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Modulating effects of famotidine and melatonin on high dose radiation induced cell lethality in normal human and cancer cell lines

H. Mozdarani[1*], O. Azadbakht[2], S. Ghorbainan Klachai[3], S. Mozdarani[3], H. Nosrati[4], S. Mozdarani[3]

[1]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2]Department of Radiology, School of Paramedicine, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Cytogenetic, Cytogenome Medical Genetic Laboratory, Chamran Medical Building, Tehran, Iran
[4]Department of Radiotherapy, Cancer Institute, Imam Khomeini Hospital, Tehran, Iran

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Corresponding authors:
Prof., Hossein Mozdarani, PhD.,
E-mail:
mozdarah@modares.ac.ir

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ABSTRACT

Background: Radioprotective effects of melatonin and famotidine were reported in previous studies. In this study, modulating effects of these agents alone or in combination were tested on high dose radiation induced cell lethality in MRC5 and Hela cells. ***Materials and Methods:*** DPPH (2,2-Diphenyl-1-picrylhydrazyl) was used to measure antioxidant property of famotidine and melatonin at different concentrations. Famotidine at a concentration of 80 µg/ml and melatonin at a concentration of 80 µg/ml was added to culture flasks containing MRC5 and Hela cells two hr prior to gamma-irradiation. Treated and untreated cells were irradiated with doses of 4 and 8 Gy gamma-rays. MTT assay was used to measure cell viability 48 and 72 hours after irradiation. Data were analyzed using nonparametric one way analysis of variance (ANOVA). ***Results:*** DPPH assay showed high antioxidant potential for melatonin. Presence of melatonin led to significant elevation of cell viability of both MRC5 and Hela cell lines after 4 and 8 Gy gamma-irradiation at both sampling times ($p<0.01$). However, for Hela cells exposed to 4 Gy, melatonin led to reduced cell viability ($p<0.05$). Famotidine, did not improve radiation induced cell lethality for both MRC5 and Hela cells exposed to 4 and 8 Gy. ***Conclusion:*** Except for 4 Gy irradiated Hela cells, presence of melatonin led to a significant radioprotection against radiation induced cell lethality of cells, Famotidine failed to improve cell viability in both cell lines. The mechanism of radioprotection of melatonin might be attributed to its radical scavenging potential.

Keywords: *Radioprotection, melatonin, famotidine, MRC5 and Hela cells, cell viability.*

INTRODUCTION

Ionizing radiation is used in various aspects of modern life from medicine to industry. There is no doubt that ionizing radiation induces various short and long terms side effects through direct or indirect action with cellular components. It is about seven decades past since the discovery of the radioprotective property of cysteine in 1949 [(1)]. Since then, a large number of compounds, mainly aminothiols, naturally occurring antioxidants and other chemicals with different mode of action were studied for their possible radioprotective potential [(2)]. Some of these compounds needed to be administered at high doses to protect against radiation induced adverse effects, hence, producing various side effects by themselves. However, it is believed combining two or more compounds with different and or complementary mechanisms of

action might lead to reduced toxicity and greater efficacy [3, 4]. Moreover, most of agents were tested on normal cells or tissues for their radioprotective potential.

In this study, radioprotective effects of famotidine and melatonin alone or in combination on cell lethality of normal and cancer cell lines exposed to relatively high doses of gamma rays was tested.

Famotidine, an orally administrable drug is a histamine H_2-receptor antagonist usually used for peptic ulcer treatment [5]. It was shown that famotidine is a potent hydroxyl radical scavenger [6] and inhibits histamine-stimulated gastric acid secretion, Hydroxyl radicals are generated mainly via indirect effect of ionizing radiation and is considered as the most damaging oxygen based free radicals [7]. Radioprotective potential of famotidine against radiation-induced lipid peroxidation was shown [8]. Other studies indicated radioprotective effect of famotidine against radiation induced micronuclei and chromosomal aberration in human peripheral blood lymphocytes and mouse bone marrow cells [9-11], DNA damage [12], radiation induced apoptosis in normal lymphocytes [13] and mouse spermatogenesis [14].

