Indian Journal of Experimental Biology
Vol. 59, March 2021, pp. 162-167

Effect of edaravone on lungs and small intestine in rats with induced radiotherapy

Hatice Sonay Yalcin Comert[1]*, Mustafa Imamoglu[1], Mustafa Emre Ercin[2], Esra Eksi[1], Mustafa Kandaz[3], Ahmet Alver[4] & Haluk Sarihan[1]

[1]Department of Pediatric Surgery; [2]Department of Pathology; [3]Department of Radiation Oncology; and [4]Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

Received 30 October 2019; *revised 17 February 2021*

Radiotherapy is a frequently used method for treatment of cancer which is regarded as one of the top two diseases causing premature death worldwide. However, radiotherapy is known to have many side effects. In this study, we evaluated biologically and histologically the possible protective effects of edaravone, the free radical scavenger and neuroprotective agent used to treat amyotrophic lateral sclerosis, on lung and small intestine against radiation-induced early side effects of 15 Gy total body irradiation in single fraction. Thirty-two rats were divided randomly into four equal groups. Groups were administered 15 Gy of external ionizing radiation to the whole body after 30 minutes of EDA administration (a dose of 500 and 50 mg/kg). Rats were sacrified at 72 h of the experiment. Tissues were separated to investigate levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPX) and malondialdehyde (MDA); and evealuate histopathological changings. The protective effect of EDA showed statistically significant in MDA, SOD and GSHPX values of lungs except CAT and statistically significant effect was observed in MDA in small intestine. Also, we showed statistically significant values with histopathological changings. Pediatric cancer patients who have a longer life expectancy are as important as their recovery from their normal life. We are expecting that EDA as a modulator of free radical scavenging pathways in many organs can reduce the side effects of radiation damage on lungs and small intestine.

Keywords: Cancer therapy, Free radical scavenging, Pediatric cancer, Tumor

Worldwide, cancer is reported to be the primary cause of premature death in every country[1]. According to the World Health Organization (WHO), cancer is either first or second cause of death in majority of the countries. With an estimated 19.3 million new cases, cancer causes 10 million deaths across the globe[2]. The global cancer burden by 2040 is estimated to be 28.4 million cases, 47% rise from 2020[2]. Currently, the average childhood cancer burden (<15 years of age) is reported to be 140 new cases per million children[1]. In USA alone, approximately, one in 285 children are diagnosed with cancer each year, and at any one time, thousands of children are living with cancer[3,4].

Radiation therapy using ionizing radiation is one of the treatment approaches for cancer. It is a local treatment and has the advantage of being non-invasive and potentially organ preserving. It is an important therapy in the multimodal treatment for mostly seen childhood solid tumours in abdomen like neuroblastoma or Wilm's tumor[5,6] and thorax metastasis of Ewing sarcoma or Hodgkin lymphoma[7]. Nevertheless, like other treatments, radiation therapy causes some side effects especially in the commonly used abdominal and thoracic region. The most early side effects of thoracic radiation therapy are difficulty of swallowing, shortness of breath, breast or nipple soreness, cough, fever, fullness of the chest, tyhroid dysfunction and radiation fibrosis[8-10]. Radiation fibrosis can engender permanent scarring of the lungs from untreated radiation pneumonitis. High radiation dose can also cause stenosis, occlusion or fistula formation and rib fractures resulting death[11-14]. Intestinal epithelium renewal is rapid and hence the small intestine is reported to be radiosensitive[15]. The most common side effects of radiation therapy to the abdomen are diarrhea, nausea, vomiting, constipation, abdominal pain, colitis, tenesmus, rectal bleeding, proctalgia, incontinence, loss of appetite and weight loss[16,17].

