

# Oral Administration of Vitamin C, Cimetidine and Famotidine on Micronuclei Induced by Low Dose Radiation in Mouse Bone Marrow Cells

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## ABSTRACT

**Background:** In many studies, chemicals and natural materials were tested to reduce the harmful effects of radiation. It is known that Famotidine and vitamin C reduce DNA damage.

**Objective:** The aim of this study was to evaluate the radioprotective effect of vitamin C, Cimetidine and Famotidine on gamma-radiation-induced damage on mouse bone marrow.

**Methods:** Six-to-seven week male NMRI mice (28 g  $\pm$  3) were randomly divided into fourteen groups: control, 2Gy irradiation, six group drugs without irradiation (Famotidine, Cimetidine, vitaminC, Fam-Cim, Fam-Vit, Cim-Vit), six groups received drugs and 2Gy radiation with a <sup>60</sup>Co  $\gamma$ -ray source at room temperature 22  $\pm$  2 °C. The mice were killed 48 hours after irradiation by cervical dislocation. Slides were prepared from bone marrow cells and stained in May-Granwald and Giemsa. Finally, the cells were counted with microscope, frequencies of polychromatic erythrocyte (PCE), normochromatic erythrocyte (NCE) and their micronucleated cell were recorded. PCE / PCE + NCE were calculated.

**Results:** There were significant differences of MNPCE/1000PCE, MNCE/1000NCE and PCE/PCE+NCE among different groups with similar radiation doses ( $p \leq 0.01$ ). Moreover, there were significant differences of MNPCE/1000PCE and PCE/PCE+NCE among different doses of radiation ( $p \leq 0.01$ ). While considering MNCE/1000NCE, there were no significant differences among similar groups with radiation dose ( $p > 0.05$ ).

**Conclusion:** Oral administration of Famotidine, vitamin C and Cimetidine demonstrate reliable and similar radioprotective effects. Additionally, the protective effect of single use of these drugs was similar to the combination form. Thus, the oral use of combination, 48 hours after irradiation cannot induce more radioprotective effect.

## Keywords

Micronuclei, Radiation, Radioprotection, Cimetidine, Vitamin C, Famotidine

## Introduction

People may be exposed to unwanted radiation during medical procedures or radiation accidents in medicine, industry or radioterrorism. Radiation causes cells clastogenic and cytotoxic damages [1], by energy transfer from radiation to macromolecules such

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as DNA, proteins and lipid membranes [2]. Free radical Proliferation leads to cell death by breaking or damaging chemical bonds in DNA [3]. The destructive effects of ionizing radiation in biological systems may be a direct interaction between radiation and target macromolecules, or indirect due to the products released during the interaction between radiation and water. Hydroxyl radical is the most destructive type of water radiolysis product [4]. Oxygen free radicals are more destructive and responsible for about 50% of the overall damages caused by free radicals, especially DNA failures [5, 6]. Radiation shielding reduces ionizing radiation damages [1]. Micronuclei test is one of the cytogenetic methods for the evaluation of radioprotective compounds [7]. It is an important indicator of protective effects of radioprotective drugs and ionizing radiation damages [8]. This test is a reliable method for assessing the clastogenic effects of materials in both in vitro and in vivo [9, 10].

In order to prevent damage to human, besides rules set by international organizations, chemicals and natural materials were tested to reduce the harmful effects of radiation. Radioprotective agents are synthetic compounds or natural products that are immediately administered before irradiation to reduce injuries caused by ionizing radiation [3]. An ideal radioprotector should provide significant protection against the effects of radiation, an acceptable route of administration, preferably oral, rapidly absorbed and distributed throughout the body, readily accessible and cheap, chemically stable to permit easy handling, storage at surrounding temperature and compatible with the wide range of other drugs which patients or personnel use. Additionally, it must have an acceptable toxicity and protective time-window effect [11, 12]. There is a continued deagerness in the identification and the development of nontoxic and effective radioprotectants that can reduce the effect of ionizing radiation [13].

