Immunohistochemical Evaluation of the Protective Effect of *Ginkgo Biloba*, Probiotic *Saccharomyces Boulardii* and N-Acetylcysteine on Radiation-Induced Small Intestine Injury

Radyasyona Bağlı İnce Bağırsak Hasarında *Ginkgo Biloba*, Probiyotik *Saccharomyces Boulardii* ve N-Asetilsistein'in Koruyucu Etkilerinin İmmünohistokimyasal Yöntemle Değerlendirilmesi

Serdar FİLİZ, MD,[a]
Şükriye Bilge GÜRSEL, MD,[b]
Pelin COŞTUR BIYIKSIZ,[a]
Savaş YÖRÜKER, MD,[c]
Süheyla GONCA, MD,[a]
Elif GELENLİ,[a]
Hakkı DALÇIK[a]

[a]Department of Histology&Embryology, Kocaeli University Faculty of Medicine, Kocaeli
Departments of
[b]Radiation Oncology,
[c]General Surgery,
Ondokuz Mayıs University Faculty of Medicine, Samsun

Geliş Tarihi/*Received:* 20.08.2009
Kabul Tarihi/*Accepted:* 13.08.2010

This study was presented in IX. National Histology and Embryology Congress (20-23 May 2009, Adana).

Yazışma Adresi/*Correspondence:*
Serdar FİLİZ, MD
Kocaeli University Faculty of Medicine, Department of Histology&Embryology, Kocaeli,
TÜRKİYE/TURKEY
serdarfiliz@yahoo.com

ABSTRACT Objective: The aim of this study was to demonstrate the radioprotective effects of ginkgo biloba (*G. biloba*, EGb 761), probiotic *Saccharomyces boulardii (S. boulardii)* and N-acetylcysteine (NAC) on radiation-induced small intestine injury via immunohistochemical evaluation using pancadherin, a cell adhesion molecule. **Material and Methods:** A total of 48 female Wistar albino rats were divided into five groups: negative and positive controls, G. biloba, *S. Boulardii* and NAC groups. The radioprotective agents were started as twice per day by gavage regimen the day before the first radiation exposure. External abdominal radiotherapy, 5Gy/day, under general anesthesia was administered on five consecutive days ending up with five fractions in total (25Gy). Radioprotective agent administration was completed to 14 days and the rats were sacrificed on the 15th day. After extraction of the jejunum, tissue morphology, integrity and the number of villi were evaluated in H&E stained slides and pancadherin immunoreactive cells were evaluated immunohistochemically. **Results:** The disruption of mucosal morphological integrity, degenerative spaces and edematous cavities were observed after radiation-induced jejunum injury. The contribution of the radioprotective effect of all agents were shown on tissue integrity. However, the most prominent effect was noted in the NAC group. In addition, the numbers of villi and pancadherin immunoreactive cells in the NAC group were significantly higher than the other groups. **Conclusion:** NAC is a good agent in protecting against and ameliorating adverse effects of radiotherapy in a rat model.

Key Words: Radiotherapy; intestine, small; radiation-protective agents; cadherins; immunohistochemistry

ÖZET Amaç: Bu çalışmada, radyasyona bağlı ince bağırsak hasarında *Ginkgo biloba* (EGb 761), probiyotik *Saccharomyces boulardii* ve N-asetilsistein (NAC)'in radyoprotektif etkilerini bir hücre adhezyon molekülü olan pankaderin ile immunohistokimyasal olarak ortaya koymak amaçlanmıştır. **Gereç ve Yöntemler:** Toplam 48 dişi Wistar albino sıçanı negatif ve pozitif kontrol grubu, G. biloba, *S. Boulardii* ve NAC grubu olmak üzere beş gruba ayrılmıştır. Radyoprotektif ajanlar, radyasyon uygulamasından bir gün önce sonda ile günde iki kez uygulanmaya başlamıştır. Eksternal abdominal radyoterapi genel anestezi altında 5Gy/gün dozundan beş gün süre ile uygulanmıştır (25Gy). Radyoprotektif ajanların uygulanması 14 güne kadar devam edilip on beşinici gün sıçanlar değerlendirilmek üzere kesilmiştir. Jejunum kesitlerinde doku morfolojisi, doku bütünlüğü ve villus sayısı H&E boyama ile değerlendirilirken pankaderin immunoreaktif hücreler immünohistokimyasal yöntemle değerlendirilmiştir. **Bulgular:** Radyasyona bağlı jejunum hasarı sonrası mukoza yapısında bozulma, dejeneratif boşluklar ve ödematöz kaviteler tespit edilmiştir. Tüm radyoprotektif ajanların doku bütünlüğüne olan desteği görülmüştür. Ancak, en belirgin etki NAC grubunda görülmüştür. Villus sayısı ve pankaderin içeren immunreaktif hücreler diğer gruplara göre NAC grubunda daha fazla tespit edilmiştir. **Sonuç:** NAC, sıçan modelindeki radyoterapiye bağlı yan etkilere karşı korumada ve radyoterapiye bağlı hasarları iyileştirmede iyi bir ajandır.

