Spectroscopic Studies of in Vitro Exposure to Low-Dose Gamma Rays from 137Cs Radioactivity on Some Human Blood Components (Plasma and Red Blood Cells)

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Research Article

Keywords: low-dose gamma rays, blood components, radioactive, radiation

Posted Date: March 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-254110/v1

Abstract

This current study was to determine the effects of *in vitro* exposure to radioactive cesium-137 on some human blood components (Plasma and red blood cells). Blood samples were given a radiation dose of 0.02, 0.05, 0.1, 0.2, and 0.3 mGy of gamma rays using a ^{137}Cs radioactive standard source. The blood samples that were exposed to 0 mGy served as sham-controls. The spectrofluoroscopic technique was used to determine the autofluorescence spectrum of protein in plasma or red blood cells by using excitation wavelength and range of emission wavelengths at 280 nm and 300-550 nm, respectively. The spectrophotometric technique was used to determine the release of hemoglobin from the red blood cells to the supernatant. This data indicated no change in the ratio of fluorescence emission intensity at 340 nm of wavelength of protein extract from irradiated whole blood or red blood cells compared to the corresponding non-irradiated control. The results did not change in the absorption intensity at 415 nm of wavelength of hemoglobin leakage from *in vitro* irradiated red blood cells when compared to the corresponding non-irradiated red blood cells. These current results suggested that there were no harmful effects of the low-dose gamma rays from radioactive ^{137}Cs on some blood components when human whole blood was exposed to gamma rays in an *in vitro* condition.

Introduction

An accident in a nuclear power plant can cause radioactive contamination to the environment. On March 2011, the radioactive cesium-137 (^{137}Cs) was released into the environment from an accident at the Fukushima Daiichi Nuclear Power Plant[1]. Consequently, the radioactive cesium-137 with a half-life of 30 years caused contamination that was a serious concern because of the deleterious effect of radioactive cesium-137 on the environment or on humans[2–4]. Radioactive cesium-137 has been found in contaminated soil[5], forest[6], seawater[7–9], and freshwater[10], In addition, there are several reports that showed that radioactive cesium-137 has contaminated seawater fisheries. Wada et al.2013 determined that radioactive cesium contamination had occurred in marine products that took place during April 2011 to October 2012. The authors showed that the exceeded regulatory limit of contaminants in Japan was found in the several commercial fishes that were caught in coastal shallow waters[11]. Consistent with Wada et al. 2013[11], other authors monitored the radioactive cesium contamination in marine products during 2011 to 2015. The authors showed that the contamination in several fishes had been found in the Fukushima Prefecture[12]. Radioactive cesium have not only contaminated seawater fish, but have also contaminated freshwater types. The authors showed that during 2015–2016, the freshwater fish found in water around the Fukushima Dai-ichi Nuclear Power Plant had been contamination with radioactive cesium[13]. Kurikami et al. 2019 have also mentioned that during October 2018, freshwater fish in rivers at Fukushima Prefecture had been found to be contaminated with radioactive cesium. This radioactive cesium was higher than the Japanese regulatory limit of 100 Bq.kg^{-1} for general foodstuffs[14]. The contamination of fishes is termed an aquatic bioaccumulation; an important pathway for the transfer of radioactive cesium from fishes to humans. Therefore, humans can potentially receive radioactive cesium by directly contact or by consumption of

contaminated fresh fishes or contaminated fish products. In addition, Kana Yamamoto et al. 2019 evaluated the radioactive cesium contamination of pregnant women over a 5-year period after the Fukushima Daiichi Nuclear Power Plant accident at Minamisoma City which was an area that was included the evacuation zone. Authors reported that a maximum annual effective dose was found in these individuals by radioactive cesium-134 and − 137 that was estimated to be at 16 μSv/year[15]. Moreover, Masahiko Matsuo et al. 2019 evaluated radioactive contamination in an evacuation area called Tomioka Town located in the Fukushima Prefecture after six years after the Fukushima Daiichi Nuclear Power Plant accident. The authors reported that the median air dose rates both for indoors and outdoors were 0.20 μSv/h and 0.26 μSv/h, respectively. The indoor radiation exposure dose rate in the area that residents had returned home to was 1.6 mSv/y. However, the median air dose rate measured outdoors in this difficult to return area was 2.3 μSv/h (20 mSv/y)[16].