In this context, the necessity to improve outcomes, particularly for children with cancer looking at the

*Correspondence:
Phone: +90 5327419246 (Mob.)
E-Mail: sonayyalcin@hotmail.com

increased risk, invites attention of researchers on treatment methods or side effects[18]. Some natural compounds and herbal extracts have also been found effective for radiation injuries[19]. Since it is not always possible to treat early and late effects caused by radiation, it has become important to develop agents that can prevent side effects. Free oxygen radicals that are exposed to ionizing radiation have a negative effect on antioxidant defense mechanisms in the cell. It reduces the activity of especially from the antioxidant enzymes present in the cell which are; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPX)[20,21]. It is possible to prevent the damage by bringing radiotherapy to free radicals in normal cells using antioxidant agents.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a free radical scavenger and neuroprotective agent used to treat dementia, mania and amyotrophic lateral sclerosis, has been reported to exhibit potential antioxidant effects[22-24]. Therapeutic effect is explained by its strong free radical scavenging property which has been proven *in vivo* and *in vitro* experimental studies[25]. Edaravone produces useful effects on oxidative stress markers including SOD, malon dialdehyde (MDA), GSHPX and CAT[22,23]. Edaravone has also been shown to have antiapoptotic, anti-necrotic and anti-inflammatory effects[26]. It is expected that edaravone will have protective effect against radiation damage as it is a substance with free radical scavenging properties.

Hence, in this study, we have made an attempt to evaluate biologically and histologically the possible protective effects of edaravone on lung and small intestine against radiation-induced early side effects of total body irradiation in single fraction.

Materials and Methods

Animals

Thirty-two female Sprague Dawley rats (10-11 weeks, weight range 250 ± 25 g) were maintained under standard conditions. All experimental studies were conducted at Karadeniz Techniquel University Surgical Application and Research Center according to the guidelines for the ethical treatment of experimental animals. Local institutional approval for researchers was obtained before initiation of the study. The experimental protocol was approved by the Animal Care and Use Committee of Karadeniz Techniquel University (Protocol No: 2016/20).

Experimental design

The rats were divided randomly into four equal groups and there were 8 animals in each group. The study groups were designed as follows. Gr. I (Sham group): Rats were given intraperitoneal (i.p.) 1.0 mL of SF and 30 min after the administration of 90 mg/kg of ketamine i.p., general anesthesia was administered, follwed by 15 Gy of external ionizing radiation to the whole body under general anesthesia; Gr. II: Under general anesthesia the solution prepared by dissolving edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one; Sigma catalog no: M70800) in saline was administered i.p. at a dose of 500 mg/kg. After 30 min of EDA administration, the whole body was exposed to 15 Gy of external ionizing radiation; Gr. III: Under general anesthesia the EDA solution prepared at a dose of 50 mg/kg in saline was administered i.p. After 30 min of EDA administration, 15 Gy of external ionizing radiation was administered to the whole body; Gr. IV (Control group): Without any intervention sacrificed at 72 h of the experiment.

After 72 h[27-29], the lungs and small intestines were removed and some of them are washed with cold isotonic saline solution and fixed in formalin for histopathological examination. The other part is reserved for biochemical measurements and stored at −80ºC.

Biochemical and Histopathologic assays

Some of the tissues were separated with the intention to investigate tissue levels of GSHPX, SOD, CAT and MDA. GSHPX activity were measured by spectrophotometric assay kit (Cayman chemical company, USA) according to the manufacturer's instructions. SOD activities in tissues were measured by Sun and Oberley method[30]. This method was based on reduction of nitroblue tetrazolium by xanthine-xanthine oxidase system. Formazon formation was assessed spectrophotometrically at 560nm. CAT activity was determined by the method of Aebi[31]. This method is based on the principle that the absorbance at 240 nm decreases because of dismutation of H_2O_2.

Other tissue samples were fixed for 48 h in 10% formol for histopathological evaluation. Tissue fragments were made transparent by passing through graded alcohol series. Paraffin blocks of the tissues were prepared and sections with thickness of 5 μM were taken from the blocks with a fully automated microtome. The sections were stained with hemetoxylene-eosin (H&E) after deparafinization, in order to evealuate inflammation, intraalveolar

thickening, edema and capillary hyperemia in lungs[32] and inflammation, epithelial spillage and necrosis and degenerative changes in the intestines[33]. The evealuation has done that there was or not without grading. All samples were scored by pathologist blinded to experimental protocol and groups.

Statistical analysis

All values will be treated as mean ± Standard Deviation. Two-way ANOVA, Independent – Samples T-Test, Chi-Squere and Duncan multiple comparison tests was used to analyze the differences between the groups. (5% Type I error, 10% Type II error, and 6% SD admission, the minimum number of rats required to achieve an 18% difference between groups was 8 for each group).

Results

The study was designed to evaluate the early radioprotective effects of EDA (high and low doses) on lungs and small intestine damage induced by total body irridation to the rats as observed within 72 hours of administration.