Anahtar Kelimeler: Radyoterapi; bağirsak, ince; radyasyondan-koruyucu ajanlar; kaderinler; immünohistokimya

Turkiye Klinikleri J Med Sci 2010;30(5):1433-40

Radiotherapy is widely used in the treatment of different types of cancer including abdominal and pelvic cancers. Although radiation is directed against the malignant tissues, its side effects in normal tissues

limit its therapeutic effectiveness. The small intestine is one of the most radiosensitive organs; thus it is the main dose-limiting factor for abdominal and pelvic radiotherapies.[1] Radiation enteritis presents as atrophy, ulceration and inflammation in the intestinal mucosa and is a serious clinical problem in abdominal radiotherapy patients.[2,3] Bacteria and endotoxins pass through the distrupted mucosal barrier and enter extra-intestinal tissues as well as the bloodstream.[4] After abdominal radiation, disruption of morphologic mucosal integrity and normal bacterial microflora lead to malabsorbtion.[5] Diarrhea, vomiting, abdominal cramps and weight loss are the complaints of the patients during and following radiation treatment of abdominal and gynecological malignancies.[6] Damage to the gastrointestinal tract remains a significant problem in spite of some technical modalities that lower the radiotherapy-related complications. Tissue injury from ionizing radiation is believed to be a consequence of a cascade of cytokine activity, which ultimately begins with oxidative stress from radiolytic hydrolysis and formation of reactive oxygen metabolites.[7,8] Furthermore, DNA, lipids and proteins are also attacked by free radicals induced by ionizing radiation which results in DNA damage, apoptosis or cell necrosis.[9-11]

Ginkgo biloba (G. biloba), extracted from the leaves of ginkgo biloba tree, is composed of flavonoid glycosides, terpenoids, ginkgolides and bilobalide.[12] G. biloba extract is a potent free radical scavenger, antioxidant and also a platelet activating factor inhibitor.[13,14] Anticlastogenic effect of G. biloba was tested at a dose of 50 and 100 mg/kg and found as 66 and 83%, respectively.[15] G. biloba was used in rats irradiated with 800 cGy whole body gamma irradiation at a dose of 50 mg/kg and showed a potential benefit in enhancing the success of radiotherapy.[16]

Probiotic is a generic term and refers to a product containing viable and defined microorganisms in a number thought to be sufficient to alter the host's microflora by implantation or colonization and thereby exert beneficial effects.[17] *Saccharomyces boulardii* (*S. boulardii*) is non-pathogenic yeast used as a biotherapeutic agent and is widely prescribed in a lyophilized form in many countries.[18] It has been found that oral administration of *S. boulardii* (1 mg/kg) after proximal enterectomy improves functional adaptation of the remnant ileum.[19]

N-acetylcysteine (NAC) is a well-known precursor of glutathione (GSH), an endogenous nonenzymatic antioxidant that plays a pivotal role in protecting mammalian cells from oxidative damage.[20] NAC also elicits beneficial effects on inflammation process.[21] The protective effect of NAC on a number of issues including fibrosis and depression of free radicals has been studied at various doses, ranging from 5 to 250 mg/kg.[22]

Cadherins are transmembrane proteins, responsible for selective cell recognition and normal tissue integrity, and they regulate morphogenesis in a variety of organs during development.[23] They are able to control the polarization of cells and hence exert direct signaling activity.[24] Interaction of cadherins with neighboring cells could intracellulary trigger a cascade of biochemical events that ultimately leads to the correct positioning, recognition, and communication of cells.[25]

Three regions of the small intestine (duodenum, jejunum, and ileum) have similar histological common features as plicae circulares, villi, and microvilli. The mucosa of the small intestine is composed of three layers: a simple columnar epithelium, the lamina propria, and the muscularis mucosae. The submucosa of the small intestine is composed of dense, irregular fibroelastic connective tissue with a rich lymphatic and vascular supply. The muscularis externa of the small intestine is composed of an inner circular layer and an outer longitudinal smooth muscle layer. Except for some parts of the duodenum, the entire small intestine is invested by a seroza. The villi of the jejunum are narrower, shorter, and sparser than those of the duodenum. The number of goblet cells per unit area is greater in the jejunum than in the duodenum.

The aim of this study was to demonstrate the histological evaluation of the radioprotective effects of G. biloba, probiotic *(S. Boulardii)* and NAC on radiotherapy induced small intestine injury. To our knowledge, this is the first experiment dealing with the effects of radioprotective agents on the cell adhesion molecule pancadherin.

MATERIAL AND METHODS

ANIMALS

A total of 48 female Wistar albino rats weighing 220-260 g were used in this study (Ondokuz Mayıs University Medical and Surgical Experimental Research Labs). Care and use of the animals were in accordance with NIH guidelines and Institute Animal Care and Use Committee regulations. The experiment protocol was approved by the Animal Experiments Local Ethical Committee of Ondokuz Mayıs University. All rats were housed in a temperature and humidity controlled environment, two per cage, with a 12 h light/12 h dark cycle at 21-24 °C. They were fed ad libitum standard rat chow and had free access to water. The rats were randomly divided into five groups: The first group (control, Group I, n= 6) received sham irradiation with table lamp. The second group (Group II, n= 6) received only irradiation. The third group (G. biloba treatment group, Group III, n= 12) received irradiation and G. biloba (Tebokan®, Abdi İbrahim, İstanbul, Turkey) 50 mg/kg/day. The fourth group (Probiotic, *S. boulardii,* treatment group, Group IV, n= 12) received irradiation and probiotic (Reflor®, Sanofi Aventis, İstanbul, Turkey) 1 mg/kg/day. The fifth group (NAC treatment group, Group V, n= 12) received irradiation and NAC (Asist®, Hüsnü Arsan, İstanbul, Turkey) 100 mg/kg/day.

