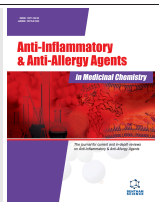
Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 2020, *19*, 149-157

RESEARCH ARTICLE



Mitigation of Radiation-induced Pneumonitis and Lung Fibrosis using Alpha-lipoic Acid and Resveratrol

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Abstract: ***Background*:** Lung is a radiosensitive organ. Studies have shown that exposure of the lung to acute and high doses of radiation following inhalation of radioactive agents or an accidental radiological event may lead to pneumonitis and fibrosis, which are associated with a risk of death. So far, some agents have been studied for mitigation of pneumonitis and fibrosis following exposure of murine lung tissues to ionizing radiation. In this study, we aimed to detect the possible mitigatory effect of alpha-lipoic acid, resveratrol and their combination on mice pneumonitis and fibrosis markers following irradiation.

***Methods*:** 25 mice were divided into 5 groups: control, radiation; radiation plus alpha-lipoic acid; radiation plus resveratrol; and radiation plus both resveratrol and alpha-lipoic acid. Mice chest regions were irradiated with 18 Gy using a cobalt-60 gamma rays source. Treatments started 24 h after irradiation and continued for two weeks. After 100 days, all mice were sacrificed and their lung tissues removed for histopathological evaluation.

***Results*:** Pathological study showed that exposure to radiation led to severe pneumonitis and moderate fibrosis after 100 days. Both resveratrol and alpha-lipoic acid, as well as their combination could mitigate pneumonitis and fibrosis markers. Although, resveratrol could not mitigate infiltration of most inflammatory cells as well as inflammation and vascular damage, alpha-lipoic acid and its combination were able to mitigate most damaged markers.

***Conclusion*:** Alpha-lipoic acid and its combination with resveratrol were able to mitigate fibrosis and pneumonitis markers in mice lung tissues following lung irradiation. Although resveratrol has a protective effect on some markers, it has a weaker effect on lung injury. In conclusion, our results suggest that the combination of resveratrol and alpha-lipoic acid has a potent mitigatory effect compared to the single forms of these agents.

ARTICLE HISTORY

Received: January 16, 2019
Revised: March 07, 2019
Accepted: March 12, 2019

DOI:
10.2174/1871523018666190319144020

Keywords: Alpha-lipoic acid, lung, mitigation, radiation, resveratrol, pneumonitis.

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1875-614X/20 $65.00+.00

1. INTRODUCTION

Accidental exposure to radiation is a threat to human because of increasing concerns about nuclear war and radiological terror activities. Whole body exposure to gamma ray doses lower than 4 Gy could lead to survival, although they may have a high risk for several disorders such as carcinogenesis, cataract, cardiovascular diseases, autoimmune diseases *etc*. [1]. In contrast, whole body exposure to doses higher than 4 Gy may lead to death after some weeks. Results of an atomic bomb explosion in Hiroshima and Nagasaki, years after this event, confirmed the incidence of several disorders [2]. In addition to hematopoietic and gastrointestinal toxicities following whole body exposure to radiation, there are concerns of organ failure to the lung, liver, kidney and heart following non-uniform body exposure [3]. In some situations, such as non-uniform whole body exposure or evaporation of radioactive iodine and cesium, which were experienced during the Chernobyl nuclear power or atomic bomb explosion, other organs including lung, kidney and liver may be exposed to a high dose of radiation, while bone marrow and gastrointestinal system may not show acute toxicity. Although radiation responses in these organs take a long time to appear, lung or kidney failures, months to years after exposure may lead to death in exposed people [4, 5].

The lung is an organ sensitive to detrimental effects of ionizing radiation. After exposure of the lung to a high dose of radiation, several cytokines and chemokines are released, leading to infiltration of inflammatory cells [6]. Macrophages, neutrophils and lymphocytes also release several cytokines which mediate the appearance of edema and pneumonitis [7]. Moreover, chronic production of free radicals including reactive oxygen and nitrogen species (ROS and RNS) leads to the stimulation of collagen deposition in extracellular space, giving rise to fibrosis [8]. Both pneumonitis and fibrosis can lead the exposed person to death [9]. Some experimental studies have been conducted to mitigate pneumonitis and fibrosis following local lung irradiation. Studies showed that some agents such as captopril and flaxseed, which suppress renin-angiotensin system and oxidative stress are able to attenuate signs of pneumonitis and lung fibrosis [10, 11].