Biochemical results

The protective effect of EDA showed statistically significant in MDA, SOD and GSHPX values of lungs compared to the control group (P <0.05). All the three groups, however, no statistically significant effect was observed in CAT results in irradiated lungs compared to the control (P >0.05).

There was only statistically significant effect was observed in MDA results in irradiated small intestine compared to the control (P <0.05). There was no statistically significant effect observed in the other oxidative stress parameters of irradiated small intestine (P >0.05). However, when we compared the groups both lungs and small intestine in term of doses of EDA (Gr. II with Gr. III), there were statistically significant results in only GSHPX (P <0.05).

Histopathological damage

The lungs of the animals in the Gr.I, II and III were affected by irridation. The analysis of the irradiated lungs of rats with low and high dose EDA stained with H&E showed evident signs of inflammation, intraalveolar thickening and edema. The irridated lungs of rats with high dose EDA results were a little better than the low dose EDA results and the photomicrographs of histological slides of rat lungs were shown in Figs 1 A-D 2 and 3. However, no statistically significant effect was observed in capillary hyperemia results in irradiated lungs of rats compared to the control (P >0.05). They all showed

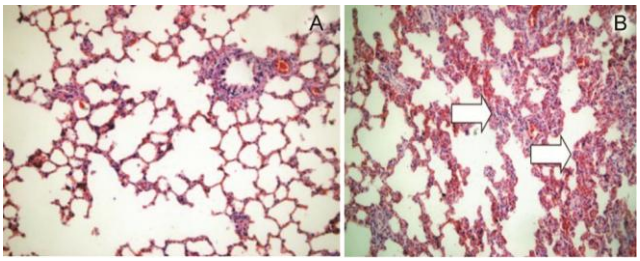

Fig. 2 — Photomicrographs of histological slides of (A) any intraalveolar thickening from Gr. II; and (B) intraalveolar thickening (marked with arrows) of rat lungs from Gr. III (H&E 200X).

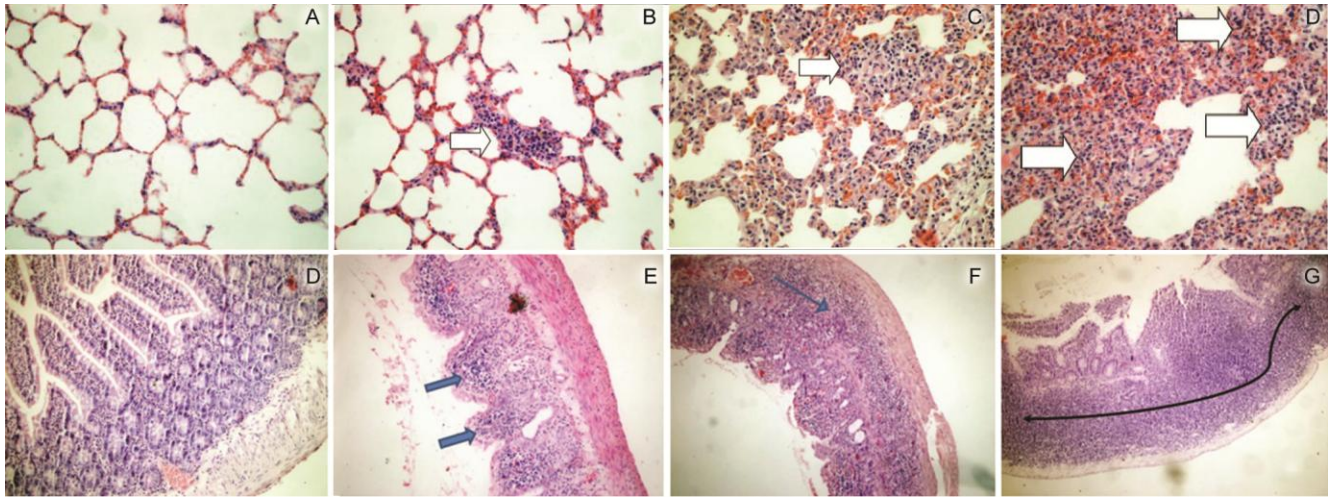

Fig. 1 — Photomicrographs of histological slides of inflammation (marked with arrows) of rat lungs and small intestine. (A) Any inflammation from Gr. IV; (B) One positive of inflammation from Gr. II; (C) Two positive of inflammation from Gr. III; (D) Three positive of inflammation from Gr. I (sham group) (H&E 400X); (E) Any inflammation from Gr. IV; (F) One positive of inflammation from Gr. II; (G) Two positive of inflammation from Gr. III; and (H) Three positive of inflammation from Gr. I (sham group) (H&E 100X).