Melatonin, on the other hand is proved to be a potent antioxidant naturally occurring in human body secreted mainly by the pineal gland during the dark hours at night. Antioxidant properties and free radical scavenging activity of melatonin has been shown in various experimental models [15]. Melatonin with oncostatic property not only acts against different neoplasia [16] but prevents the promotion and growth of mammary tumours [17]. Moreover, as an adjuvant therapy, reduces the side effects of chemotherapeutic drugs [18]. Melatonin acts in different pathways related antioxidant properties [19]. Experimental evidences indicate the radio protective effect of melatonin against radiation induced genetic damage in blood, bone marrow and mortality of mice [20]. However, different results were obtained for cells irradiated in G_0 cell cycle or G_2 phase of the cell cycle in the presence of melatonin [21, 22].

The aim of this study was therefore to evaluate modulating effects of melatonin and famotidine alone or in combination against high dose radiation induced cell lethality in normal or cancer cells. MTT assay was used to measure cell viability after different time intervals following irradiation of cells alone or in the presence of the drugs.

MATERIALS AND METHODS

Drug preparation and treatment

Pure famotidine and melatonin powder was provided by Chemidarou Pharmaceutical Co. (Tehran, Iran). Various doses of famotidine (10, 20, 40 and 80 µg/ml), melatonin (100, 200, 400 and 800 µg/ml) was prepared by dissolving the powders in sterile distilled water for their potential for free radical scavenging by DPPH (2, 2- diphenyl-1-picrylhydrazyl) assay. An optimum dose of each chemical was used to treat cell lines before irradiation.

DPPH assay

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay was conducted as described by Brand-Williams methods previously [23]. This assay is widely used and is known as an easy, economic and valid procedure for evaluating radical scavenging properties of antioxidants, since there is no need to generate free radicals. In summary, different concentration of melatonin (100, 200, 400 and 800 µg/ml) and famotidine (10, 20, 40 and 80 µg/ml) reacted with a stable DPPH radical solution (Sigma Aldrich, Germany). Each reaction included; 0.5 ml of either melatonin or famotidine solution, 3 ml of absolute ethanol and 0.3 ml of DPPH radical solution (0.5 mM in absolute ethanol). The control solution was prepared by adding 3.5 ml of ethanol and 0.3 ml of DPPH radical solution. Each reaction was mixed, covered and left in the dark for 30 min at room temperature, and then the absorbance was measured at 517 nm by UV–Vis spectrophotometer (ELX 800, USA). For each concentration of melatonin or famotidine, DPPH assay was repeated three times.

The inhibition percentage was calculated by using the equation 1 [24]:

% Inhibition = [(Control OD – Sample OD) / Control OD]×100 (1)

Cell lines and cell culture

Human fetal lung fibroblast cells (MRC-5) (as a normal cell line) and Hela cells originated from cervical carcinoma (as a cancer cell line) were obtained from the Cell Bank of Pasteur Institute of Iran (Tehran, Iran). Cells were cultured in Eagle's minimal essential medium (MEM) (Gibco, BRL), supplemented with 10% fetal bovine serum (Gibco, BRL) and antibiotics (100µg /ml penicillin and 100µg /ml streptomycin, (Gibco, BRL). Cells were seeded in 75 cm^3 culture flasks (Nunc) in 10 ml complete culture medium, then left in a 37°C incubator with an atmosphere of 5% CO2 and 95% air. Cells were trypsinized with trypsin/EDTA (Merck, Germany) when 80% of the flasks were covered to maintain exponential growth. For each cell treatment group, three flasks were prepared.

Irradiation

Cells were irradiated with a dose of 4 and 8 Gy of γ- radiation generated from a therapeutic cobalt -60 source (Theratone 780 ACEL, Canada) at room temperature (23 ˚C±2) and a source to sample distance (SSD) of 80 cm at a dose rate of 0.85 cGy/min.