Resveratrol and alpha-lipoic acid are two potent antioxidants that have shown appropriate radioprotective effect. Resveratrol is not a direct antioxidant but it is able to stimulate the activities of antioxidant enzymes in cells such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [12]. Also, *via* stimulation of sirtus-1 (Sirt-1), it induces DNA repair and ameliorates cell death in oxidative stress conditions. Studies have revealed that treatment with resveratrol before exposure to radiation alleviates radiation toxicity [13, 14]. In contrast to resveratrol, alpha-lipoic acid is a potent antioxidant which neutralizes free radicals through recycling of ascorbic acid and alpha-tocopherol [15]. Some studies have revealed the potential radioprotective effect of alpha-lipoic acid in some murine organs such as intestine and thyroid [16, 17]. In the present study, we aimed to investigate the potential mitigatory effect of resveratrol, alpha-lipoic acid and their combination on the development of radiation-induced pneumonitis and fibrosis. We hypothesized that both resveratrol and alpha-lipoic acid are able to neutralize endogenous free radical production, which is involved in oxidative injury as well as late effects in irradiated lung tissues. Interestingly, both resveratrol and alpha-lipoic acid have inhibitory effects on renin-angiotensin system. We hypothesized that these properties of resveratrol and alpha-lipoic acid improve the management of pneumonitis and lung fibrosis in mice.

2. MATERIALS AND METHODS

2.1. Drugs and Irradiation

Resveratrol and alpha-lipoic acid were purchased from Nano-Kimia Company, Tehran, Iran. Alpha lipoic acid was dissolved in 20% ethanol at a concentration of 6 mg per milliliter. Each mouse was treated with 1 ml of alpha-lipoic acid solution (equaling 200 mg per kg body weight). Similar to alpha-lipoic acid, resveratrol was mixed in 20% ethanol and diluted with distilled water. The concentration of resveratrol was 3 mg per milliliter. Each mouse was treated with 1 ml resveratrol solution (equaling 100 mg per kg body weight). For the combinational form of resveratrol and alpha-lipoic acid, both agents were dissolved in 20% ethanol. Each milliliter of this solution was made up of 3 mg resveratrol and 6 mg alpha-lipoic acid,

which were equal to 100 mg/kg and 200 mg/kg, respectively. The non-toxic doses of resveratrol and alpha-lipoic acid were chosen based on the previous studies. A resveratrol dose of 100 mg/kg has been proposed for alleviating inflammatory responses in some organs such as spinal cord and lung [18]. This dose has also been proposed for attenuation of fibrosis [19]. Alpha-lipoic acid is a low toxic antioxidant, with no toxicity observed even for longer time of administration [20]. Alpha-lipoic acid dose of 200 mg/kg has shown anti-inflammatory and anti-fibrosis effects in animal studies [21, 22]. Treatments with resveratrol, alpha-lipoic acid or their combination began 24 h after irradiation, as oral gavage and continued for 2 weeks. The time and duration of treatment were selected according to the previous studies, which gave the most effective time for mitigation of lung toxicity [23].

Irradiation was performed using a cobalt-60 gamma ray (1.25 MeV) source. Before irradiation, mice were anesthetized with ketamine and xylazine at 20 and 5 mg per kg body weight. Mice were placed supine on the treatment table before exposure to 18 Gy gamma rays to their lung tissues at a source to skin distance (SSD) of 80 cm and a dose rate of 60 cGy per min. The total dose of radiation was chosen based on a previous study.

2.2. Experimental Design

This experimental study was in accordance with the ethical laws of animal care, by Kermanshah University of Medical Sciences, Kermanshah, Iran. Mice were kept under standard conditions such as: temperature (25°C) and humidity (55%). Furthermore, same light/dark cycle (light: 5 AM-5 PM, dark: 5 PM-5 AM) was observed.

All 25 mice were allotted to five groups (5 mice in each). Group 1 was selected as control. This group did not receive radiation or treatment except ketamine and xylazine for anesthesia. Group 2 was chosen as radiation group. Each mouse in this group received 18 Gy gamma rays to lung tissues after anesthesia with ketamine and xylazine. Group 3 received 18 Gy gamma rays similar to group 2 and after 24 h, treatment with alpha-lipoic acid began for 2 weeks. Groups 4 received 18 Gy gamma rays similar to group 2 and after 24 h, oral treatment with resveratrol began for 2 weeks (5 days per week). Groups 5 mice received 18 Gy gamma rays similar to group 2 and after 24 h, treatment with resveratrol and alpha-lipoic acid commenced. For this group, administration of alpha-lipoic acid was similar to group 3 and for resveratrol, it was similar to group 4. 100 days after irradiation, all mice were killed and their lung tissues were removed after chest opening. Thereafter, the tissues were fixed in 10% normal buffer formalin for histopathological evaluation.

2.3. Histopathological Evaluation

After complete fixation of all samples, lung tissues were embedded in paraffin blocks. With the aid of a microtome, two layers with 4 micron thicknesses were prepared from each sample. After cutting, lung samples were located on the slides for staining. One slide from each sample was stained using hematoxylin and eosin (H&E) while another slide was stained using Masson's trichrome (MTC). Slides with H&E staining showed morphological changes such as congestion, infiltration of inflammatory cells, edema, vascular and alveolar changes, *etc*. MTC staining was used for detecting collagen deposition in intracellular spaces showing signs of fibrosis.