IRRADIATION

The rats were anesthetized with a subcutaneous injection of 0.05 mg/g ketamine hydrochloride (50 mg/ml, Ketalar® Pfizer, Turkey) before all irradiation applications. After anesthesia, all extremities of the animals were fixed to a special frame with adhesive bands and the abdominal regions were shaved. Under the xiphoid process, one anterior field of 4 x 5 cm (width X length) was simulated in SSD 80 technique (source skin distance 80 cm) (Nucletron System 300). External abdominal radiotherapy, 5 Gy/day, was administered for five consecutive days ending up with a total of five fractions (25 Gy). The animals were irradiated with Cobalt-60 photons (Theratronics, Theratron 780 C) at skin dose, via one anterior field.

The radioprotective agents were started as twice per day by gavage regimen the day before the first radiation exposure and completed within 14 days. The rats were sacrified on the 15th day.

H&E STAINING AND IMMUNOHISTOCHEMISTRY

After extraction of the jejunum segments, the samples were fixed in 4% paraformaldehide, washed in water, dehydrated with alcohols, cleared in xylene and embedded in paraffin. Five μm thick paraffin sections were removed from tissues and then deparafinized and rehydrated. H&E staining was applied to sections in order to evaluate tissue morphology, integrity and villus number. Immunohistochemistry was performed using a monoclonal antibody against pancadherin using avidin-biotin-peroxidase method (Zymed®, San Francisco CA, USA). The monoclonal anti-pancadherin antibody (clone CH-19), which was generated by using a synthetic peptide corresponding to the C-terminal amino acids of chicken N-cadherin with an extra N-terminal lysine residue as immunogen showing reaction with a distinct 135 kD band in a wide variety of tissue and species was obtained from Sigma (St. Louis MO, USA). Sections were washed several times with phosphate buffered saline-triton X-100 (PBS-Tx) and they were pre-treated with 3% hydrogen peroxide for 10 min to eliminate endogenous peroxidase activity. To reduce non-specific staining, sections were pretreated with normal rabbit serum for 20 min. Sections were then incubated with a 1/200 dilution of anti-pancadherin antibody for 24 h at 4 °C in a humidified chamber. After washing in PBS-Tx, biotinylated secondary antibody (Histostain plus kit, Zymed®) was applied for 15 min at room temp. Following washing in PBS-Tx, streptavidin-peroxidase conjugate (Histostain plus kit, Zymed®) was applied for 15 min at room temp. After washing in PBS-Tx, 0.6% H_2O_2 and 3, 3'-diaminobenzidine (DAB) was applied as chromogen for 3-5 min at room temperature. Sections were examined by light microscopy (BX5OF-3; Olympus, Tokyo, Japan). In the absence of primary antibody, sections were incubated with PBS for immunohistochemical controls.

COUNTING

Counting procedunes were performed independently by two observers who were blinded for the evaluated slides (SF and PCB). The number of villi was determi-

ned at five separate microscopic fields of the jejunum in 5 sections for each animal (total 25 microscopic fields) and recorded as the mean value by using light microscopy at an original magnification x 200.

Quantification of pancadherin immunoreactive cells was performed in randomly selected 10 villus of the jejunum in 5 sections for each animal (total 50 villi) at an original magnification x 400.

STATISTICAL ANALYSIS

SPSS 10.0 statistical software was used for statistical analysis of the data using Kruskal-Wallis one-way analysis of variance test. All data were expressed as mean ± SD. A p value of <0.05 was considered statistically significant.

RESULTS

Histological analysis of intestinal sections demonstrated the hazardous effects of irradiation in all groups when compared to the control group. In Group I (control), the number of villi and pancadherin immunoreactive (IR) cells in the villus epithelium were greater than the other groups ($p< 0.05$; Graph 1). Pancadherin immunoreactivity was also present in the lamina propria, muscularis mucosa, muscularis externa, myenteric plexus, and around all vessels (Figure 1A, B). In H&E staining of Group I, intestinal structures are normal (Figure 1C).

The intestinal mucosa of Group II (irradiation alone) rats showed superficial mucosal ulcerations, villus loss, cryptic dilatations, increased vascularity and dilated vessels in the mucosa, submucosa, and muscular layers. Lamina propria destruction was also seen in some villi. In addition, mucosa was separated from the underlying submucosa in some areas (Figure 2A, B, and for H & E staining C). The number of villi and pancadherin IR cells in the villus epithelium of Group II were smaller than the other therapeutic groups ($p< 0.05$; Graph 1).

In Group III (irradiated and received *G. biloba*), the number of villi and pancadherin IR cells were greater than Group II ($p< 0.05$), but smaller than Group I, IV and V ($p< 0.05$; Graph 1). Mucosal ulceration, cryptic dilatations and dilated vessels were present more notably than the other groups, except for Group II. Lymph vessel congestion was also present in the sections of Group III. Neverthe-