Vitamin C or ascorbic acid is one of the best

defenses against the effects of cell damaging free radicals, demonstrated to have significantly radioprotective effects [14-17]. Also, the radioprotective effect of vitamin C on the internal and external radiation and a wide range of DRF have been reported [15, 18-20]. Administration of vitamin C prior to irradiation stops micronuclei [14, 21] and apoptosis [22]. Radiation induced wounds improved with administration of vitamin C [18]. It can lead to the repair of DNA failure and improved cell survival [23] and the reduction of DNA damage in normal cells [24]. Cimetidine and Famotidine are antagonists of histamine type II receptors used clinically for treating peptic ulcers, have been reported to be radioprotective due to its radical scavenging ability [25, 26] and stimulation of the immunosystem [27, 28]. Famotidine prevents DNA damage [29], apoptosis [22, 30] and the formation of micronuclei [7]. Besides, the inhibition of gastric acid secretion induced by histamine is a powerful hydroxyl radical sweeper [25]. These drugs with a dose-dependent effect prevent the production of O<sub>2</sub>- and H<sub>2</sub>O<sub>2</sub> in neutrophils [31].

According to previous studies and given the importance of following a diet of radioprotective drugs, this study investigated and compared radioprotective effects of combination and single oral administration of vitamin C, Cimetidine and Famotidine at micronuclei test.

## Material and Methods

### Experimental Animals

Seven or six week male NMRI mice (Pasture Institute, Amol, Iran) were housed five per cage (for one week) in climate-controlled, circadian rhythm-adjusted rooms, and they were allowed free access to food and water. The animals were, on average, seven to eight weeks old at the time of radiation and weighed between 28±3g. All experimental protocols and procedures were in agreement with the guidelines for animal experiments and the law

of Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran.

### Drug Treatments

Cimetidine and Famotidine powders were provided as gifts from ChemiDaru Co. (Tehran, Islamic Republic of Iran). Vitamin C powder was also as a gift from Osveh Co. (Tehran, Islamic Republic of Iran). All drugs were dissolved in distilled water and were freshly prepared for each use. All drugs were divided in three doses. Cimetidine, Famotidine and vitamin C (respectively, 15, 1.5 and 100mg/kg) were dissolved in distilled water, and were tested with gavage method along with 2 Gy of gamma rays; Gavigated three days before irradiation each 12h and also 2h before irradiation. The route of administration was per oral for in vivo studies in animals. All drugs were administered orally by gavages.

### Irradiation

Irradiation was carried out with a  $^{60}\text{Co}$   $|\gamma|$ -ray source (Theratron II, 780 C, Canada) at a dose of 2 Gy. The source-to-subject distance (SSD) was 80 cm and the dose rate was 0.98Gy/min. Mice were placed in individual ventilated Plexiglas chambers and simultaneously given a single whole-body exposure to  $\gamma$  -rays. Each mouse was placed in a separate and ventilated Plexigla chamber, in which they could not move. Each chamber size is 12×3×3 cm. Sham irradiation included comparable immobilization in the same irradiation chamber. The irradiation was performed without anesthesia of the mice.

### Sampling, Mn Preparation and Staining

The mice were killed by cervical dislocation 48 hours after irradiation. Control groups were also killed 48 hours after the latest demo gavages. Bone marrow cells were flushed from both femurs with Fetal Bovine Serum, and a cell suspension was duly prepared. The sus-

pension was centrifuged for 7 minutes at 1000 rpm. After centrifuging, the supernatant was removed and cells were resuspended in the remaining serum and a smear was prepared, fixed and stained by May Grunwald-Giemsa method (Schmid 1975). In this method of staining, polychromaticerythrocytes (PCEs) are stained blue-violet; while, normochromatic erythrocytes (NCEs) are stained yellow-range.