It should be noted that the environmental and human contamination from radioactive cesium after several years since the Fukushima Daiichi Nuclear Power Plant accident have still been found to be in the low-dose radiation range. It is known that high doses of radiation induce damage to cells or tissues[17–21]. However, information on the potential health risks from exposure to low-dose radiation is still unclear[22].

There are works that have studied the effect of gamma ray at the radiation dose level that might be found in a radiation accident or environmental radiation dose. Rithidech et al. 2005 studied the effect of ^{137}Cs gamma ray on bone marrow cells of mice after an *in vivo* exposure to 0.05, 0.1, or 1.0 Gy. The authors found that NF-κB activation occurred in bone marrow cells of mice after an *in vivo* exposure to 0.1 and 1.0 Gy (but not 0.05 Gy) of ^{137}Cs gamma ray[23]. Similar findings came from another publication, Jangiam et al. 2018 determined the late effects of ^{137}Cs gamma ray on the bone marrow, lungs, and testis of mice after an *in vivo* exposure to 0.05, 0.1, or 1.0 Gy. The authors found that the number of cell death, DNA damage, and inflammation response did not significantly change in bone marrow, lungs, and testis of mice that were exposed to 0.05 Gy of ^{137}Cs gamma rays, when compared to a sham control. In contrast, significant changes in the number of cell death, DNA damage, and inflammation responses were shown in bone marrow, lungs, and testis of mice that had been exposed to 0.1 and 1.0 Gy of ^{137}Cs gamma rays[24]. It should be noted that there was a difference in biological response to 0.05 Gy of gamma rays, when compared to 0.1 or 1.0 Gy exposure.

This current study has focused on the effect of low-dose gamma rays that might result in radioactive cesium contamination in humans. Red blood cells and plasma were used to study radiation effects because red blood cells and plasma are major components of blood. In addition, the damage of red blood cells might cause insufficient oxygen consumption in tissue or organs, resulting in increases in the risk of deleterious effects on the human body[25]. Moreover, there were evidences suggested the effect of low-dose fast neutrons on the blood components[26–28]. The objective of this current study was to determine the effects of *in vitro* exposure to radioactive cesium-137 on some human blood components.

Materials And Methods

Blood samples

Blood samples (n = 5 male and 5 female) were collected from normal blood test groups (age 40–65 years old) at the Associated Medical Sciences Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. Blood sample collections were performed under the approved guidelines set by the Institutional Committees on Research Involving Human Subjects and approval was obtained from the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (No. AMSEC-61EM-001).

Irradiation

Blood samples were given a radiation dose of 0.02, 0.05, 0.1, 0.2, and 0.3 mGy of gamma rays (at the dose rate of 0.048 mGy/hr) using a ^{137}Cs radioactive standard source located in the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. The blood samples that were exposed to 0 mGy served as sham-controls. The experimental design is shown in Fig. 1.

Determination of low-dose gamma rays effect on protein in plasma

The blood samples were performed with some modify from previously report [29].The blood samples were centrifuged at 2,500 rpm for 30 minutes. Next, the supernatant plasma was completely collected. Plasma samples (0.15 mL) were incubated with 0.15 mL of analytical grade acetone and were mixed thoroughly. The mixture was centrifuged at 2,500 rpm for 30 minutes. The clear supernatant of mixture was collected and kept at -20 ^{o}C before use in experiment. The luminescence spectrometer (Perkin-Elmer LS-55) was used to determine the autofluorescence spectrum of protein in plasma by using excitation and emission wavelength at 280 nm and 300–550 nm, respectively. Of note, peak of excitation fluorescence spectrum at 280 nm and of emission fluorescence spectrum at 340–350 nm are defined as the tryptophan molecule (Fig. 2A).

Determination of low-dose gamma rays effect on protein in red blood cells

The method to determine low-dose gamma rays effect on protein in red blood cells was similar to the method used for determining low-dose gamma rays effect on protein in plasma. Blood samples were centrifuged at 2,500 rpm for 30 minutes. The supernatant plasma was then completely removed. Red blood cells (0.15 mL) were incubated with 0.15 mL of analytical grade acetone and were thoroughly mixed. The mixture was then centrifuged at 2,500 rpm for 30 minutes. The clear supernatant of mixture was collected and kept at -20 ^{o}C before use in experiment. The luminescence spectrometer (Perkin-Elmer

LS-55) was used to determine the autofluorescence spectrum of protein in red blood cells by using excitation and emission wavelength at 280 nm and 300–550 nm, respectively (Fig. 2A).