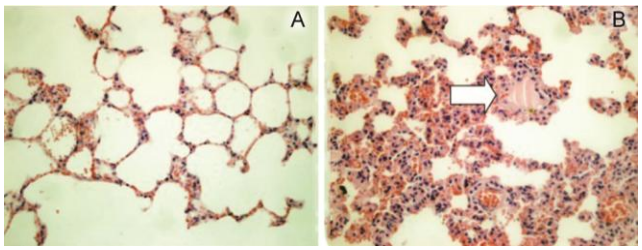

Fig. 3 — Photomicrographs of histological slides of (A) any edema from Gr. II; and (B) edema (marked with arrow) of rat lungs from Gr. III (H&E 400X).

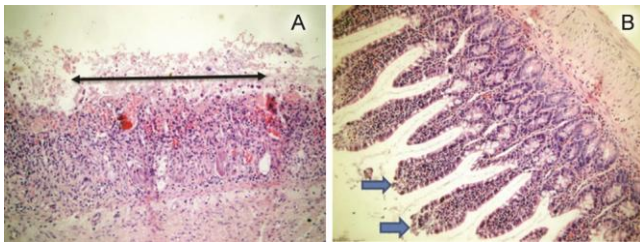

Fig. 4 — Photomicrographs of histological slides of epithelial spillage and necrosis (marked with arrows) of rat small intestines from (A) Gr. I, and (B) Gr. III (H&E 200X).

Table 1 — Histopathological results of lungs and small intestine

Groups	Lungs								Small intestine			
	Inflammation (n=8)		Intraalveolar thickening (n=8)		Edema (n=8)		Capillary hyperemia (n=8)		Inflammation (n=8)		Epithelial spillage and necrosis (n=8)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
I (n=8)	8	0	8	0	8	0	8	0	8	0	7	1
II (n=8)	5	3	3	5	4	4	8	0	3	5	3	5
III (n=8)	6	2	1	7	6	2	8	0	7	1	4	4
IV (n=8)	1	7	2	6	2	6	7	1	2	6	0	7
	P <0.05		P <0.05		P <0.05		P >0.05		P >0.05		P <0.05	

statistically significant findings compared to the control group (P <0.05) as showen in Table 1.

The analysis of the irradiated small intestine of rats with low and high dose EDA stained with H&E showed evident signs of epithelial spillage and necrosis from Gr. I and III shown in Fig. 4. Also again high dose EDA results were beter than the low dose EDA results. Unfortunately no statistically significant effect was observed in inflammation results in irradiated small intestine of rats compared to the control (P >0.05). All groups had inflammation and there was any enough response from EDA to the small intetines of rats (Fig. 1 E-H).

Discussion

In this study, we evaluated the early (within 72 h of administration) radioprotective effects of EDA (high and low doses) on lungs and small intestine damage induced by total body irridation to the rats. The effect of EDA (low and high doses) therapy on the activities of antioxidant enzymes of GSHPX, SOD, CAT and MDA in the irridated rat lungs was investigated. Our results have shown that EDA reduced the increased activities of GSHPX, SOD and MDA on lungs except CAT especially with high doses. However, only MDA results have shown statistically significant differance in irradiated small intestine of rats. MDA is considered to be the index of lipid peroxidation and as expected, the first and early impact was seen in MDA results here in this study. The ionized radiation causes an increase in free radicals in the biological system. These radicals act on the DNA, proteins and membrane lipids of the cell to form cell damage. In addition, our histopathological results of lungs have shown statistically significant effect in lungs and small intestine except capillary hyperemia (in lungs) and inflammation (in small intestine). In our histopathological observation of lung, we also evaluated in terms of alveolar thickness, edema and capillary hyperemia besides inflammation, since inflammation could also occur in normal healthy animals.