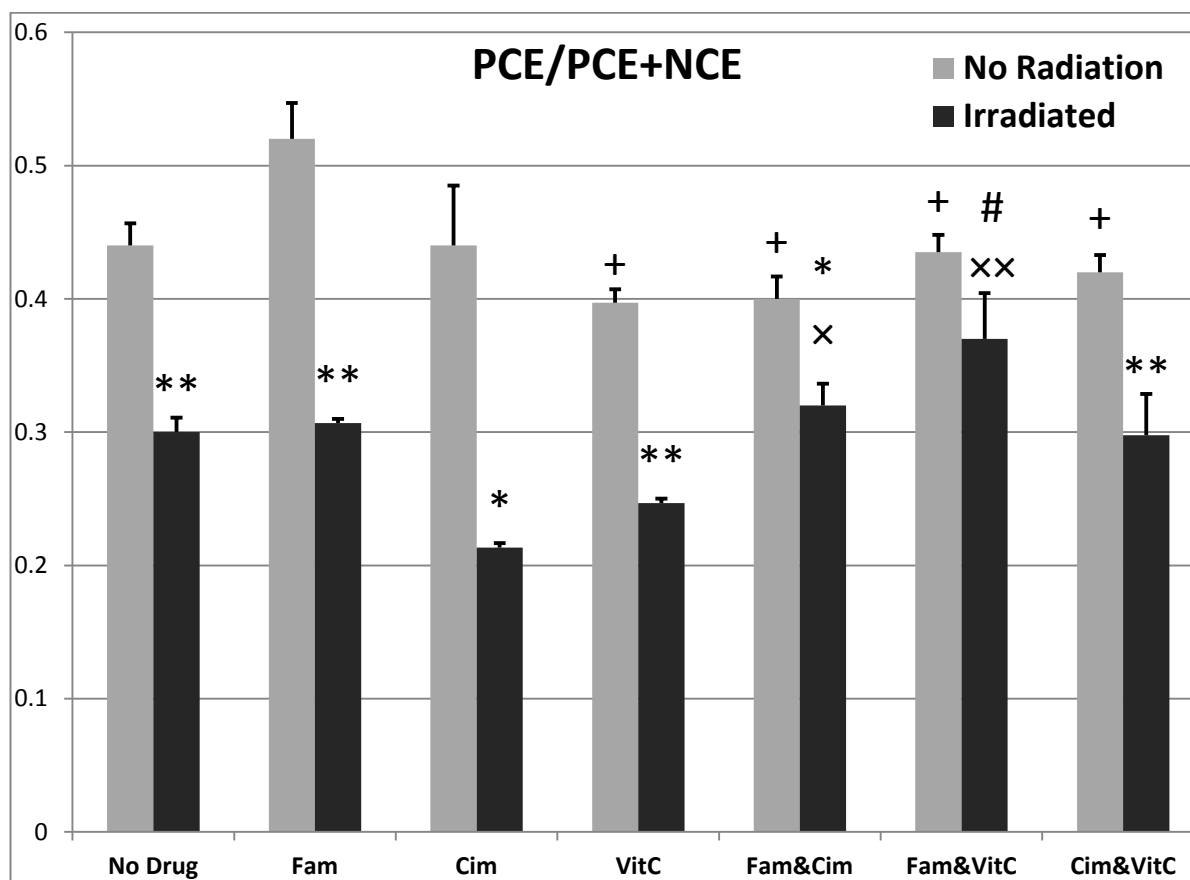
### Microscopic and Statistical Analysis

OLYMPUS microscope with ×100 objective lens was used for counting the cells. For each mouse, 1000 PCEs were scored. At the same time, NCEs/1000 PCEs as well as PCEs and NCEs containing micronuclei were counted and recorded. In order to study the cytotoxic effects of gamma rays on the proliferation of the bone marrow cells, the ratio of PCEs/PCEs+NCEs was calculated. Data were expressed as mean±SEM for each experimental group. Statistical analysis was performed using SPSS 16.0 software. The significance of any intergroup differences in the number of micronucleated PCEs and NCEs as well as the ratio of PCEs/PCEs+NCEs were tested. Multiple groups were tested with analysis of variance (one-way ANOVA) followed by Tukey's, and Two group comparisons were performed using t-test. P-value of less than  $p \leq 0.05$  was considered significant.

### Results

#### MNPCE/1000PCE after 48 hours: comparison of different groups

Data related to MNPCE/1000PCE after 48 hours are illustrated in Figure 1. There were significant differences of MNPCE/1000PCE among different groups without irradiation (0 Gy) [ $F(6, 29) = 4.25, p \leq 0.005$ ], and with irradiation (2 Gy) [ $F(6, 24) = 302.74, p \leq 0.001$ ].