Cell viability assay

Viability of MRC5 and Hela cells after different treatments (control, different doses of radiation, combined treatment with melatonin, famotidine or both) was measured by MTT assay. Cells were treated with either famotidine or melatonin alone or in combination 2 hours prior to irradiation. Each treated or untreated culture vessel was irradiated with different doses of gamma radiation. Soon after irradiation, cultures were transferred to the lab and cells were trypsinised and counted using trypan blue exclusion dye for viable cells under a light microscope. Cells were seeded into 96 well culture plates containing complete culture medium at a density of 10^4 cells/well. Cells were incubated for 48 h and 72 h at 37 °C in a humidified CO_2 incubator. Each treatment was replicated in 3 wells. After the incubation time, the cell proliferation and cytotoxicity were determined by the use of MTT assay kits (Sigma, USA). According to the manufacturer's instruction, 100 µL of Formazan solubilization solution was added to each well and mixed thoroughly. After 4 hours, the content of each well was replaced with 200 µl of dimethyl sulfoxide (DMSO, Merck, Germany) incubated for another 30 min. Color change of each well was measured at 630 nm with a microplate reader (Awareness Technology, USA).

Statistical analysis

Data were analyzed using SPSS (version 17) software (SPSS Inc. Chicago, IL, USA). The significance of any inter-group differences was evaluated by one-way analysis of variance (ANOVA). A p value of less than 0.05 was considered as significant difference.

RESULTS

Results of DPPH assay

The results for antioxidant properties of famotidine and melatonin is shown in figure 1. As seen, famotidine has shown very poor radical scavenging property even at high doses. However, based on this result, the highest dose used in this study (80 µg/ml) was selected for using in combination with radiation. The situation for melatonin was different. Radical scavenging potential for Melatonin at doses between 100-400 µg/ml was nearly similar but slightly and significantly different when the dose of 800 µg/ml was used. Therefore, this dose was selected for using in combination with radiation.

Viability results of MRC5 cells

Results of MRC5 cells exposed to 4 Gy gamma rays in the absence or the presence of famotidine and melatonin or combination of both is shown in figure 2A. As seen, 48 hours after irradiation, viability of irradiated cells was not decreased significantly, but there was a significant reduction in viable cells after 72 hours ($p<0.01$). Presence of melatonin increased cellular viability both after 48 h and 72 hour

sampling times ($p<0.05$). Presence of famotidine alone could not improve cell viability at 48 hours sampling time ($p>0.05$) but it was more potent than melatonin in increasing cell viability after 72 hours sampling time ($p<0.01$). Combination of melatonin and famotidine had no significant effect on radiation induced lethality at 48 hours sampling time, but had significantly different effect on irradiated cells after 72 hours ($p<0.01$) (figure 1A).

Results obtained with cells irradiated with 8 Gy gamma rays (figure 2B) were similar with that of the 4 Gy. Presence of melatonin was more effective for cells irradiated with 8 Gy after 48 and 72 hours post-irradiation sampling times ($p<0.01$). Presence of famotidine alone led to slight improve but not statistically significant in cell viability at 48 hours sampling time ($p>0.05$). Combination of melatonin and famotidine had no significant effect on radiation induced lethality at 48 hours sampling time, but had significantly different effect on 8 Gy irradiated cells after 72 hours ($p<0.01$) (figure 2B).