Determination of low-dose gamma rays effect on hemolysis in red blood cells

Blood samples (0.3 mL) were centrifuged at 2,500 rpm for 30 minutes. The supernatant plasma was then completely discarded. Red blood cell elements were re-suspended in phosphate buffer saline followed by centrifuging at 2,500 rpm for 30 minutes. Next, the clear supernatant was collected and kept at -20 ^{o}C before use in experiment. The UV–vis spectrophotometer (Agilent 8453) was used to determine the release of hemoglobin from the red blood cells to the supernatant. The peak of absorption spectrum at 415 nm is defined as a hemoglobin molecule (Fig. 2B).

Statistical analysis

The authors expressed the results as mean ± standard error of the mean (S.E.). The student's t-test was used independently to evaluate statistical differences in the mean values between each irradiated group and the corresponding control group. A p-value of less than 0.05 was considered as statistically significant.

Results

Effect of low-dose gamma ray on protein in plasma

For male sample group (Fig. 3A): this data indicated no change in the ratio of fluorescence emission intensity at 340 nm of wavelength of plasma protein extract from irradiated whole blood compared to the corresponding non-irradiated whole blood (Student's t-test, p-values ranging from 0.10–0.69). The results suggested that these radiation doses did not have any hazardous effect on protein in plasma for *in vitro* irradiated whole blood. However, these ratios were increased statistically significantly in plasma protein for whole blood that had exposure to 0.2 and 0.3 mGy of gamma rays. For female sample group (Fig. 3B): In ways similar to male sample group, this data showed no change in the ratio of fluorescence emission intensity at 340 nm of wavelength of plasma protein extract from irradiated whole blood when compared to the corresponding non-irradiated whole blood (Student's t-test, p-values ranging from 0.15–0.77). The results suggested that these radiation doses did not have any hazardous effect on protein in plasma for *in vitro* irradiated whole blood.

Effect of low-dose gamma rays on protein in red blood cells

The results were similar to the effect on protein in plasma. For male sample group (Fig. 4A): this data indicated no change in the ratio of fluorescence emission intensity at 340 nm of wavelength of protein extract from irradiated red blood cells when compared to the corresponding non-irradiated red blood cells (Student's t-test, p-values ranging from 0.33–0.77). The results suggested that these radiation doses did

not have any harmful effect on protein of *in vitro* irradiated red blood cells. For female sample group (Fig. 4B): In ways similar to the male sample group, this data showed no change in the ratio of fluorescence emission intensity at 340 nm of wavelength of protein extract from irradiated red blood cells compared to the corresponding non-irradiated red blood cells (Student's t-test, p-values ranging from 0.34–0.67). The results suggested that these radiation doses did not have any harmful effect on protein of *in vitro* irradiated red blood cells.

Effect of low-dose gamma ray on hemolysis in red blood cells

The results were similar to the effect on protein in plasma and in red blood cells. For male sample group (Fig. 5A): the results did not change in the absorption intensity at 415 nm of wavelength of hemoglobin leakage from *in vitro* irradiated red blood cells when compared to the corresponding non-irradiated red blood cells (Student's t-test, p-values ranging from 0.13–0.84). The results suggested that these radiation doses did not have any deleterious effect on *in vitro* irradiated red blood cells. For female sample group (Fig. 5B): Again, in ways similar to male sample group, this data showed no change the absorption intensity at 415 nm of wavelength of hemoglobin leakage for *in vitro* irradiated red blood cells compared to the corresponding non-irradiated red blood cells (Student's t-test, p-values ranging from 0.05–0.91). The results suggested that these radiation doses did not have any deleterious effect on *in vitro* irradiated red blood cells.