2.4. Statistical Analysis

Results of histopathological evaluation were scored as follows: 0=normal, 1=mild changes, 2=moderate changes, and 3=severe changes. Histopathological parameters including collapse, erythrocyte hemorrhage, congestion, inflammation, macrophages infiltration, lymphocyte infiltration, neutrophil infiltration, vascular wall thickness, vascular damage, alveolar thickness, edema and fibrosis, were scored for all groups same as the above mentioned method. Afterwards, results for each group were reported as mean ± standard deviation (SD). For analyses of differences between groups, we used SPSS version 22 (IBM, Chicago, USA). Data were analyzed using Mann-Whitney non-parametric test. P value <0.05 was considered statistically significant.

3. RESULTS

Results from histological evaluation showed a significant increase in inflammation and fibrosis

Table 1. Histopathological results evaluating the mitigatory effect of alpha-lipoic acid (ALA), resveratrol (RES) and their combination on mice lung tissues following exposure to 18 Gy gamma rays.

-	Control	RAD	RAD+ALA	RAD+RES	RAD+ALA+RES
Collapse	0.25 ± 0.43	1.00 ± 00[a]	1.25 ± 0.43	0.66 ± 0.47	1.00 ± 00
Erythrocyte hemorrhage	0.00 ± 00	3.00 ± 0.70[a]	1.00 ± 0.70[b]	0.00 ± 00[b]	0.00 ± 00[b,c]
Congestion	0.00 ± 00	3.50 ± 0.86[a]	1.50 ± 0.50[b]	1.00 ± 00[b]	0.00 ± 00[b,c,d]
Inflammation	0.25 ± 0.43	3.25 ± 0.43[a]	2.50 ± 0.50	3.33 ± 0.47	1.75 ± 0.89[b]
Macrophages infiltration	0.25 ± 0.43	3.00 ± 00[a]	0.50 ± 0.50[b]	3.00 ± 0.81	1.75 ± 0.83[b]
Lymphocyte infiltration	0.00 ± 00	2.75 ± 0.83[a]	1.75 ± 0.43	3.66 ± 0.47	1.50 ± 0.50
Neutrophil infiltration	0.00 ± 00	3.00 ± 0.70[a]	1.00 ± 00[b]	1.00 ± 00[b]	0.00 ± 00[b,c,d]
Vascular wall thickness	0.00 ± 00	1.25 ± 0.43[a]	0.00 ± 00[b]	1.00 ± 00	0.00 ± 00[b,d]
Vascular damage	0.25 ± 0.43	2.25 ± 0.43[a]	1.00 ± 00[b]	2.00 ± 00	0.75 ± 0.83[b]
Alveolar thickness	0.00 ± 00	1.00 ± 00[a]	0.00 ± 00[b]	0.00 ± 00[b]	0.00 ± 00[b]
Edema	0.00 ± 00	3.00 ± 00[a]	0.75 ± 1.29[b]	1.00 ± 0.82[b]	1.50 ± 1.65[b]
Fibrosis	0.00 ± 00	2.00 ± 00[a]	0.00 ± 00[b]	0.00 ± 00[b]	0.00 ± 00[b]

a: significant compared to control group; b: significant compared to radiation group; c: significant compared to alpha-lipoic acid group; d: significant compared to resveratrol group (Mann-Whitney Test, $p < 0.05$).

RAD = Radiation; ALA = Alpha-Lipoic Acid; RES = Resveratrol; ALA+RES = Resveratrol Plus Alpha-Lipoic Acid.

markers. It further showed that that irradiation of mice lung tissues with 18 Gy gamma rays led to severe infiltration of inflammatory cells including macrophages, lymphocytes and neutrophils, as well as severe inflammation, edema, congestion and erythrocyte. However, a mild to moderate collapse, vascular and alveolar injuries, in addition to fibrosis were also revealed. Treatment with alpha-lipoic acid or its combinational form could mitigate all mentioned markers except for collapse and lymphocyte infiltration. Treatment with resveratrol could mitigate alveolar thickness, congestion, erythrocyte hemorrhage, edema, neutrophil infiltration, as well as fibrosis (Figs. **1** and **2**). However, it could not mitigate other injury parameters such as vascular damage, inflammation as well as infiltration of macrophages and lymphocytes. The detailed results have been presented in Table **1**.

4. DISCUSSION

Lung injury is one of the major causes of death after some radiation disasters. Moreover, in patients with chest cancer such as non-small cell lung carcinoma, breast cancer or in some situations such as head and neck cancer, there is a risk of pneumonitis or fibrosis. Pneumonitis in human occurs some months after exposure to an acute dose, while the incidence of fibrosis may take years. So far, some experimental studies have been conducted to evaluate several agents for mitigation of radiation-induced pneumonitis and fibrosis. Genistein, a soy isoflavone and eukarion, which is a potent ROS scavenger have shown partial mitigatory effects on radiation pneumonitis and fibrosis in murine models [24, 25]. Treatment with other antioxidants including BIO 300 (a nanosuspension of genistein) and flaxseed, 24 h after irradiation,