Viability results of Hela cells

Results of Hela cells exposed to 4 Gy gamma rays in the absence or the presence of famotidine and melatonin or combination of both is shown in figure 3A. As seen, 48 hours after irradiation, viability of irradiated cells was not decreased significantly, but there was a significant reduction in viable cells after 72 hours ($p<0.01$). Presence of melatonin significantly decreased cellular viability both after 48 h and 72 hour sampling times ($p>0.05$). Presence of famotidine alone could not improve cell viability at 48 hours sampling time ($p>0.05$) but it was more potent than melatonin in increasing cell viability after 72 hours sampling time ($p<0.01$). Combination of melatonin and famotidine had no significant effect on radiation induced lethality at 48 hours sampling time, but had significantly different effect on irradiated cells after 72 hours ($p<0.01$) (figure 3A).
Results obtained with cells irradiated with 8 Gy gamma rays were similar with that of the 4 Gy. Presence of melatonin was more effective for cells irradiated with 8 Gy after 48 and 72 hours post-irradiation sampling times ($p<0.01$) (figure 3B). Presence of famotidine alone led to slight decrease but not statistically significant in cell viability at 48 hours sampling time ($p>0.05$). Combination of melatonin and famotidine had no significant effect on radiation induced lethality at 48 and 72 hours sampling time ($p<0.01$) (figure 3B).

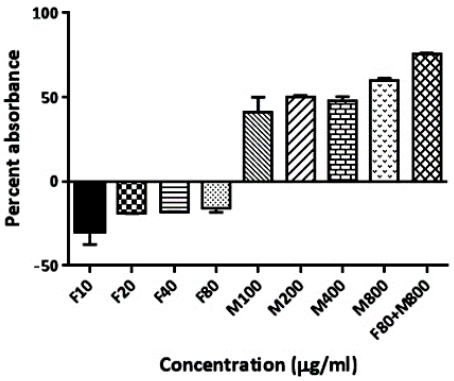

Figure 1. Free radical scavenging activity of famotidine and melatonin alone or in combination in different concentrations. Error bars indicate standard deviation from the mean values.

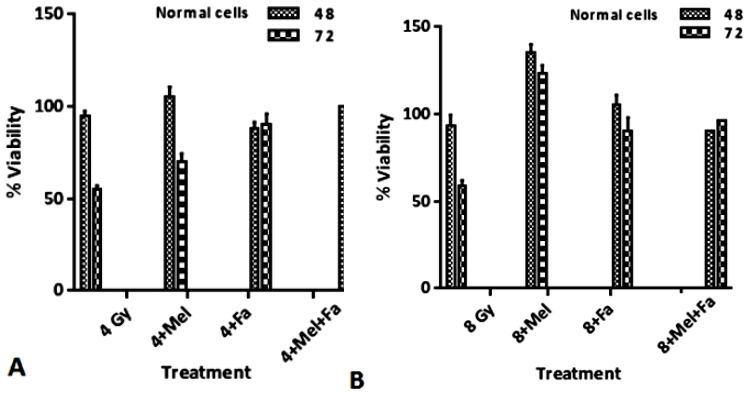

Figure 2. A: Percent viability of MRC5 (normal) cells after irradiation with 4 Gy gamma rays in the absence or presence of melatonin (800 µg/ml), famotidine (80 µg/ml) or combination of both agents. **B:** Percent viability of MRC5 (normal) cells after irradiation with 8 Gy gamma rays in the absence or presence of melatonin, famotidine or combination of the two agents with the same concentration. Error bars indicate standard deviation of the mean values.