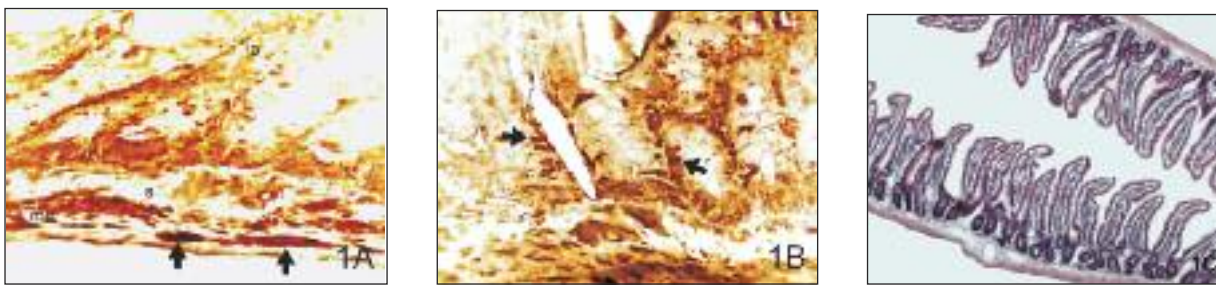


FIGURE 1: Photomicrograph of pancadherin immunostaining in jejunum of the control rat (Group I). (A) Intestinal structures are normal. Pancadherin immunoreactivity is present in the lamina propria (lp), submucosa (s), muscularis externa (me), and myenteric plexus (arrows). (B) Pancadherin immunoreactive cells are present in the crypts (arrows). (C) H & E staining of Group I. Original magnifications x400 for A, B, and x40 for C.

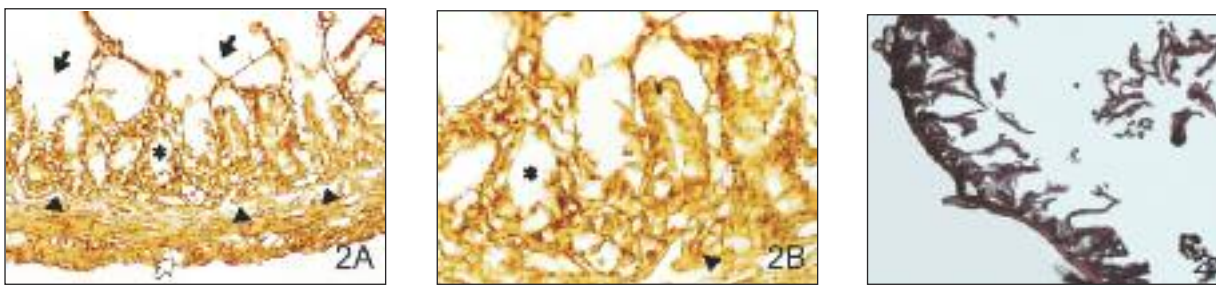


FIGURE 2: Photomicrographs of Group II (irradiation alone). Superficial mucosal ulcerations (solid arrows), villus loss, cryptic dilatations (asterisks), dilated vessels (open arrow), and increased vascularity are present. Notice the separated mucosa from underlying submucosa (arrowheads). Original magnifications x200 for A, x400 for B, and x40 for C (H & E staining).

less, lamina propria in the villus was relatively well protected (Figure 3A, B, for H&E staining C).

In Group IV (irradiated and received probiotic) superficial mucosal ulceration was present in patches. Cryptic dilatations and lymph vessel congestions were relatively more frequent than Group III. Although dilated vessels and edematous cavities were seen, the tissue integrity was well in comparison with Group III (Figure 4A, B, and for H&E staining C). The number of villi and pancadherin IR cells were greater in number when compared to Group II and III ($p< 0.05$), but smaller than Group V and similar to Group V (Graph 1).

In Group V (irradiated and received NAC), mucosal integrity was well protected. Cryptic dilatations and dilated vessels were significantly less when compared to Group II, III and IV (Figure 5A, B, and for H&E staining C). The number of villi and pancadherin IR cells were greater than Group II and III ($p< 0.05$; Graph 1).

In all groups, pancadherin immunoreactivity was seen in the lamina propria, muscularis mucosa, around the vessels and in the muscularis externa. The intensity of the immunoreactivity was dense in epithelial cells located towards the tip of the villi compared with the cells located at the base. In ad-

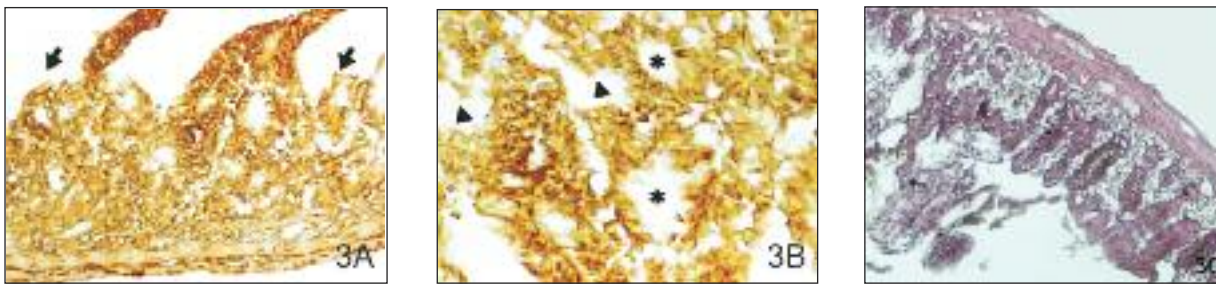


FIGURE 3: Photomicrographs of Group III (irradiation plus G. biloba). Superficial mucosal ulcerations (arrows), cryptic dilatations (asterisks), and edematous cavities (arrowheads) are seen. Pancadherin immunoreactivity increased in the villus epithelium, lamina propria, and around the vessels. Original magnifications x200 for A, x400 for B, and x100 for C (H & E staining).