We observed that EDA reduced the inflammation, intraalveolar thickening and edema on lungs with high doses. Nevertheless we could not show the same effect on capillary hyperemia. Tajima *et al.*[34] have shown that EDA alleviates the progression of lung injury and fibrosis. Also, Asai *et al.*[35] demonstrated same effect of EDA on rabbit lungs by reflecting its ability to scavenge free radicals, which inhibits apoptosis and overexpression of transforming growth factor (TGF)-β. Our results have also shown statistically significant average on epithelial spillage and necrosis of small intestine with high doses of EDA between all experimental groups. On the other hand, inflammation of small intestine results have not shown any ststistically significant average between the groups.

EDA clears various free radicals, and the irridation produces hydroxyl, peroxyl and superoxide radicals. Free radicals are removed by antioxidant enzymes in living cells and antioxidant defences SOD, GPX,

CAT and glutathione. Many studies have shown that protective effect of EDA was associated with the increased SOD activitiy and CAT[36]. EDA applies a protective effect in animal models of myocardial, lung, intestinal, liver, bladder, testis, pancreatic and renal injuries[26,37]. EDA has also anti-apoptotic, anti-necrotic and anti-cytokine effects in many different diseases and reduces the relase of adhesion molecules and markers of tumorigenesis[37]. Hassan *et al*.[38] suggested that EDA showed a potential protective effect in ISO (Isoproterenol)-induced myocardical infarction. Yoshizaki *et al*.[39] have observed the benefical effects of EDA on fibrosis and eveluated for a potential use as an antifibrotic agent in systemic sclerosis. Ito *et al*.[40] have found a protective effect of EDA againist lung injury induced intestine ischemia-reperfusion injury.

Conclusion

The pediatric cancer patients who have a longer life expectancy are as important as their recovery from their normal life. We found the protective effect of EDA showed statistically significant in MDA, SOD and GSHPX values of lungs except CAT and statistically significant effect was observed in MDA in small intestine of rats. Also we showed improvement in histopathological changings against radiation-induced in both the lung and the small intestines of rats. Finally edaravone is an effective drug for multiple organs and we expected to be able to reduce the side effects of radiation damage.

Conflict of Interest

Authors declare no conflict of interest.

References

1 Jemal A, Torre L, Soerjomataram I & Bray F (Eds). The Cancer Atlas. Third Ed. Atlanta, GA: American Cancer Society, 2019. Also available at: www.cancer.org/canceratlas.

2 Hyuna Sung, Jacques Ferlay, Rebecca L. Siegel Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal & Freddie Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 0 (2021) 1. https://doi.org/10.3322/caac.21660.

3 Snaman JM, Kaye EC, Baker JN & Wolfe J, Pediatric Palliative Oncology: The State of the Science and Art of Caring for Children With Cancer. *Curr Opin Pediatr*, 30 (2018) 40.

4 Global Burden of Disease Cancer Collaboration, Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol*, 5 (2019) 1749.

5 Guerreiro F, Seravalli E, Janssens GO, Ven CP, Heuvel-Eibrink MM & Raaymakers BW, Intra- And Inter-Fraction Uncertainties During IGRT for Wilms' Tumor. *Acta Oncol*, 57 (2018) 941.

6 Charlton J, Irtan S, Bergeron C & Pritchard-Jones K, Bilateral Wilms Tumour: A Review of Clinical and Molecular Features. *Expert Rev Mol Med*,19 (2017) e8.

7 Demoor-Goldschmidt C, Chiavassa S, Josset S, Mahé MA & Supiot S, Respiratory-gated Bilateral Pulmonary Radiotherapy for Ewing's Sarcoma and Nephroblastoma in Children and Young Adults: Dosimetric and Clinical Feasibility Studies. *Cancer Radiother*, 21 (2017) 124.

8 Hanania AN, Mainwaring W, Ghebre YT, Hanania NA & Ludwig M, Radiation-Induced Lung Injury: Assessment and Management. *Chest*, 156 (2019) 150.

9 Kalman NS, Hugo GD, Mahon RN, Deng X, Mukhopadhyay ND & Weiss E, Diabetes Mellitus and Radiation Induced Lung Injury After Thoracic Stereotactic Body Radiotherapy. *Radiother Oncol*, 129 (2018) 270.