**Figure 1:** Data related to effects of vitamins C, Famotidine and Cimetidine on PCE/PCE+NCE after irradiation at the doses of 0 and 2Gy. Values represent the mean  $\pm$  SEM.

\* $p \leq 0.05$ , \*\* $p \leq 0.001$  significantly different among similar groups (0 Gy with 2 Gy).

Between different doses: #  $p \leq 0.01$  with Vit C 2Gy, x  $p \leq 0.05$ , xx  $p \leq 0.001$  with Cim 2 Gy expect Fam&Cim, +  $p \leq 0.001$  with Fam0Gy.

#### MNPCE/1000PCE after 48 hours: comparison of similar groups, 0 and 2 Gy

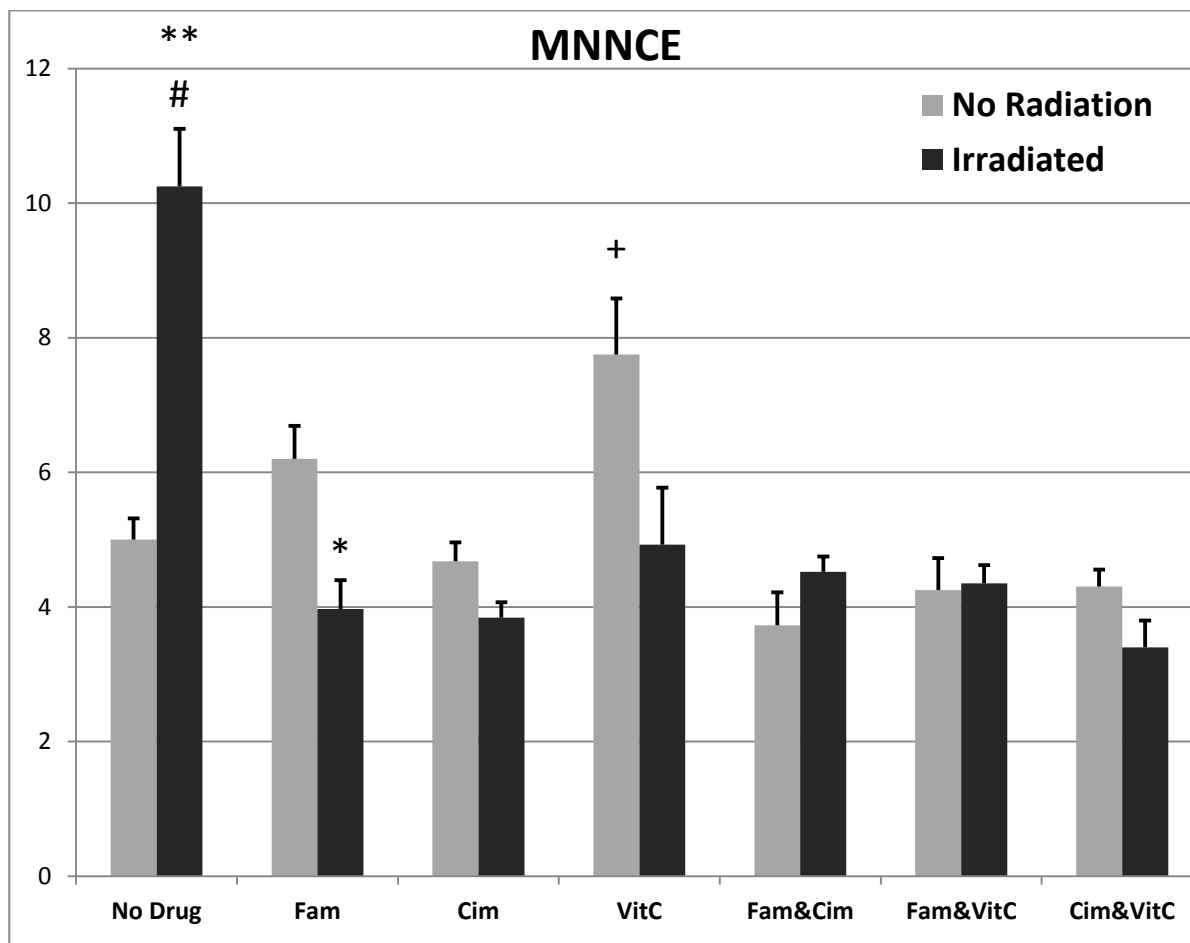
Data related to MNPCE/1000PCE after 48 hours are illustrated in Figure 1. There were significant differences of MNPCE/1000PCE among different doses of irradiation; between control groups ( $p \leq 0.001$ ), between vitamin groups ( $p \leq 0.001$ ), between Famotidine groups ( $p \leq 0.001$ ), between Cimetidine groups ( $p \leq 0.001$ ), Famotidine +Cimetidine groups ( $p \leq 0.001$ ), Famotidine +vitamin C groups ( $p \leq 0.001$ ) and Cimetidine +vitamin C groups ( $p \leq 0.001$ ).