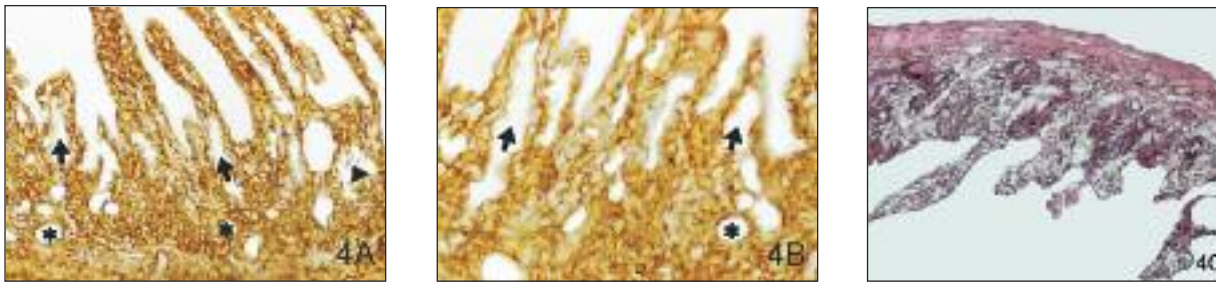


FIGURE 4: Photomicrographs of Group IV (irradiation plus probiotic). Fewer superficial mucosal ulcerations are seen. Cryptic dilatations (asterisks) and lymph vessel congestions (arrows) are prominent. Edematous cavity (arrowhead) is also observed. Pancadherin immunoreactivity is intense in the villus epithelium and lamina propria. Original magnifications x200 for A, x400 for B, and x100 for C (H & E staining).

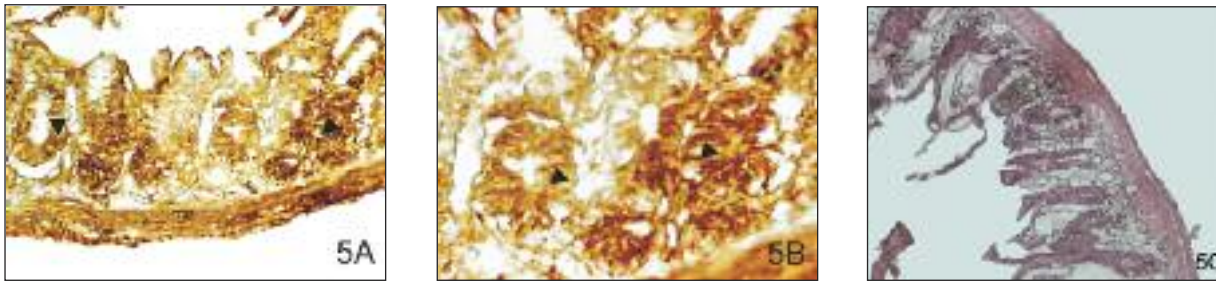


FIGURE 5: Photomicrographs of Group V (irradiation plus NAC). Mucosal integrity is well protected. Superficial mucosal ulcerations, cryptic dilatations and dilated vessels are significantly fewer. Lymph vessel congestion is absent. Pancadherin immunoreactivity in the villus epithelium, crypts (arrowheads) and muscular layer (me) are more intense. Original magnifications x200 for A, x400 for B, and x100 for C (H & E staining).

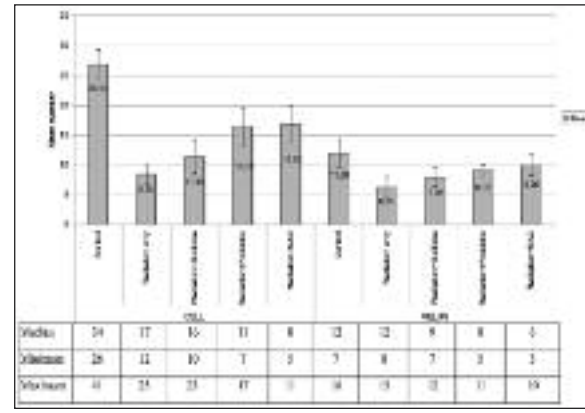
dition, epithelial cell shape changes were observed in all groups when compared to the control rats.

DISCUSSION

Ionizing radiation induces various morphological, functional and biochemical changes in small intestine. The intestinal epithelium undergoes rapid and continuous self-renewal along the crypt-villus axis ensured by the mitotic activity of its stem cells. Stem cells differentiate into columnar epithelium and migrate toward the villus.[26] This rapid cycling constitutes the basis for the high sensitivity of the gastrointestinal mucosa to radiation.[27] The results of the present study demonstrated the destructive effects of radiation on intestinal mucosa and the efficacy of radioprotective agents to minimize the side effects of radiotherapy. However, in this study antioxidant levels at baseline were not assessed, which in part might have contributed to the observed radioprotective effect.

In the present study, ionizing radiation caused intestinal mucosal ulcerations, villus loss, cryptic dilatations, and increased vascularity and dilated vessels in the mucosa, submucosa, and muscular layers which ascertained inflammation. Besides, separated mucosa from the underlying submucosa was seen in some areas. Similar findings were also demonstrated in other studies.[4,5,28,29] The localization of pancadherin immunoreactivity was shown in normal intestinal tissues in our previous studies.[30,31] For the first time, the present study determined the localization of pancadherin immunoreactivity in small intestine of irradiated rats, as well as the effects of radioprotective agents on pancadherin immunoreactivity.