Discussion

There are several effects that may be shown for proteins after exposure to radiation such as fragmentation, cross-linking, structural changes, and aggregation[30–33]. This current research, reveals that the results from protein in plasma of whole blood of male sample showed significantly increased fluorescence emission intensity at 340 nm of wavelength after whole blood exposure to 0.2 or 0.3 mGy of gamma rays, when compared to corresponding non-irradiated control (0 mGy), but not in 0.02, 0.05, or 0.1 mGy levels of exposure. These finding suggested that gamma rays (0.2 or 0.3 mGy) changed the fluorescence emission intensity of tryptophan molecule, resulting in a possible effect on the structure of tryptophan molecules following protein in plasma. Chinnathambi et al. 2015 reported that the absorbance of bovine serum albumin protein had increased in maximum wavelength at 278 nm after bovine serum albumin protein exposure to UVC radiation. The increment of absorbance was showed as a dose-dependent effect. The authors suggested that bovine serum albumin protein was an aggregation[34]. Although, some of our results agreed with the research that was conducted by Chinnathambi et al 2015[34], those results were a negative effect of radiation on protein. However, there were differences in the experimental design between current research and the study conducted by Chinnathambi et al 2015.[34]; i.e., the radiation types and protein sample used to study. The current research used gamma radiation and protein in plasma, while UV radiation and bovine serum albumin solution were used in the study conducted by Chinnathambi et al 2015[34]. In contrast, these current

results from protein in plasma of whole blood of female samples showed no change in fluorescence emission intensity at 340 nm of wavelength after whole blood exposure to gamma rays, when compared to corresponding non-irradiated control. It should be noted that there was a difference in radiation response between male and female samples. Similar to the results from protein in plasma of whole blood of female samples, the results from protein in red blood cells of male and female samples showed no change in fluorescence emission intensity at 340 nm of wavelength after red blood cell exposure to gamma rays, when compared to corresponding non-irradiated control. Of note, protein in plasma of whole blood sample in this current research might be albumin protein, because excitation wavelength of protein in plasma was at 280 nm which is similar to the absorption of albumin solution in the study conducted by Chinnathambi et al 2015[34].

The results from the hemolysis methods in this current research i.e.; determination of hemolysis in red blood cells for detecting the damage of cell membrane or membrane integrity of red blood cells after exposure to low doses of gamma rays. Results from radioactive ^{137}Cs indicated a non-significant change in the level of hemoglobin leakage from irradiated red blood cells to 0.02, 0.05, 0.1, 0.2, and 0.3 mGy, when compared to that of a corresponding non-irradiated control (0 mGy). Hemolysis results in this current research is in agreement with our previous publication[35]. Our previous publication reported the hemolysis and osmotic fragility in red blood cells after exposure to medical diagnostic X-rays at a total dose of 0.03, 0.05, and 0.1 mGy. This same previous publication showed that the low dose X-rays did not induce hemolysis and osmotic fragility in exposed red blood cells, when compared to the corresponding non-exposed red blood cells[35]. Of note, there was a difference in the radiation type used to study between this current research and the previous research conducted by Tungjai et al 2019[35]. Gamma rays were used in this current research in contrast to the X-rays that were used in previous research conducted by Tungjai et al 2019[35]. In addition, with respect to radiation doses (0.05 or 0.1 mGy), the results in the current research showed similar outcomes with our previously published data with X-rays[36]. The previously published results showed that the cell cycle, apoptotic cells, and mitochondrial membrane potential did not change in lymphocytes after an *in vitro* exposure to 0.03, 0.05, and 0.1 mGy of X-rays when compared to the non-exposed lymphocytes [36].

There are strengths in this current research. The radiation dose ranges are not possibly found in environmental contamination only but also maybe find in occupational radiation. The samples or cells used in this research were collected from humans. It was not a simulation model nor was results taken from cell lines. Therefore, the results in this current research should be strongly relevant to humans. Moreover, the sample was collected from both genders, i.e. male and female subjects. The results in this current research could reflect the factor that is involved in terms of the gender of subject. However, there is a particular limitation in this current research. The radiation sensitivity is dependent on age of subject. It has been suggested that adults are less sensitive to radiation than children[37, 38]. The subjects in this current research were adults. We suggest that future research should include children or adolescent subjects.

Conclusion

These current results suggested that there were no harmful effects of the low-dose gamma rays from radioactive ^{137}Cs on some blood components when human whole blood was exposed to gamma rays in an *in vitro* condition. This current data will help improve understanding of the fundamental radiation biology of low-dose gamma rays.

Declarations

Funding

This research was partially supported by Chiang Mai University.

Conflicts of interest/Competing interests

The authors declare no conflicts of interest/competing interests.

Ethics approval

Blood sample collections were performed under the approved guidelines set by the Institutional Committees on Research Involving Human Subjects and approval was obtained from the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (No. AMSEC-61EM-001).