#### MNNCE/1000NCE after 48 hours: comparison of different groups

Data related to MNNCE/1000NCE after 48 hours are illustrated in Figure 2. There were significant differences of MNNCE/1000NCE among different groups without irradiation [F (6, 29) = 7.77,  $p \leq 0.001$ ], and with irradiation [F (6, 25) = 21.76,  $p \leq 0.001$ ].

#### MNNCE/1000NCE after 48 hours: comparison of similar groups, 0 and 2 Gy

Data related to MNNCE/1000NCE after 48 hours are illustrated in Figure 2. There were significant differences of MNNCE/1000NCE



**Figure 2:** Data related to effects of vitamins C, Famotidine and Cimetidine on MNNCE/1000NCE after irradiation at the doses of 0 and 2Gy. Values represent the mean  $\pm$  SEM.

\* $p \leq 0.05$ , \*\* $p \leq 0.001$  significantly different among similar groups (0 Gy with 2 Gy).

Between different doses: #  $p \leq 0.001$  with other groups 2Gy, +  $p \leq 0.005$  with other groups 2 Gy except Fam.

among different doses of irradiation; between control groups ( $p \leq 0.001$ ), between vitamin C groups ( $p > 0.05$ ), between Famotidine groups ( $p \leq 0.05$ ), between Cimetidine groups ( $p > 0.05$ ), Famotidine +Cimetidine groups ( $p > 0.05$ ), Famotidine +vitamin C groups ( $p > 0.05$ ) and Cimetidine +vitamin C groups ( $p > 0.05$ ).