In this study, the number of pancadherin IR cells in the villus epithelium was considerably low in Group II (irradiation alone). During radiotherapy, ionizing irradiation particles interact with biological systems to induce a cascade of cytokine activity and excessive oxygen free radicals or reactive oxygen species, which attack various cellular components including DNA, proteins and membrane lipids, thereby leading to significant cellular damage.[7,32] In relatively few instances, free radicals may perform beneficial functions, such as in the destruction of



GRAPH 1: Mean number of pancadherin immunoreactive cells and villi.
*Error bars indicate ± standard deviation.

pathogens during phagocytic activation or in the regulation of vascular tone.[33] Under normal conditions, free radicals generated during cell metabolism are rapidly scavenged by endogenous antioxidant enzymes, however, in tissue injury, free radicals increase excessively. Acute radiation to the small intestine causes immediate and potentially reversible effect on the sensitive regenerative epithelium of the intestinal mucosa while repeated exposure to radiation therapy may lead to a progressive disease through irreversible obliterative vasculitis changes.[34] Cadherins play a major role in maintaining tissue integrity and induce epithelial polarity, plasticity and survival during epithelial remodeling.[35,36] The integrity of polarized intestinal epithelia is ensured by a series of junctions, tight junctions, adherent junctions, and desmosomes.[37] So, it can be assumed that the low levels of pancadherin IR especially at the tip of the villus may indicate the destruction of epithelial cells and their integrity.

In Group III (irradiated and received G. biloba), the number of villi and pancadherin IR cells were greater than the irradiation alone group, but smaller than the other groups. In addition, mucosal ulceration, cryptic dilatations and dilated vessels were more compared to the other treatment groups. G. biloba extract, particularly the flavonoid glycoside component scavenges the excess free radicals.[38] It was shown that severe epithelial loss of the villi and inflammatory cell invasion in the lamina propria due to irradiation was replaced to regular tissue integrity with G. biloba pretreatment for

15 days.[16] In our study, although G. biloba treatment was effective on the protection of villi and their lamina propria, this effect was less than the ones in the other treatment groups.

In Group IV (irradiated and received probiotic), there were superficial mucosal ulcerations in patches. Cryptic dilatations and lymph vessel congestions were relatively more frequent compared to Group III. We determined that the number of villi and pancadherin IR cells were greater than those in Group II and III, but there was no statistically significant difference with Group V. It has been shown that *S. boulardii* is resistant to acidity, proteases and naturally to all antibacterial antibiotics and furthermore, has no known adverse effects.[18,39] After treatment with *S. boulardii*, significant increases in several brush border microvillus enzymes were found in humans and produced a marked stimulation of secretory IgA production in intestinal crypt cells which is the main immunologic defense barrier of the intestinal epithelium.[18,40] Previous studies demonstrated that use of probiotic organisms may effectively down modulate the severity of intestinal inflammation through altering the composition and the metabolic and functional properties of gut indigenous flora.[41] Our results were in parallel with Demirer et al. who used *L. bulgaricus* and concluded that the administration of probiotics in the treatment of radiation-induced enteritis exerted a radioprotective effect on small intestinal mucosa which may be related to their anti-inflammatory actions.[5] Other studies carried out with different species and strains *L. acidophilus, L. helveticus, L. casei, L. plantarum, L. delbrueckii subsp. bulgaricus, B. longum, B. breve, B. infantis* probiotics have also shown a radioprotective effect. However, such effect cannot be extrapolated to probiotics in general.[34,42]

The present study clearly demonstrated that mucosal integrity was well protected in Group V (irradiated and received NAC). Cryptic dilatation and dilated vessels were less when compared to other treatment groups. Furthermore, number of villi and pancadherin IR cells were greater when compared to all other treatment groups, except for the control group. This difference was significant for Group II and III, but insignificant for Group IV. NAC is one of the most widely investigated pharmacological agents among free radical scavengers. NAC exerts its antioxidant effect in two ways: first, as a source of sulphydryl groups it indirectly facilitates GSH biosynthesis and, thereby, increases GSH supply for glutathione peroxidase. Secondly, it directly reacts with reactive oxygen species.[43] Thiol and its derivatives constitute the most effective class of radioprotection compounds and NAC is a thiol reducing agent which has antioxidant, antiangiogenic, and anticarcinogenic properties.[44,45] The radioprotective effects of NAC on cochlear cells and ovary cells were attributed to a mechanism operating over the thiol pathway.[46,47] NAC treatment maintained the intestinal mucosal barrier integrity after burn injury and had a radioprotective effect on liver tissue.[32,48,49] E-cadherin is one of the major cadherins in the intestinal epithelium and an alteration of its expression has been observed in a number of diserders involving loss of cell polarizations and differentiation.[50] The expression of pancadherin in the intestinal epithelium in NAC treatment was increased. This may indicate the protective effect of NAC on formation of cellular junctional complexes between intestinal cells which was more pronounced than that of G. biloba and probiotic treatment groups.

In the present study, the intensity of the immunoreactivity was dense in epithelial cells located towards the tip of the villi compared with those located in the base. Continuously generated epithelial cells in the crypt region are pushed up to higher levels on the villi. Thus it can be assumed that the low levels of immunoreactivity in the cells located at the base may be due to proliferation, differentiation and migration of epithelial cells, and adhesion complexes established at the tip of the villi. We also observed epithelial cell shape changes in all groups when compared to the control group. It is known that irradiation alters tight and adherent junctions. The destruction of tight junctions may lead to alterations of barrier function reflected by changes in paracellular pathway when the disruption of adherent junctions implicated in the intimate cell-cell contact may lead to the shape change of epithelial cells.[51]

In conclusion, our results indicated that abdominal irradiation caused severe intestinal mucosal destruction and although, G. biloba and probiotics may

have constructive effects against irradiation-induced intestinal damage; NAC may have beneficial effects. However, further studies are needed for implications of the use of such agents in clinical treatment.