10 Morgan TM, Danish H, Nanda RH, Esiashvili N & Meacham LR, Whole Lung Irradiation in Stage IV Wilms Tumor Patients: Thyroid Dosimetry and Outcomes. *Pediatr Blood Cancer*, 65 (2018) e26843.

11 Duijm M, Schillemans W, Aerts JG, Heijmen B & Nuyttens JJ, Dose and Volume of the Irradiated Main Bronchi and Related Side Effects in the Treatment of Central LungTumors With Stereotactic Radiotherapy. *Semin Radiat Oncol*, 26 (2016) 140.

12 Kimsey F, Mckay J, Gefter J, Milano MT, Moissenko V, Grimm J & Berg R, Dose-Response Model for Chest Wall Tolerance of Stereotactic Body Radiation Therapy. *Semin Radiat Oncol*, 26 (2016) 129.

13 Bucknell NW, NicholasHardcastle N, Bressel M, Hofman MS, Kron T, Ball D & Siva S, Functional lung imaging in radiation therapy for lung cancer: A systematic review and meta-analysis. *Radiother Oncol*, 129 (2018) 196.

14 Scobioala S & Eich HT, Risk Stratification of Pulmonary Toxicities in the Combination of Whole Lung Irradiation and High-Dose Chemotherapy for Ewing Sarcoma Patients With Lung Metastases: A Review. *Strahlenther Onkol*, (2020) doi: 10.1007/s00066-020-01599-8. Online ahead of print.

15 Musa AE, Shabeeb D & Alhilfi HSQ, Protective Effect of Melatonin Against Radiotherapy-Induced Small Intestinal Oxidative Stress: Biochemical Evaluation. *Medicina* (*Kaunas*), 55 (2019) 308.

16 Kalanxhi E, Risberg K, Barua IS, Dueland S, Waagene S, Andersen SN, Pettersen SJ, Lindvall JM, Redalen KR, Flatmark K & Ree AH, Induction of Apoptosis in Intestinal Toxicity to a Histone Deacetylase Inhibitor in a Phase I Study With Pelvic Radiotherapy. *Cancer Res Treat*, 49 (2017) 374.

17 Franco RD, Borzillo V, Ravo V, Ametrano G, Falivene S, Cammarota F, Rossetti S, Romano FJ, D'Aniello C, Cavaliere C, Iovane G, Piscitelli R, Berretta M, Muto P & Facchini G, Rectal/urinary Toxicity After Hypofractionated *vs*. Conventional Radiotherapy in Low/Intermediate Risk Localized Prostate Cancer: Systematic Review and Meta Analysis. *Oncotarget*, 8 (2017) 17383.

18 Forrest SJ, Geoerger B & Janeway KA, Precision Medicine in Pediatric Oncology. *Curr Opin Pediatr*,30 (2018) 17.

19 Maurya DK, Devasagayam TP & Nair CK, Some novel approaches for radioprotection and the beneficial effect of natural products. *Indian J Exp Biol*, Feb;44(2) (2006) 93

20 Takei K, Watanabe K, Yuki S, Akimoto M, Sakata T & Palumbo J, Edaravone and Its Clinical Development for Amyotrophic Lateral Sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*, 18 (2017) 5.

21 Sawada H, Clinical Efficacy of Edaravone for the Treatment of Amyotrophic Lateral Sclerosis. *Expert Opin Pharmacother*, 18 (2017) 735.

22 Ibrahim A. Alhaider, Effects of Edaravone on Scopolamine Induced-dementia in Experimental Rats. *Int J Pharm*, 9 (2013) 271.

23 Arslan FC, Tiryaki A, Yıldırım M, Özkorumak E, Alver A, Altun İK, İnce İ & Gedikli Ö, The effects of edaravone in ketamine-induced model of mania in rats. *Acta Neurobiol Exp* (*Wars*), 76(3) (2016) 192.

24 Jaiswal MK, Riluzole and Edaravone: A Tale of Two Amyotrophic Lateral Sclerosis Drugs. *Med Res Rev*, 39 (2019) 733.

25 Bhandari R, Kuhad A & Kuhad A, Edaravone: A New Hope for Deadly Amyotrophic Lateral Sclerosis. *Drugs Today* (*Barc*), 54 (2018) 349.

