damage in the gastrointestinal epithelium and infiltration of lamina propria with activated inflammatory cells. Radiation exposure can alter the balance of endogenous protective systems (Samarnath and Kumar, 2003). GSH provides major protection in oxidative injury (Ross, 1988). Several studies indicate that tissue damage induced by radiation is coupled with GSH depletion (Pratheeshkumar and Kuttan, 2011; Sener *et al*., 2003; Hepgul *et al*., 2010). In the present study, the decrease in small intestinal tissues' GSH levels after irradiation may be due to their consumption during oxidative stress caused by irradiation. Vit E and Se treatments were found to increase the levels of GSH decreased after irradiation. Yanardag and Koc (2002-2003) have shown in their study that when several substances were given to the organism, total protein values have been reported to increase. In our study, no significant change was

observed in the serum total protein levels in the radiation-only group compared to the control group, whereas, administration of Vit E and Se yielded an elevated levels of serum proteins both in the irradiated and control group. The observation of serum total protein values being increased, has shown that Vit E and Se-given radiation group had elevated protein synthesis levels. The findings of this study led us to conclude that Vit E and Se have a protective effect against radiation-induced small intestinal damage. As a result, Vit E and Se as supportive agents during radiotherapy can protect intestine against radiation.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Akpolat, M., Kanter, M. and Uzal, M.C., 2009. Protective effects ofcurcumin against gamma radiation-induced ileal mucosal damage. *Arch. Toxicol.*,**83**: 609–617.

Bancroft, J.D. and Stevens, A., 1982. Carbohydrates. In:*Theory and practice of histological techniques*. Churchill Livingstone, Edinburgh, pp. 188-190.

Berthrong, M. and Fajardo, L.F.,1981. Radiation injury in surgical pathology. Part II. Alimentary tract. *Am. J. Surg. Pathol.*,**5**: 153-78.

Beutler, E., Duron, O. and Kelly, B.M., 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*,**61**: 882-888.

Chow, C.K., 1991. Vitamin E and oxidative stress. *Free Radic. Biol. Med.*,**11**: 215–232.

Chung-Ta, C., Ho, L., Tın-Yun, H., Chıa-Cheng, L., Hsın-Yi, L., Shıh-Lu, W., Yi-Fang, H., Ji-An, L. and Chıen-Yun, H.,2011. Comprehensive assessment of host responses to ıonizing radiation by nuclear factor-kb bioluminescence imaging-guided transcriptomic analysis. *PLoS One*,**6**: 1-11.

Dahlquıst, A., Olsson, I. and Norden, A.,1965. The periodate schiff reaction: specifity, kinetics and reaction products with pure substrate. *J Histochem. Cytochem.*,**13**: 423-430.

Dokmeci, D., Akpolat, M., Aydogdu, N., Uzal, M.C. and Turan, F.N., 2006. The protective effect of L-carnitine on ionizing radiation-induced free oxygen radicals. *Scan. J. Lab. Anim. Sci.*,**33**:75-83.

Donald, P., Carroll, M.D., Rıchard, T., Zera, M.D. and Jeanesse, C.R., 1995. Efficacy of radioprotective agents in preventing small and bowel radiation injury. *Dis. Colon Rectum*,**65**: 716-725.

Droge, W., 2002. Free radicals in the physiological control of cell function. *Physiol. Rev*.,**82**: 47-95.

Felemovicius, I., Bonsack, M.E., Baptista, M.L. andDelaney, J.P., 1995. Intestinal radioprotection by vitamin E (alpha-tocopherol). *Annals Surg.,* **222**: 504–508.

Grdina, D.J., Murley, J.S. and Kataoka, Y., 2002. Radioprotectants, current status and new directions. *Oncology*,**63**: 2-10.

Halliwell, B. and Whıteman, M.,2004. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *Br. J. Pharmacol.*,**142:** 231-255.