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

BS., WV. and SW. ; sample collection, acquisition and analysis of data, SK.; acquisition, analysis, interpretation of data and provided critical discussions. MT.; study conception and design, acquisition, analysis, and interpretation of data, provided critical discussions, drafting the manuscript and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

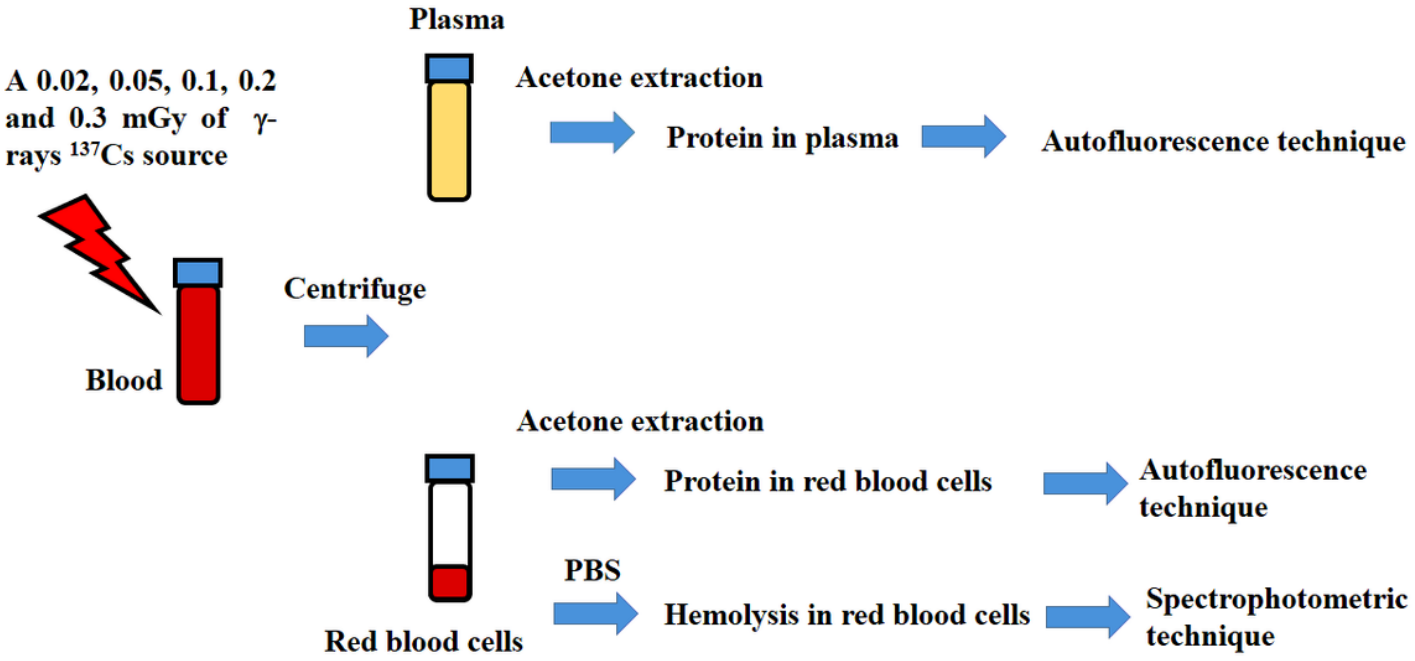

Figure 1

The experimental design

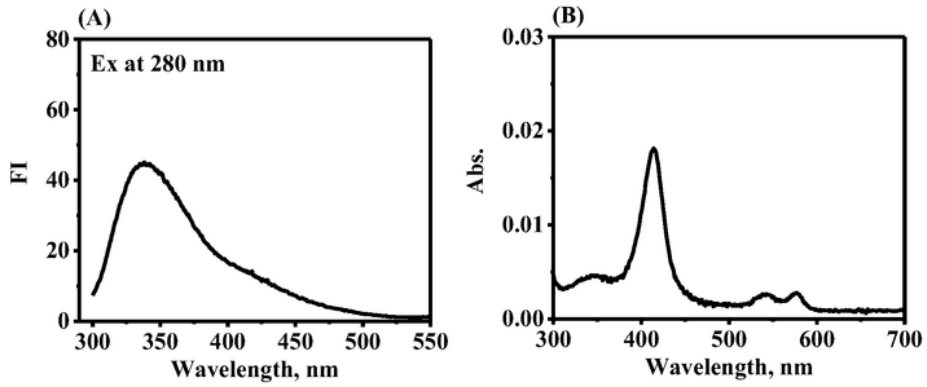

Figure 2

(A) Fluorescence emission intensity (FI), excitation wavelength (Ex) at 280 nm of protein extract from blood samples. (B) Absorption intensity (Abs) of hemoglobin leakage from blood samples.