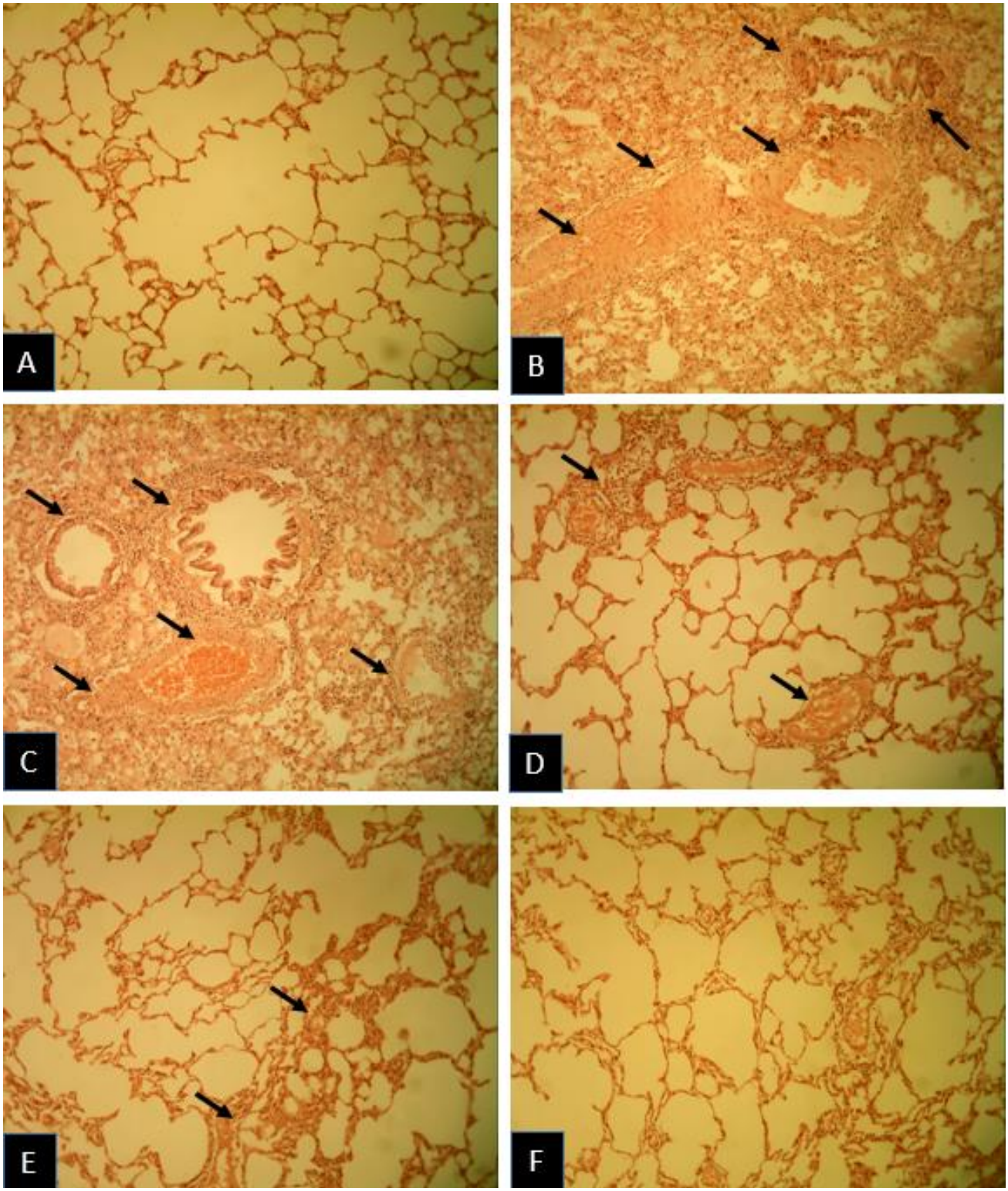

Fig. (1). Histopathological investigation of the radio-mitigatory effects of ALA and RES, and radiation damage after 100 days. (**A**) Control: alveolar space, bronchioles, and vascular bed are seen normal. (**B**) RAD: severe interstitial inflammation and pulmonary edema are observed. (**C**) RAD: severe inflammation of bronchial wall and wall thickness are seen with destruction of bronchus. (**D**) ALA: moderate to mild inflammation was observed. (**E**) RES: moderate inflammation was observed. (**F**) ALA+RES: mild inflammation was observed. The arrows indicate an accumulation of lymphocytes, macrophages, and neutrophils in lung tissue (H and E, ×100).