Harapanhalli, R.S., Narra, V.R., Yaghmai V., Azure, M.T., Goddu, S.M., Howell, R.W. and Rao, D.V., 1994. Vitamins as radioprotectors *in vivo*. II. Protection by vitamin A and soybean oil against radiation damage caused by internal radionuclides. *Radiat. Res.*,**139**: 115-122.

Hepgul, G., Tanrıkulu, S., Unalp, H.R., Akguner, T., Erbil, Y., Olgac, V. and Ademoglu, E., 2010. Preventive effect of pentoxifylline on acute radiation damage via antioxidant and anti-inflammatory pathways. *Dig. Dis. Sci.*,**55:** 617-625.

Howarth, G.S., Francis, G.L., Cool, J.C., Xu, X., Byard, R.W. and Read, L.C., 1996. Milk growth factors enriched from cheese whey ameliorate intestinal damage by methotrexate when administeredorally to rats. *J. Nutr.*,**126**: 2519-2530.

Humanson, G.L., 1972. *Animal tissue techniques*. H.W. San Francisco, USA: ISBN:61-17383. Freeman and Company.

Kanter, M.and Akpolat, M., 2008. Vitamin C protects against ionizing radiation damage to goblet cells of the ileum in rats. *Acta Histochem.*,**110**: 481-490.

Kennedy, M., Brunınga, K., Mutlu, E.A., Losurdo, J., Choudhary, S. and Keshavarzian, A., 2001. Successful and sustained treatment of chronic radiation proctitis with antioxidant vitamins E and C. *Am. J. Gastroenterol*.,**96**:1080-1084.

Klimberg, S., 1991. Prevention of radiogenic side effects using glutamine - enriched elemental diets. *Recent Result Cancer Res.*,**121**: 283-285.

Klimberg, V.S., Souba, W.W., Dalson, D.J., Salloum, R.M., Dean Hautamakı, R.D., Plumley, D.A., Mendenhall, W.M., Bova, F.J., Khan, S.R.and Hackett, R.L.,1990. Prophylactic glutamine protects the intestinal mucosa from radiation injury. *Cancer*,**66**:62-68.

Kobashigawa, S., Kashıno, G., Suzukı, K., Yamashıta, S.and Morı, H.,2015**.** Ionizing radiation-induced cell death ıs partly caused by ıncrease of mitochondrial reactive oxygen species in normal human fibroblast cells. *Radiat. Res.*,**183**: 455-464.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurements with the folin phenol reagent. *J. biol. Chem.*,**193**: 265-275.

McArdle, A.H., Reıd, E.C., Laplante, M.P. and Freeman, C.R., 1986**.** Prophylaxis against radiation injury: the use of elemental diet prior and during radiotherapy for invasive bladder cancer and early postoperative feeding following radical cystectomy and ileal conduit. *Arch. Surg.*,**121**: 879-885.

Monti, P., Wysocki, J., Van Der Meeren, A. and Griffiths,

N.M., 2005. The contribuiıon of radiation-induced injury to the gastrointestinal tract in the development of multi organ dysfunction syndrome or failure. *Br. J. Radiol.*,**27:** 89-94.

Mutlu-Turkoglu, U., Erbil, Y., Oztezcan, S.,Olgac, V., Toker, G. and Uysal, M., 2000. The effect of selenium and/or vitamin E treatments on radiation-induced ıntestinal ınjury in rats. *Life Sci.*,**66**: 1905-1913.

Neoman, E., Zahran, A.M., Kamal, A.M. and Omran, M.F., 2002. Vitamin E and selenium administration as a modulator of antioxidant defence system: biochemical assesment and modification. *Biol. Trace Elem. Res.*,**86**: 55-64.