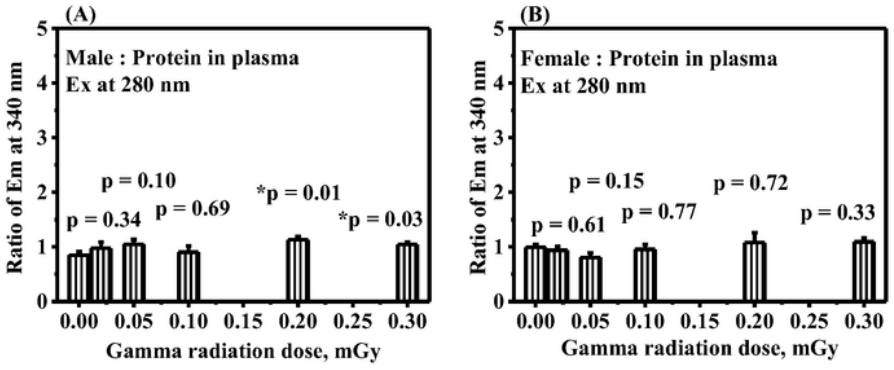

Figure 3

Ratio of Em (Emission fluorescence) at 340 nm (Excitation wavelength, Ex at 280 nm) of plasma protein extract from in vitro irradiated whole blood to the corresponding non-irradiated control groups. (A) Male and (B) Female.

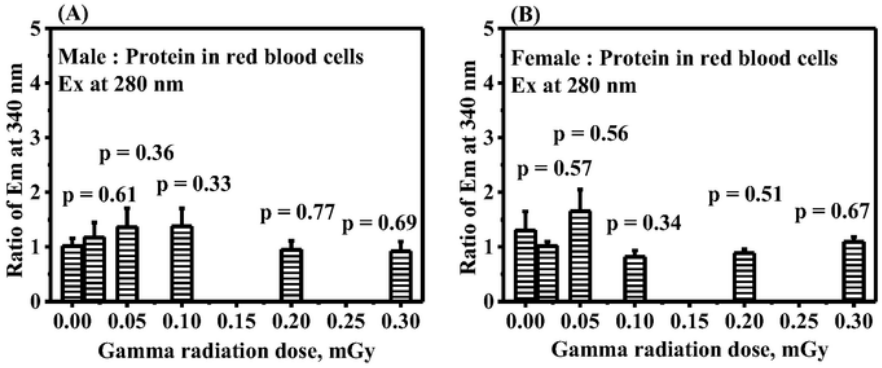

Figure 4

Ratio of Em (Emission fluorescence) at 340 nm (Excitation wavelength, Ex at 280 nm) of protein extract from in vitro irradiated red blood cells to the corresponding non-irradiated control groups. (A) Male and (B) Female.

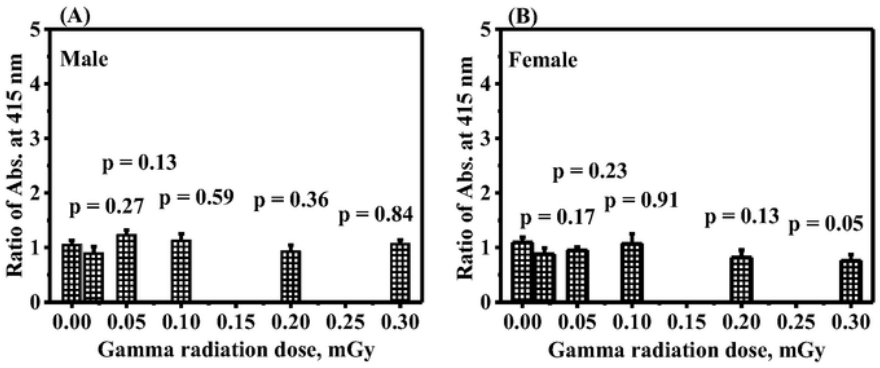

Figure 5

Absorption intensity (Abs) at 415 nm of wavelength of hemoglobin leakage from in vitro irradiated red blood cells and the corresponding non-irradiated control groups. (A) Male and (B) Female.