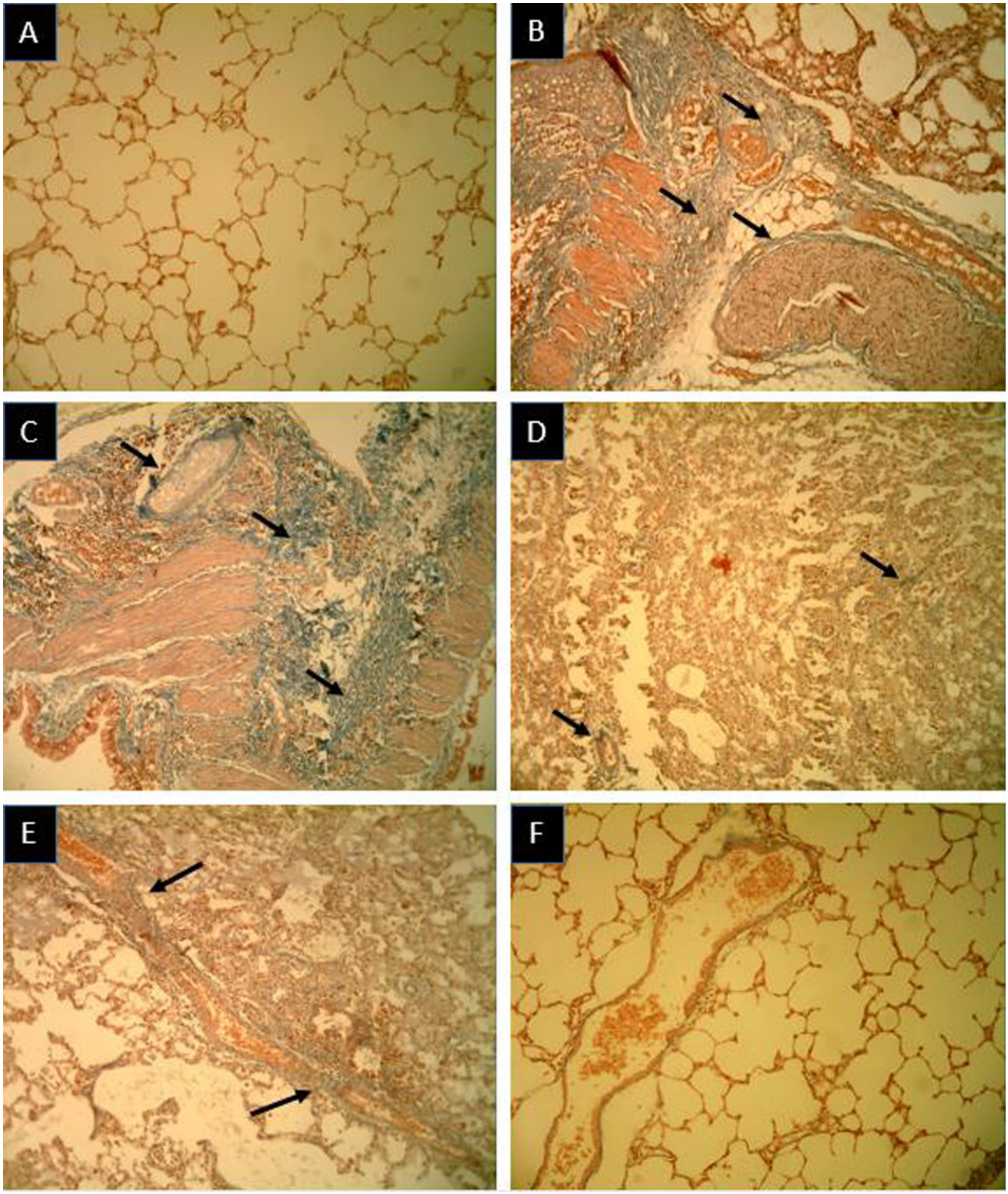

Fig. (2). Histopathological investigation of the radio-mitigatory effects of ALA and RES, and radiation damage after 100 days in the lung tissue. (**A**) Control: alveolar space, bronchioles and vascular bed are seen normal. (**B** & **C**) Radiation: severe collagen deposition was observed. (**D**) RES: collagen deposition was moderate around the vascular bed and alveolar space. (**E**) ALA: moderate to mild collagen deposition was observed. (**F**) ALA+RES: mild collagen deposition was revealed. The arrows indicate the collagen deposition in the lung tissue. Collagen deposition is depicted with light blue. (Masson's trichrome staining: magnification ×100).

have shown similar results [26]. These agents have been shown to mitigate lung pneumonitis and fibrosis *via* scavenging free radicals and alleviation of inflammatory mediators [11, 23]. Suppression of renin-angiotensin system by captopril also showed potent mitigation of lung injury, even when treatment commenced 1 week after irradiation [4, 27, 28]. Kma *et al*. showed that inhibition of renin-angiotensin system by captopril, enalapril and fosinopril has a direct relation with reduced collagen deposition and fibrosis [29]. In addition to accidental exposure to radiation, mitigation of lung injury can be very useful for cancer patients which show a high risk for pneumonitis and fibrosis. At the moment, a clinical trial study for patients with lung cancer is ongoing (NCT02809456).

Till date, no study has been conducted to understand the protective role of resveratrol and alpha-lipoic acid against radiation-induced lung injury. Resveratrol and alpha-lipoic acid are two potent antioxidants which have shown abilities to attenuate radiation injury. Resveratrol inhibits continuous production of ROS and hematopoietic injury *via* suppression of NOX4, upregulation of Sirt1 as well as stimulation of SOD2 and GPx1 in mice bone marrow cells [14, 30]. Similar effects have been revealed in mice intestine [31]. Alpha-lipoic acid stimulates glutathione, suppresses inflammatory cytokines and mediators such as NF-Kb. Furthermore, it attenuates apoptosis induction, leading to amelioration of radiation-induced salivary glands, thyroid and intestinal toxicities as well as oral mucositis [16, 32, 33].