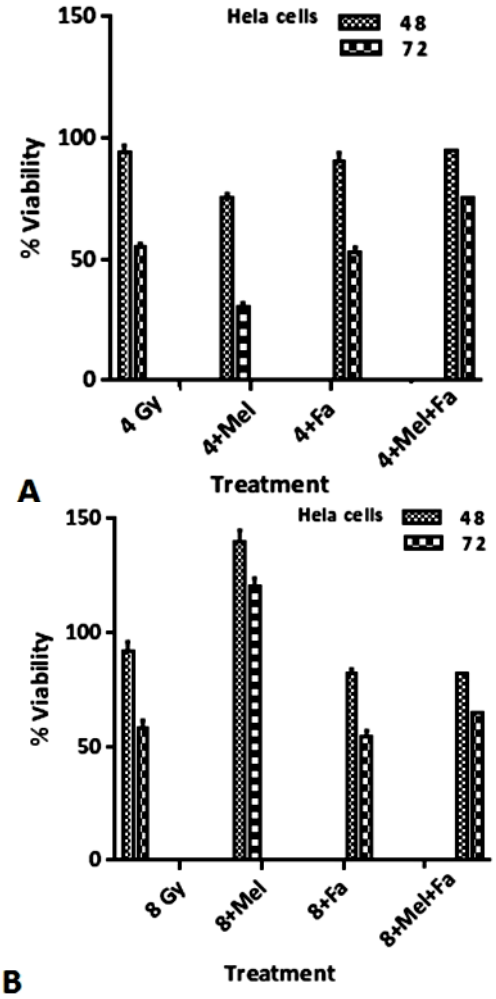

Figure 3. A: Percent viability of Hela (cancer) cells after irradiation with 4 Gy gamma rays in the absence or presence of melatonin (800 µg/ml), famotidine (80 µg/ml) or combination of both agents. **B:** Percent viability of Hela (cancer) cells after irradiation with 8 Gy gamma rays in the absence or presence of melatonin, famotidine or combination of the two agents with the same concentration. Error bars indicate standard deviation of the mean values.

DISCUSSION

Viability of normal human (MRC5) and cancer (Hela) cell lines with different relatively high doses of gamma rays in the presence of optimum concentrations of famotidine and melatonin or both was measured using the MTT assay. The obtained results with famotidine are somehow and generally consistent with our previous reports indicating radioprotective capacity of this drug. Although DPPH assay did not prove radical scavenging potential of famotidine (figure 1) but it was previously shown that it could be a potent radioprotector both *in vitro* and *in-vivo* [9-14, 25, 26]. Presence of famotidine led to an increase in cell viability after both 4 and 8 Gy gamma irradiation (figure 2) after 72 hours incubation time. This effect might be attributed to the anti-clastogenic effect of famotidine as proposed by Lapenna *et al*. (1994) [27]. Unlike normal cells, presence of famotidine in culture medium of Hela cells at the time of irradiation, no significant change in cell viability was observed. Both after 4 and 8 Gy irradiation at either 48 or 72 hour sampling time. This observation might indicate different mode of action of famotidine on radiation induced cellular damage in normal and cancer cells.

The antioxidant activity of melatonin as shown in figure 1 was significantly higher at all concentrations used in this study compared to famotidine. Several other studies have shown the antioxidant and free radical scavenging potential of melatonin [28, 29]. As seen in figure 1 A and B, presence of melatonin led to significant increase in cell viability after irradiation of 4 and 8 Gy for both sampling times. These results are consistent with our previous report concerning the protective effects of melatonin on radiation induced micronucleus in MRC5 and Hela cells [21] and other studies about antioxidant and radio-protective effects of melatonin [30, 31]. Radioprotective potential of melatonin was confirmed in 1995 [32]. Concerning Hela cells irradiated with different doses of gamma rays in the presence of melatonin, two different cellular response was observed. Presence of melatonin in cellular environment of Hela cells during 4 Gy gamma radiation led to statistically significant decrease in cell viability ($p<0.01$) for both sampling times (figure 3A); whereas for 8 Gy irradiation a distinct statistically significant increase in cell viability was observed for both sampling times ($p<0.01$) (figure 3B). The protective effects seen for cells irradiated with 8 Gy is justified with the radioprotection properties of melatonin reported earlier for different test systems and end points [32]. The reason for observation of radiosentising effect of melatonin following 4 Gy irradiation of Hela

cells is not clearly understood, although there are results indicating that melatonin could act as a radiosensitizer when used on G_2 cells of normal and breast cancer cell lines [22]. Moreover, it was shown that pre-treatment of breast cancer cells with melatonin lead to radiosensitization by down regulation of proteins (RAD51, DNA-PKs) involved in DNA double strand break repair [33]. Combined treatment of melatonin and famotidine did not improve viability of MRC5 and Hela after irradiation compared to famotidine or melatonin treatment alone (figures 2 and 3).