Acknowledgement

We are grateful to Assoc. Prof. Dr. Müge Filiz for her contribution in statistical analysis and proofreading.

REFERENCES

1. Emami B, Lyman J, Brown A, Coia L, Goitein M, Munzenrider JE, et al. Tolerance of normal tissue to therapeutic irradiation. Int J Radiat Oncol Biol Phys 1991;21(1):109-22.
2. Waddell BE, Rodriguez-Bigas MA, Lee RJ, Weber TK, Petrelli NJ. Prevention of chronic radiation enteritis. J Am Coll Surg 1999;189 (6):611-24.
3. Howarth GS, Fraser R, Frisby MB, Schrmer MB, Yeoh EK. Effects of insulin-like growth factor-I administration on radiation enteritis in rats. Scand J Gastroenterol 1997;32(11): 1118-24.
4. Alexandrides TH, Spiliotis J, Mylonas P, Melachrinou M, Kardamakis D, Spiliopoulou C, et al. Effect of growth hormone and insulin-like growth factor-I on radiation enteritis. Eur Surg Res 1998;30(5):305-11.
5. Demirer S, Aydıntug S, Aslım B, Kepenekci I, Sengül N, Evirgen O, et al. Effects of probiotics on radiation-induced intestinal injury in rats. Nutrition 2006;22 (2):179-86.
6. Yeoh E, Horowitz M. Radiation enteritis. Surg Gynecol Obstet 1987;165(4):373-9.
7. Rubin P, Johnston CJ, Williams JP, McDonald S, Finkelstein JN. A perpetual cascade of cytokines postradiation leads to pulmonary fibrosis. Int J Radiat Oncol Biol Phys 1995;33(1): 99-109.
8. Agrawal A, Chandra D, Kale RK. Radiation induced oxidative stress: II studies in liver as a distant organ of tumor bearing mice. Mol Cell Biochem 2001;224(1-2):9-17.
9. Isabelle V, Prevost C, Spotheim-Maurizot M, Sabattier R, Charlier M. Radiation induced damage in single and double-stranded DNA. Int J Radiat Biol 1995;67(2):169-76.
10. Potten CS, Merritt A, Hickman J, Hall P, Faranda A. Characterization of radiation induced apoptosis in the small intestine and its biological implications. Int J Radiat Biol 1994;65 (1):71-8.
11. Mylonas PG, Matsouka PT, Papandoniou EV, Vagianos C, Kalfarentzos F, Alexandrides TK. Growth hormone and insulin-like growth factor I protect intestinal cells from radiation induced apoptosis. Mol Cell Endocrinol 2000; 160(1-2):115-22.
12. Van Beek TA. Ginkgolides and bilobalide: their physical, chromatographic and spectroscopic properties. Bioorg Med Chem 2005;13 (17):5001-12.
13. Maitra I, Marcocci L, Droy-Lefaix MT, Packer L. Peroxyl radical scavenging activity of Ginkgo biloba extract EGb 761. Biochem Pharmacol 1995;49(11): 1649-55.
14. Yoshikawa T, Naito Y, Kondo M. Ginkgo biloba leaf extract: Review of biological actions and clinical applications. Antioxid Redox Signal 1999;1(4): 469-80.
15. Alaoui-Youssefi A, Lamproglou I, Drieu K, Emerit I. Anticlastogenic effects of Ginkgo biloba extract (EGb 761) and some of its constituents in irradiated rats. Mutat Res 1999;445 (1):99-104.
16. Sener G, Kabasakal L, Atasoy BM, Erzik C, Velioglu-Ogunc A, Cetinel S, et al. Ginkgo biloba extract protects against ionizing radiation-induced oxidative organ damage in rats. Pharmacol Res 2006;53(3): 241-52.
17. Famularo G, De Simone C, Matteuzzi D, Pirovano F. Traditional and high potency probiotic preparations for oral bacteriotherapy. BioDrugs 1999;12(6):455-70.
18. Buts JP, DeKeyser N. Effects of Saccharomyces boulardii on intestinal mucosa. Dig Dis Sci 2006;51(8):1485-92.
19. Buts JP, De Keyser N, Marandi S, Hermans D, Sokal EM, Chae YH, et al. Saccharomyces boulardii upgrades cellular adaptation after proximal enterectomy in rats. Gut 1999;45(1): 89-96.
20. Anderson ME. Glutathione and glutathione delivery compounds. Adv Pharmacol 1997;38: 65-78.
21. Tsuji F, Miyake Y, Aono H, Kawashima Y, Mita S. Effects of bucillamine and N-acetylcysteine on cytokine production and collagen-induced arthritis. Clin Exp Immunol 1999;115 (1):26-31.
22. Ferreira FR, Biojone C, Joca SR, Guimarães FS. Antidepressant-like effects of N-acetyl-L-cysteine in rats. Behav Pharmacol 2008;19(7): 747-50.
23. Takeichi M. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development 1988;102(4):639-55.
24. Vleminckx K, Kemler R. Cadherins and tissue formation: integrating adhesion and signaling. Bioessays 1999;21(3):211-20.
25. Behrens J. Cadherins as determinants of tissue morphology and suppressors of invasion. Acta Anat 1994;149(3):165-9.
26. Maj JG, Paris F, Haimovitz-Friedman A, Venkatraman E, Kolesnick R, Fuks Z. Microvascular function regulates intestinal crypt response to radiation. Cancer Res 2003;63 (15):4338-41.