In the present study, our results showed that post-exposure treatment with alpha-lipoic acid, resveratrol, or their combination can mitigate pneumonitis and fibrosis in a mice model. Results also showed a moderate induction of fibrosis by irradiation of mice lung tissues, which was reversed completely by all reagents. Alpha-lipoic acid and its combinational form could attenuate infiltration of inflammatory cells, while resveratrol did not show any remarkable effect. Neither resveratrol nor alpha-lipoic acid was able to mitigate inflammation, while their combination could attenuate it significantly. Overall results showed that alpha-lipoic acid is a potent radiation mitigator for lung tissues, however, its combination with resveratrol could lead to more effective results.

CONCLUSION

This study was conducted to evaluate the possible mitigatory effects of resveratrol, alpha-lipoic acid and their combination after lung irradiation with gamma rays. Results showed that treatment with resveratrol alleviated fibrosis as well as alveolar and vascular injuries, while it could not mitigate inflammatory markers. Alpha-lipoic acid has a potent effect on lung injury and mitigated the infiltration of most inflammatory cells, edema, alveolar and vascular injuries as well as fibrosis. However, the combination of both resveratrol and alpha-lipoic acid may offer potent mitigatory effect compared with the single forms of these drugs. In conclusion, using alpha-lipoic acid or its combination with resveratrol is a good strategy for mitigation of both pneumonitis and fibrosis following exposure of the lung to a high dose of radiation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by ethics committee of Kermanshah University of Medical Sciences, Iran, with ethics code: IR.KUMS.REC.1397.296.

HUMAN AND ANIMAL RIGHTS

No humans were used in the study. All the animal procedures were in accordance with the guidelines of Ethical Laws of Animal Care, by Kermanshah University of Medical Sciences, Kermanshah, Iran.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

FUNDING

The study was funded by Kermanshah University of Medical Sciences, Iran, project number: 97299.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Little, M.P. Cancer and non-cancer effects in Japanese atomic bomb survivors. *J. Radiol. Prot.,* **2009**, *29*(2A), A43-A59. http://dx.doi.org/10.1088/0952-4746/29/2A/S04 PMID: 19454804

[2] Barnaby, F. The effects of the atomic bombings of Hiroshima and Nagasaki. *Med. War,* **1995**, *11*(3), 1-9. http://dx.doi.org/10.1080/07488009508409217 PMID: 7565301

[3] Mahmood, J.; Jelveh, S.; Calveley, V.; Zaidi, A.; Doctrow, S.R.; Hill, R.P. Mitigation of lung injury after accidental exposure to radiation. *Radiat. Res.,* **2011**, *176*(6), 770-780. http://dx.doi.org/10.1667/RR2562.1 PMID: 22013884

[4] Molthen, R.C.; Wu, Q.; Fish, B.L.; Moulder, J.E.; Jacobs, E.R.; Medhora, M.M. Mitigation of radiation induced pulmonary vascular injury by delayed treatment with captopril. *Respirology,* **2012**, *17*(8), 1261-1268. http://dx.doi.org/10.1111/j.1440-1843.2012.02247.x PMID: 22882664

[5] Moulder, J.E.; Cohen, E.P.; Fish, B.L. Mitigation of experimental radiation nephropathy by renin-equivalent doses of angiotensin converting enzyme inhibitors. *Int. J. Radiat. Biol.,* **2014**, *90*(9), 762-768. http://dx.doi.org/10.3109/09553002.2014.938375 PMID: 24991882

[6] Medhora, M.; Gao, F.; Jacobs, E.R.; Moulder, J.E. Radiation damage to the lung: mitigation by angiotensin-converting enzyme (ACE) inhibitors. *Respirology,* **2012**, *17*(1), 66-71. http://dx.doi.org/10.1111/j.1440-1843.2011.02092.x PMID: 22023053

[7] Zhao, W.; Robbins, M.E. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr. Med. Chem.,* **2009**, *16*(2), 130-143. http://dx.doi.org/10.2174/092986709787002790 PMID: 19149566

[8] Jiang, F.; Liu, G.-S.; Dusting, G.J.; Chan, E.C. NADPH oxidase-dependent redox signaling in TGF-β-mediated fibrotic responses. *Redox Biol.,* **2014**, *2*, 267-272. http://dx.doi.org/10.1016/j.redox.2014.01.012 PMID: 24494202

[9] Carver, J.R.; Shapiro, C.L.; Ng, A.; Jacobs, L.; Schwartz, C.; Virgo, K.S.; Hagerty, K.L.; Somerfield, M.R.; Vaughn, D.J. American Society of Clinical Oncology clinical evidence review on the ongoing care of adult cancer survivors: cardiac and pulmonary late effects. *J. Clin. Oncol.,* **2007**, *25*(25), 3991-4008. http://dx.doi.org/10.1200/JCO.2007.10.9777 PMID: 17577017