Pereira, H.M., Leadley, P.W., Poenca, V., Alkemade, R.,Scharlemann, J.P.W., Fernandez-Manjarrés, J.F., Mıguel, B., Araújo, M.B., Balvanera, P., Bıggs, R. Cheung, W.W., Chini, L., Cooper, H.D., Gilman, E.L., Guénette, S., Hurtt, G.C., Huntington, H.P., Mace, G.M., Oberdorff, T., Revenga, C., Rodrigues, P., Scholes, R.J., Sumaila, U.R. and Walpole, M., 2010. Scenarios for global biodiversity in the 21st century. *Science*,**330**: 1496-1501.

Prasad, R.N., Menon, V.P., Vasudev, V. and Pugalendi, K.V., 2005. Radioprotective effect of sesamol on Y-radiation induced DNA damage, lipid peroxidation and antioxidants level in cultured human lymphocytes. *Toxicology*,**209**: 225-335.

Pratheeshkumar, P. and Kuttan, G.,2011**.** Protective role of Vernonia cinerea L. against gamma radiation induced immunosupression and oxidative stress in mice. *Hum. Exp. Toxicol.*,**30**: 1022-1038.

Roche, M., Kemp, F.W., Agrawal, A., Attanasio, A., Neti, P.V., Howell, R.W. and Ferrarıs, R.P.,2011.Marked changes in endogenous antioxidant expression precede vitamin A, C-, and E-protectable, radiation-induced reductions in small intestinal nutrient transport. *Free Radic. Biol. Med.*, **1**: 55–65.

Ross, D., 1988. Glutathione, free radicals and chemotherapetic agents. *Pharmacol. Ther.*,**37:** 231-249.

Saada, H.N., Rezk, R.G. and Eltahawy, N.A.,2010. Lycopene protects the structure of the small ıntestine against gamma-radiation-induced oxidative stress. *Phytother. Res.*,**24**: 204-208.

Salama, S.F., 2011. Beta-glucan ameliorates gamma-rays induced oxidative injury in male swiss albino rats. *Pakistan J. Zool.*, **43**: 933-951.

Samarth, R.M. and Kumar, A., 2003. Mentha piperiat leaf extract provides protection against radiation induced chromosomaal damage in bone marrow of mice. *Indian J. exp. Biol.*,**41**: 229-237.

Sarma, L. and Kesavan, P.C., 1993. Protective effects of vitamins C and E against gamma-ray-induced chromosomal damage in mouse. *Int. J. Radiat. Biol.*,**63**: 759-64.

Sener, G., Jahovic, N., Tosun, O., Atasoy, B.M. and Yegen, B.C., 2003. Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rats. *Life Sci.*,**74**: 563-572.

Serafini, M. and Del Rio, D., 2004**.** Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool? *Redox Rep.*,**9**: 145-152.

Smıth, D.H. and De Cosse, J.J., 1986. Radiation damage to the small intestine. *World J. Surg.*,**10**: 189-194.

Somosy, Z., Horvath, G., Telbisz, A., Rez, G. and Palfia, Z., 2000. Morphological aspects of ionizing radiation response of small intestine. *Micron*, **33**: 67–178.

Taunk, N.K., Haffty, B.G. and Kostis, J.B., 2015. Radiation-induced heart disease: pathologic abnormalities and putative mechanisms. *Goyal S.Front. Oncol.*,**5**: 1-8.

Tomas-Zapıco, C. and Coto-Montes, A., 2006. Melatonin as antioxidant under pathological processes. Recent patents on endocrine, *Metab. Immune Drug Discov.*,**1:** 63-82.

Warren, S., Patel., S. and Kapron, C.M.,2000. The effect of vitamin E exposure on cadmium toxicity in mouse embryo cells *in vitro*. *Toxicology*, **142**: 119-126.

Yanardag, R. and Koc, L., 2002-2003. The effects of pentachloronitrobenzen and pentachloronitrobenzen +vitamin E on serum total protein level and transaminase activities in rats. *J. Fac. Pharm.*, **3555**: 133-138.

Yanardag, R. and Orak, H., 2001. Total selenium concentration in various waters of Turkey. *Environ. Technol.*, **22**: 237-246.