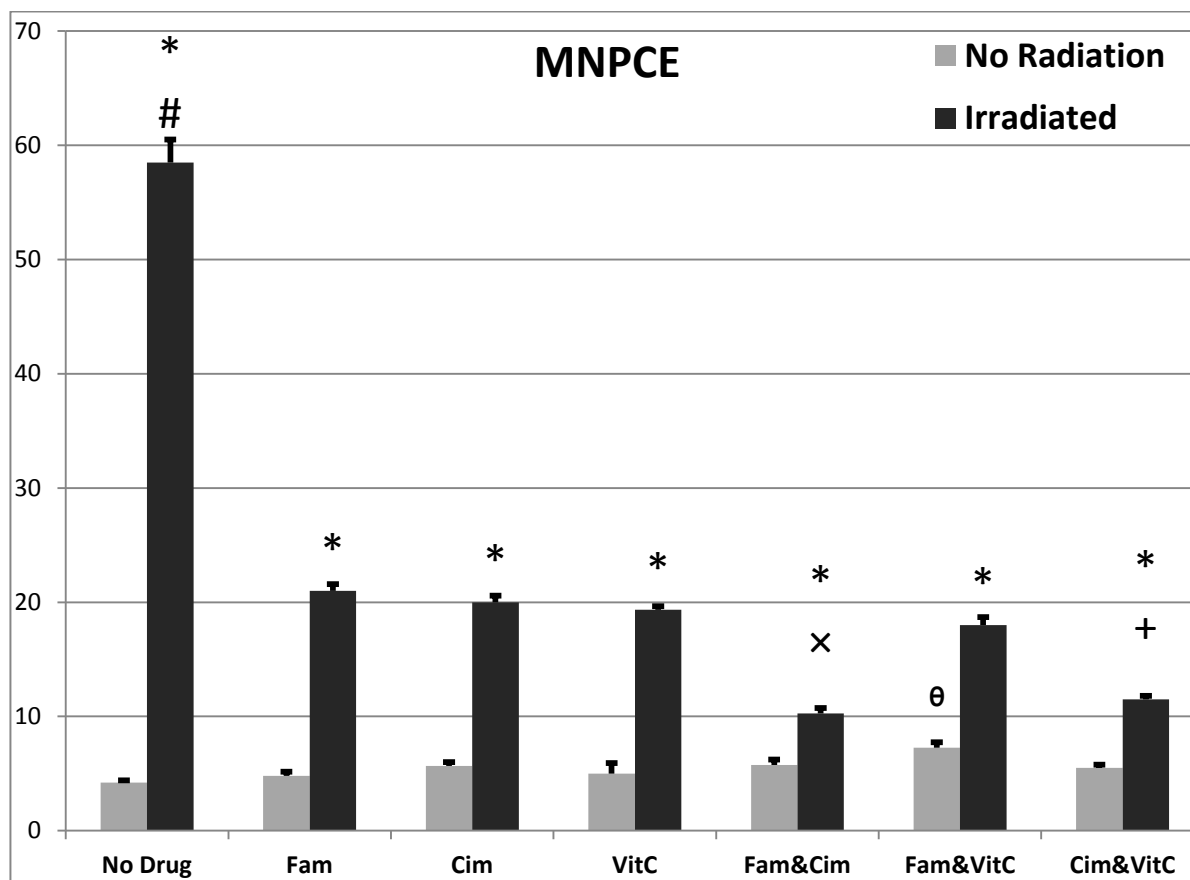
#### PCE/PCE+NCE after 48 hours: comparison of different groups

Data related to PCE/PCE+NCE after 48 hours are illustrated in Figure 3. There were significant differences of PCE/PCE+NCE among different groups without irradiation [F (6, 29) = 4.14,  $p \leq 0.01$ ], and with irradiation

[F (6, 25) = 5.71,  $p \leq 0.005$ ].

#### PCE/PCE+NCE after 48 hours: comparison of similar groups, 0 and 2 Gy

Data related to PCE/PCE+NCE after 48 hours are illustrated in Figure 3. There were significant differences of PCE/PCE+NCE among different doses of irradiation; between control groups ( $p \leq 0.001$ ), between vitamin C groups ( $p \leq 0.001$ ), between Famotidine groups ( $p \leq 0.001$ ), between Cimetidine groups ( $p \leq 0.05$ ), Famotidine+Cimetidine groups ( $p \leq 0.05$ ), Famotidine +vitamin C groups ( $p > 0.05$ ), Cimetidine+vitamin C groups ( $p \leq 0.001$ ).



**Figure 3:** Data related to effects of vitamins C, Famotidine and Cimetidine on MNPCE/1000MNPCE after irradiation at the doses of 0 and 2Gy. Values represent the mean ± SEM.

\*p ≤ 0.001 significantly different among similar groups (0 Gy with 2 Gy).

Between different doses: # p ≤ 0.001 with other groups 2Gy, + p ≤ 0.005 with other groups 2 Gy except Fam&Cim, x p ≤ 0.001 with other groups 2 Gy except Cim&Vit C, θ p ≤ 0.05 with control, Fam, and Cim groups 0Gy.

## Discussion

Ionizing radiation induces clastogenic and cytotoxic damages especially several damages in DNA, while radiation shielding reduces them [1]. Micronuclei test is an effective method for the evaluation of clastogenic effects of physical and chemical agents [14, 21]. It is a reliable method to measure the protective effect of radioprotective drugs [8]. So, this study investigated the comparison of radioprotective effects of combination and single oral administration of vitamin C, Cimetidine and Famotidine by micronuclei test. These results are consistent with previous studies; they showed Gamma radiation increased the number of mi-

cronuclei in PCE and NCE (MnPCE, MnNCE) in the mouse bone marrow cells, and the PCE/PCE+NCE significantly reduced, which indicates cytotoxic gamma rays effects on the proliferation of mouse bone marrow cells, while oral use of vitamin C, Cimetidine and Famotidine did not create additional micronuclei and did not improve radiation lethality [15, 16, 20, 27, 32]. According to the present study, oral use of these drugs caused a remarkable decrease in the number of MnPCEs and MnNCEs compared to control groups at the dose of 2Gy gamma radiation. These findings are consistent with a previous study that showed decrease in the cell proliferation [33].