[10] Cohen, E.P.; Fish, B.L.; Moulder, J.E. Mitigation of radiation injuries *via* suppression of the renin-angiotensin system: emphasis on radiation nephropathy. *Curr. Drug Targets,* **2010**, *11*(11), 1423-1429. http://dx.doi.org/10.2174/1389450111009011423 PMID: 20583975

[11] Pietrofesa, R.; Turowski, J.; Tyagi, S.; Dukes, F.; Arguiri, E.; Busch, T.M.; Gallagher-Colombo, S.M.; Solomides, C.C.; Cengel, K.A.; Christofidou-Solomidou, M. Radiation mitigating properties of the lignan component in flaxseed. *BMC Cancer,* **2013**, *13*, 179. http://dx.doi.org/10.1186/1471-2407-13-179 PMID: 23557217

[12] Rubiolo, J.A.; Mithieux, G.; Vega, F.V. Resveratrol protects primary rat hepatocytes against oxidative stress damage: activation of the Nrf2 transcription factor and augmented activities of antioxidant enzymes. *Eur. J. Pharmacol.,* **2008**, *591*(1-3), 66-72. http://dx.doi.org/10.1016/j.ejphar.2008.06.067 PMID: 18616940

[13] Mortezaee, K.; Najafi, M.; Farhood, B.; Ahmadi, A.; Shabeeb, D.; Musa, A.E. Resveratrol as an adjuvant for normal tissues protection and tumor sensitization. *Curr. Cancer Drug Targets,* **2020**, *20*(2), 130-145. http://dx.doi.org/10.2174/1568009619666191019143539 PMID: 31738153

[14] Zhang, H.; Zhai, Z.; Wang, Y.; Zhang, J.; Wu, H.; Wang, Y.; Li, C.; Li, D.; Lu, L.; Wang, X.; Chang, J.; Hou, Q.; Ju, Z.; Zhou, D.; Meng, A. Resveratrol ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice. *Free Radic. Biol. Med.,* **2013**, *54*, 40-50. http://dx.doi.org/10.1016/j.freeradbiomed.2012.10.530 PMID: 23124026

[15] Shay, K.P.; Moreau, R.F.; Smith, E.J.; Smith, A.R.; Hagen, T.M. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim. Biophys. Acta,* **2009**, *1790*(10), 1149-1160. http://dx.doi.org/10.1016/j.bbagen.2009.07.026 PMID: 19664690

[16] Jeong, B.K.; Song, J.H.; Jeong, H.; Choi, H.S.; Jung, J.H.; Hahm, J.R.; Woo, S.H.; Jung, M.H.; Choi, B.-H.; Kim, J.H.; Kang, K.M. Effect of alpha-lipoic acid on radiation-induced small intestine injury in mice. *Oncotarget,* **2016**, *7*(12), 15105-15117. http://dx.doi.org/10.18632/oncotarget.7874 PMID: 26943777

[17] Jung, J.H.; Jung, J.; Kim, S.K.; Woo, S.H.; Kang, K.M.; Jeong, B.-K.; Jung, M.H.; Kim, J.H.; Hahm, J.R. Alpha lipoic acid attenuates radiation-induced thyroid injury in rats. *PLoS One,* **2014**, *9*(11), e112253.

http://dx.doi.org/10.1371/journal.pone.0112253
PMID: 25401725

[18] Liu, J.; Yi, L.; Xiang, Z.; Zhong, J.; Zhang, H.; Sun, T. Resveratrol attenuates spinal cord injury-induced inflammatory damage in rat lungs. *Int. J. Clin. Exp. Pathol.,* **2015**, *8*(2), 1237-1246.
PMID: 25973008

[19] Inanaga, K.; Ichiki, T.; Matsuura, H.; Miyazaki, R.; Hashimoto, T.; Takeda, K.; Sunagawa, K. Resveratrol attenuates angiotensin II-induced interleukin-6 expression and perivascular fibrosis. *Hypertens. Res.,* **2009**, *32*(6), 466-471.
http://dx.doi.org/10.1038/hr.2009.47
PMID: 19373235

[20] Cremer, D.R.; Rabeler, R.; Roberts, A.; Lynch, B. Long-term safety of α -lipoic acid (ALA) consumption: a 2-year study. *Regul. Toxicol. Pharmacol.,* **2006**, *46*(3), 193-201.
http://dx.doi.org/10.1016/j.yrtph.2006.06.003 PMID: 16899332

[21] Lee, J.E.; Yi, C.O.; Jeon, B.T.; Shin, H.J.; Kim, S.K.; Jung, T.S.; Choi, J.Y.; Roh, G.S. α-Lipoic acid attenuates cardiac fibrosis in Otsuka Long-Evans Tokushima fatty rats. *Cardiovasc. Diabetol.,* **2012**, *11*(1), 111.
http://dx.doi.org/10.1186/1475-2840-11-111 PMID: 22992429