26 Kikuchi K, Uchikado H, Miyagi N, Morimoto Y, Ito T, Tancharoen S, Miura N, Miyata K, Sakamoto R, Kikuchi C, Iida N, Shiomi N, Kuramoto T & Kawahara KI, Beyond neurological disease: new targets for edaravone (Review). *Int J Mol Med*, 28 (2011) 899.

27 Sener G, Jahovic N, Tosun O, Atasoy BM & Yegen BC, Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rats. *Life Sci*, 74 *(*2003) 563.

28 Picard C, Wysocki J, Linard C, Garcia-Villar R, Bueno L, Griffiths NM & Fioramonti J, Absence of protective role of afferent nerves in early intestinal mucosal alterations induced by abdominal irradiation in rats. *Int J Radiat Biol*, 77 (2001) 349.

29 Olgac V, Erbil Y, Barbaros U, Oztezcan S, Giris M, Kaya H, Bilge H, Guler S & Toker G, The efficacy of octreotide in pancreatic and intestinal changes: radiation-induced enteritis in animals. *Dig Dis Sci*, 51 (2006) 227.

30 Teoh ML, Sun W, Smith BJ, Oberley LW & Cullen JJ, Modulation of reactive oxygen species in pancreatic cancer. *Clin Cancer Res*, 15 (24) (2007) 7441.

31 Aebi HE, *Catalase*. Methods of Enzymatics Analysis 3 (1987) 273.

32 Karpathiou G, Giatromanolaki A, Koukourakis MI, Mihailidis V, Sivridis E, Bouros D & Froudarakis ME, Histological changes after radiation therapy in patients with lung cancer: a prospective study. *Anticancer Res*, 34 (2014) 3119.

33 Wang J, Zheng H, Ou X, Fink LM & Hauer-Jensen M, Deficiency of microvascular thrombomodulin and up-regulation of protease-activated receptor-1 in irradiated rat intestine: possible link between endothelial dysfunction and chronic radiation fibrosis. *Am J Pathol*, 160 (2002) 2063.

34 Tajima S, Bando M, Ishii Y, Hosono T, Yamasawa H, Ohno S, Takada T, Suzuki E, Gejyo F & Sugiyama Y, Effects of edaravone, a free-radical scavenger, on bleomycin-induced lung injury in mice. *Eur Respir J*, 32 (2008) 1337.

35 Asai T, Ohno Y, Minatoguchi S, Funaguchi N, Yuhgetsu H, Sawada M, Takemura G, Komada A, Fujiwara T & Fujiwara H, The specific free radical scavenger edaravone suppresses bleomycin-induced acute pulmonary injury in rabbits. *Clin Exp Pharm Physiol*, 34 (2007) 22.

36 Zhou X, Zhu L, Wang L, Guo B, Zhang G,Sun Y,Zhang A, Lee SMY, Yu P & Wang Y, Protective Effect of Edaravone in Primary Cerebellar Granule Neurons against Iodoacetic Acid-Induced Cell Injury. *Oxidative Med Cell Longevity*, (2015) 606981. doi: 10.1155/2015/606981 8 pages.

37 Kikuchi K, Takeshige N, Miura N, Morimoto Y, Ito T, Tancharoen S, Miyata K, Kikuchi C, Iida N, Uchikado H, Miyagi N, Shiomi N, Kuramoto T, Maruyama I, Morioka M & Kawahara KI, Beyond free radical scavenging: Beneficial effects of edaravone (Radicut) in various diseases (Review). *Exp Ther Med*, 3 (2012) 3.

38 Hassan Q, Akhtar S, Akhtar M, Ali J, Haque SE & Najmi AK, Edaravone, a potent free radical scavenger and a calcium channel blocker attenuate isoproterenol induced myocardial infarction by suppressing oxidative stress, apoptotic signaling and ultrastructural damage. *Ther Adv Cardiovasc Dis*, 10 (2016) 214.

39 Yoshizaki A, Yanaba K, Ogawa A, Iwata Y, Ogawa F, Takenaka M, Shimizu K, Asano Y, Kadono T & Sato S, The specific free radical scavenger edaravone suppresses fibrosis in the bleomycin-induced and tight skin mouse models of systemic sclerosis. *Arthritis Rheum*, 63 (2011) 3086.

40 Ito K, Ozasa H & Horikawa S, Edaravone protects against lung injury induced by intestinal ischemia/reperfusion in rat. *Free Radic Biol Med*, 38 (2005) 369.