In conclusion, this study indicated that melatonin is a better radioprotector agent in terms of cell viability after irradiation f high doses of gamma rays compared to famotidine. The reason for this radioprotective potential might reside in the potent radical scavenging property of melatonin. There was essentially no difference in the effects of these two drugs in response to radiation of normal and cancer cells. However, there was significant difference for the effect of melatonin following irradiation of Hela cells with 4 Gy that should be resolved in future studies.

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***Conflicts of interest*: Declared none.**

REFERENCES

1. Patt HM, Tyree EB, Straube RL, Smith DE (1949) Cysteine protection against X irradiation. *Science,* ***110(2852):*** *213–214.*
2. Seed TM (2005) Radiation protectants: current status and future prospects. *Health Phys,* ***89:*** *531.*
3. Maisin JR (1988) Protection against ionizing radiation by combinations of radioprotectors. *Pharmacol Ther,* ***39:*** *189–193*.
4. Uma Devi P and Thomas B (1988) Bone marrow cell protection and modification of drug toxicity by combination of protectors. *Pharmacol Ther,* ***39:*** *213–214.*
5. Deakin M, Williams JG (1992) Histamine H2-receptor antagonists in peptic ulcer disease: efficacy in healing peptic ulcers. *Drugs,* ***44:*** *709–719.*
6. Ching TL, Haenen GR, Bast A (1993) Cimetidine and other H2 receptor antagonists as powerful hydroxyl radical scavengers. *Chem Biol Interact,* ***86:*** *119–127*.
7. Imlay JA and Linn S (1988) DNA damage and oxygen radical toxicity. *Science,* ***240(4857):*** *1302.*
8. Della Rovere F, Broccio M, Granata A, Zirilli A, Brugnano L, Artemisia A, Broccio G (1996) Anti free radical action of calcium antagonists and H1 and H2 receptors antagonists in neoplastic disease. *Anticancer Res,* ***16(4A):*** *1749–1754.*
9. Ghorbani M and Mozdarani H (2003) *In-vitro* radioprotective effects of histamine H2 receptor antagonists against gamma-rays induced chromosomal aberrations in human lymphocytes. *Int J Radiat Res,* ***1(2)****: 99-104*.
10. Mozdarani H and Shahidi M (2003) Potent radioprotective effect of therapeutic doses of ranitidine and famotidine against gammarays induced micronuclei *in-vivo*. *Int J Radiat Res,* ***1:*** *29–35*.
11. Mozdarani H (2003) Radioprotective properties of histamine H2 receptor antagonists: present and future prospects*. J Radiat Res,* ***44:*** *145–149.*
12. Mozdarani H, Nasirian B, Haeri SA (2007) In vivo gamma-rays induced initial DNA damage and the effect of famotidine in mouse leukocytes as assayed by the alkaline comet assay. *J Radiat Res* ***48:*** *129–134.*
13. Mozdarani H and Ghoraeian P (2008) Modulation of gamma-ray-induced apoptosis in human peripheral blood leukocytes by famotidine and vitamin C. *Mutat Res,* ***649:*** *71–78.*
14. Mahdavi M and Mozdarani H (2011) Protective effects of famotidine and vitamin C against radiation induced cellular damage in mouse spermatogenesis process. *Int J Radiat Res,* ***8:*** *223–230.*
15. Karbownik M, Lewinski A, Reiter RJ (2001) Anticarcinogenic actions of melatonin which involve antioxidative processes: comparison with other antioxidants. *Int J Biochem Cell Biol,* ***33****: 735-53*.
16. Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, *et al.* (2010) Basic mechanisms involved in the anti-cancer effects of melatonin. *Curr Med Chem,* ***17****: 4462-81*.
17. Cos S and Sánchez-Barceló EJ (2000) Melatonin and mammary pathological growth. *Neuroendocrinol,* ***21****: 133–170*.
18. Oz E, Erbas D, Surucu HS, *et al.* (2006) Prevention of doxorubicin- induced cardiotoxicity by melatonin. *Mol Cell Biochem,* ***282****: 31-7*.
19. Sanchez-Barcelo EJ, Mediavilla MD, Alonso-Gonzalez C, Reiter RJ (2012) Melatonin uses in oncology: Breast cancer prevention and reduction of the side effects of chemotherapy and radiation. *Expert Opin Inv Drug,* ***21****: 819-831*.
20. Vijayalaxmi Meltz ML, Herman TS (1999) Melatonin and protection from genetic damage in blood and bone marrow: Whole-body irradiation studies in mice. *J Pineal Res,* ***27 (4)****; 221-225.*
21. Ghorbanian Kelachayeh S, Sangtarash MH, Mozdarani H (2020) The effects of melatonin on the frequency ofmicro-