[22] Jung, T.S.; Kim, S.K.; Shin, H.J.; Jeon, B.T.; Hahm, J.R.; Roh, G.S. α-lipoic acid prevents non-alcoholic fatty liver disease in OLETF rats. *Liver Int.,* **2012**, *32*(10), 1565-1573.
http://dx.doi.org/10.1111/j.1478-3231.2012.02857.x
PMID: 22863080

[23] Christofidou-Solomidou, M.; Tyagi, S.; Tan, K.S.; Hagan, S.; Pietrofesa, R.; Dukes, F.; Arguiri, E.; Heitjan, D.F.; Solomides, C.C.; Cengel, K.A. Dietary flaxseed administered post thoracic radiation treatment improves survival and mitigates radiation-induced pneumonopathy in mice. *BMC Cancer,* **2011**, *11*, 269.
http://dx.doi.org/10.1186/1471-2407-11-269 PMID: 21702963

[24] Mahmood, J.; Jelveh, S.; Calveley, V.; Zaidi, A.; Doctrow, S.R.; Hill, R.P. Mitigation of radiation-induced lung injury by genistein and EUK-207. *Int. J. Radiat. Biol.,* **2011**, *87*(8), 889-901.
http://dx.doi.org/10.3109/09553002.2011.583315
PMID: 21675818

[25] Mahmood, J.; Jelveh, S.; Zaidi, A.; Doctrow, S.R.; Hill, R.P. Mitigation of radiation-induced lung injury with EUK-207 and genistein: effects in adolescent rats. *Radiat. Res.,* **2013**, *179*(2), 125-134.
http://dx.doi.org/10.1667/RR2954.1 PMID: 23237541

[26] Jackson, I.L.; Zodda, A.; Gurung, G.; Pavlovic, R.; Kaytor, M.D.; Kuskowski, M.A.; Vujaskovic, Z. BIO 300, a nanosuspension of genistein, mitigates pneumonitis/fibrosis following high-dose radiation exposure in the C57L/J murine model. *Br. J. Pharmacol.,* **2017**, *174*(24), 4738-4750.
http://dx.doi.org/10.1111/bph.14056
PMID: 28963717

[27] Mahmood, J.; Jelveh, S.; Zaidi, A.; Doctrow, S.R.; Medhora, M.; Hill, R.P. Targeting the Renin-angiotensin system combined with an antioxidant is highly effective in mitigating radiation-induced lung damage. *Int. J. Radiat. Oncol. Biol. Phys.,* **2014**, *89*(4), 722-728.
http://dx.doi.org/10.1016/j.ijrobp.2014.03.048 PMID: 24867538

[28] Ghosh, S.N.; Zhang, R.; Fish, B.L.; Semenenko, V.A.; Li, X.A.; Moulder, J.E.; Jacobs, E.R.; Medhora, M. Renin-angiotensin system suppression mitigates experimental radiation pneumonitis. *Int. J. Radiat. Oncol. Biol. Phys.,* **2009**, *75*(5), 1528-1536.
http://dx.doi.org/10.1016/j.ijrobp.2009.07.1743
PMID: 19931735

[29] Kma, L.; Gao, F.; Fish, B.L.; Moulder, J.E.; Jacobs, E.R.; Medhora, M. Angiotensin converting enzyme inhibitors mitigate collagen synthesis induced by a single dose of radiation to the whole thorax. *J. Radiat. Res. (Tokyo),* **2012**, *53*(1), 10-17.
http://dx.doi.org/10.1269/jrr.11035 PMID: 22302041

[30] Carsten, R.E.; Bachand, A.M.; Bailey, S.M.; Ullrich, R.L. Resveratrol reduces radiation-induced chromosome aberration frequencies in mouse bone marrow cells. *Radiat. Res.,* **2008**, *169*(6), 633-638.
http://dx.doi.org/10.1667/RR1190.1 PMID: 18494544

[31] Zhang, H.; Yan, H.; Zhou, X.; Wang, H.; Yang, Y.; Zhang, J.; Wang, H. The protective effects of Resveratrol against radiation-induced intestinal injury. *BMC Complement. Altern. Med.,* **2017**, *17*(1), 410.
http://dx.doi.org/10.1186/s12906-017-1915-9 PMID: 28814292

[32] Kim, J.H.; Kim, K.M.; Jung, M.H.; Jung, J.H.; Kang, K.M.; Jeong, B.K.; Kim, J.P.; Park, J.J.; Woo, S.H. Protective effects of alpha lipoic acid on radiation-induced salivary gland injury in rats. *Oncotarget,* **2016**, *7*(20), 29143-29153.
http://dx.doi.org/10.18632/oncotarget.8661
PMID: 27072584

[33] Kim, J.H.; Jung, M.H.; Kim, J.P.; Kim, H.J.; Jung, J.H.; Hahm, J.R.; Kang, K.M.; Jeong, B.K.; Woo, S.H. Alpha lipoic acid attenuates radiation-induced oral mucositis in rats. *Oncotarget,* **2017**, *8*(42), 72739-72747.
http://dx.doi.org/10.18632/oncotarget.20286 PMID: 29069822