27. Potten CS, Booth C, Pritchard DM. The intestinal epithelial stem cell: the mucosal governor. Int J Exp Pathol 1997;78(4):219-43.
28. Erbil Y, Dibekoglu C, Turkoglu U, Ademoglu E, Berber E, Kizir A, et al. Nitric oxide and radiation enteritis. Eur J Surg 1998;164(11):863-8.
29. Erbil Y, Oztezcan S, Giris M, Barboros U, Olgac V, Bilge H, et al. The effect of glutamine on radiation-induced organ damage. Life Sci 2005;78(4):376-82.
30. Filiz S, Dalcik H, Yardimoglu M, Gonca S, Ceylan S. Localization of pan-cadherin immunoreactivity in adult rat tissues. Cell Biol Int 2002;26(11):985-91.
31. Dalçik C, Filiz S, Filiz TM, Dalçik H. Immunohistochemical analysis of neural cell adhesion molecule (N-CAM) and pan-cadherin in the small intestine of intrauterine growth-retarded newborn rats caused by maternal protein malnutrition. Acta Histochem 2003;105(2):183-90.
32. Mansour HH, Hafez HF, Fahmy NM, Hanafi N. Protective effect of N-acetylcysteine against radiation induced DNA damage and hepatic toxicity in rats. Biochem Pharmacol 2008;75(3):773-80.
33. Mantle D, Wilkins RM, Gok MA. Comparison of antioxidant activity in commercial Ginkgo biloba preparations. J Altern Complement Med 2003;9(5):625-9.
34. Seal MG, Naito Y, Barreto R, Lorenzetti A, Safran P, Marotta F. Experimental radiotherapy-induced enteritis: a probiotic interventional study. Dig Dis 2007;8(3): 143-7.
35. Oberg S, Peters JH, De Meester TR. Inflammation and specialized intestinal metaplasia of the cardiac mucosa is a manifestation of gastroesophageal reflux disease. Ann Surg 1997;226(4):522-30.
36. Erguler G, Demir N, Demir R. [Structural properties and functions of adhesion molecules]. Turkiye Klinikleri J Med Sci 2002;22(3):313-27.
37. Denker BM, Nigam SK. Molecular structure and assembly of the tight junction. Am J Physiol 1998;274(1 Pt 2): F1-9.
38. Pincemail J, Dupuis M, Nasr C, Hans P, Haag-Berrurier M, Anton R, et al. Superoxide anion scavenging effect and superoxide dismutase activity of Ginkgo biloba extract. Experientia 1989;45(8):708-12.
39. Buts JP, Bernasconi P, Van Craynest MP, Maldague P, De Meyer R. Response of human and rat small intestinal mucosa to oral administration of Saccharomyces boulardii. Pediatr Res 1986;20(2):192-6.
40. Buts JP, Bernasconi P, Vaerman JP, Dive C. Stimulation of secretory IgA and secretory component of immunoglobulin in small intestine of rats treated with Saccharomyces boulardii. Dig Dis Sci 1990;35(2):251-6.
41. Famularo G, Mosca L, Minisola G, Trinchieri V, De Simone C. Probiotic lactobacilli: a new perspective fort he treatment of inflammatory bowel disease. Curr Pharm Des 2003;9(24): 1973-80.
42. Delia P, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, et al. Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol 2007;13(6): 912-5.
43. Aruoma OI, Halliwel B, Hocy BM, Butler J. The antioxidant action of N-Acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Radic Biol Med 1989;6(6): 593-7.
44. Hosseinimehr SJ, Shafiee A, Mozdarani H, Akhlagpour S. Radioprotective effects of 2-iminothiazolidine derivatives against lethal doses of gamma radiation in mice. J Radiat Res 2001;42(4):401-8.
45. Tosetti F, Ferrari N, De Flora S, Ablini A. Angioprevention: angiogenesis is a common and key target for cancer chemopreventive agents. FASEB J 2002;16(1):2-14.
46. Low WK, Sun L, Tan MG, Chu AW, Wang DY. L-N-Acetylcysteine protects against radiation-induced apoptosis in a cochlear cell line. Acta Otolaryngol 2008;128(4):440-5.
47. Wu W, Abraham L, Ogony J, Matthews R, Goldstein G, Ercal N. Effects of N-acetylcysteine amide (NACA), thiol antioxidant on radiation-induced cytotoxicity in Chinese hamster ovary cells. Life Sci 2008;82(21-22): 1122-30.
48. Ocal K, Avlan D, Cinel I, Unlu A, Ozturk C, Yaylak F, et al. The effect of N-acetylcysteine on oxidative stress in intestine and bacterial translocation after thermal injury. Burns 2004;30(8):778-84.
49. Liu Y, Zhang H, Zhang L, Zhou Q, Wang X, Long J, et al. Antioxidant N-acetylcysteine attenuates the acute liver injury caused by X-ray in mice. Eur J Pharmacol 2007;575(1-3):142-8.
50. Ahuja V, Dieckgrafe BK, Anant S. Molecular biology of the small intestine. Curr Opin Gastroenterol 2006;22(2):90-4.
51. Dublineau I, Lebrun F, Grison S, Griffiths NM. Functional and structural alterations of epithelial barrier properties of rat ileum following X-irradiation. Can J Physiol Pharmacol 2004;82(2):84-93.