Other previous findings have shown that gamma ray induced micronuclei in bone marrow erythrocytes [34, 35]. Histamine plays an increasing role on the bone marrow cell proliferation rate [36]. Famotidine, as a histamine H<sub>2</sub> receptor antagonist, is a powerful hydroxyl radical sweepers [25]. Ranitidine and Famotidine have a high scavenging power for OH•, HOCl and NH<sub>2</sub>Cl [37]. Famotidine does not have immunomodulatory role in immune system, but is capable of exerting scavenging oxygen radicals [38]. Famotidine prevents DNA damage [5], inducing chromosomal aberrations [38, 38], and micronuclei formation [7]. These results are consistent with Famotidine radioprotective effects which is against the induction of micronuclei in both in vitro and in vivo conditions [38, 39], and also Cimetidine, as another histamine H<sub>2</sub> receptor antagonist [28]. The most important protection mechanism of vitamin C is free radical and reactive oxygen scavenging [14, 21]. Vitamin C modulates apoptosis in bone marrow cells increasing antiapoptosis gene expression and works against pro-apoptosis gene expression [40]. It has a direct role in the differentiation and proliferation of bone marrow cells [41, 42]. Vitamin C treatment before irradiation causes the reduction of micronuclei in bone marrow, leukocyte and peripheral blood lymphocyte [14, 43], and causes to prevent apoptosis in peripheral blood leukocytes [22]. Vitamin C can lead to repair in a double-stranded DNA failure and improves cell survival [23] indirectly through the restoration of glutathione help in radioprotection [44]. The results of this study showed that combination use of these drugs cannot create additional micronuclei. As was shown in comparison with the control group, these drugs combination reduced MnPCE and increased PCE/PCE+NCE at similar a manner to using in no-combination route (single use). Thus, it seems that the sweeping effect of these drugs caused the reduction of radiation harms.

Due to increased use of ionizing radiation in human life such as radiotherapy of cancer,

industry, power generation and also high cost, side effects and toxicity of current available radioprotectors, we need to develop an effective and non-toxic radioprotector. Search for alternative sources such as plants, has been ongoing for several decades [45]. There are approaches to locate a potent radioprotector including mimics of antioxidant enzymes, nitroxides, gene therapy, growth factors, hyperthermia apart from natural products [46]. Although natural radioprotector sources especially medicinal plants or herbs are regarded as non-toxic even at higher concentrations, there is a growing interest among ethnomedicines and people [47]. Radioprotective activity of pure compounds isolated from the plants was previously evaluated, for example efficient superoxide radical scavenging ability of ellagitannins from *Phyllanthusamarus* [48], potent antioxidant activity of Ethanol extract of *Ficusracemosa* [49], radioprotective potential of quercetin and ethanolic extract of propolis in mice exposed to a single radiation [50]. But further investigation is needed to clarify the underlying protection mechanisms of natural radioprotectors. According to above studies and our results, these drugs in combination with natural radioprotectors could help scientists find a more effective radioprotective drug.

In conclusion, based on the results of this study, oral administration of Famotidine, vitamin C and Cimetidine showed reliable and similar radioprotective effects. Moreover, protective effect of single use of these drugs was similar to combination form. So, the oral combinational use 48 hours after irradiation cannot induce more radioprotective effects. The reduction of the incidence of micronuclei observed by the action of vitamin C, Cimetidine and Famotidine might be due to their antioxidant and radical scavenging properties. Further investigation with different organs or plans is necessary to evaluate the protective effect of these drugs, individual or in combination with natural radioprotectors, against radi-

ation-induced genotoxicity and cytotoxicity in radiation accident or radiation therapy

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## Conflict of Interest

None Declared

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