nuclei induced by ionizing radiation in cancerous and normal cell lines. *Int J Radiat Res,* ***18(1):*** *57-64.*

22. Samei E, Mozdarani H, Samiei F, Javadi GR (2020) Radiosensitizing effects of melatonin on radiation induced chromosomal aberration in G2-lymphocytes of breast cancer patients. *Int J Radiat Res,* ***18(4):*** *657-662*.
23. Brand-Williams W, Cuvelier ME, Berset CL (1995) Use of a free radical method to evaluate antioxidant activity. *LWTFood Science and Technology,* ***28(1)****: 25-30*.
24. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature,* ***181(4617)****: 1199.*
25. Razaghdoust A, Mozdarani H, Mofid B, Aghamiri SMR, Heidari AH (2014) Reduction in radiation-induced lymphocytopenia by famotidine in patients undergoing radiotherapy for prostate cancer. *Prostate,* ***74:*** *41–47*.
26. Zangeneh M, Mozdarani H, Mahmoudzadeh A (2015) Potent radioprotective effects of combined regimens of famotidine and vitamin C against radiation-induced micronuclei in mouse bone marrow erythrocytes. *Radiat Environ Biophys,* ***54:*** *175–181.*
27. Lapenna D, De Gioia S, Mezzetti A, Grossi L, Festi D, Marzio L, Cuccurullo F (1994) H2-receptor antagonists are scavengers of oxygen radicals. *Eur J Clin Invest,* ***24:*** *476–481*.
28. Chen W, Jia Z, Zhu H, Zhou K, Li Y, Misra HP (2010) Ethyl pyruvate inhibits peroxynitrite-induced DNA damage and hydroxyl radical generation: implications for neuroprotection. *Neurochemical Research,* ***35(2)****: 336-342*.
29. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2002) On the *in vitro* antioxidative properties of melatonin. *J Pineal Res,* ***33(3)****: 167-171*.
30. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L (2016) Melatonin as an antioxidant: under promises but over delivers. *Journal of Pineal Research,* ***61(3)****: 253-278*.
31. Gitto E, Aversa S, Reiter RJ, Barberi I, Pellegrino S (2011) Update on the use of melatonin in pediatrics. *Journal of Pineal Research,* ***50(1)****: 21-28*.
32. Vijayalaxmi, Reiter RJ, Sewerynek E, Poeggeler B, Leal BZ, Meltz ML (1995) Marked reduction of radiation-induced micronuclei in human blood lymphocytes pretreated with melatonin. *Radiation Research,* ***143(1)****: 102-106*.
33. Alonso-Gonzalez C, Gonzalez A, Martinez-Campa C, Gomez - Arozamena J, Cos S (2015) Melatonin sensitizes human breast cancer cells to ionizing radiation by downregulating proteins involved in double-strand DNA break repair. *J Pineal Res,* ***58****: 189-197.*