# Modulation of Ionizing Radiation-Induced Apoptosis by Taurine in Human Peripheral Blood Lymphocytes: Flow Cytometry-based Quantification

Shahab Faraji (MSc)[1], Mohsen Rajaeinejad (MD)[2], Hamed Bagheri (PhD)[1,2]*, Mohammad Afshar Ardalan (MD)[2], Hossein Moutabian (PhD)[2], Faramarz Ehsani (BSc)[2], Mohammad Pourarjmand (MSc)[2], Samira Sadat Mirshafieyan (MSc)[1], Farshid Alazamani (PhD)[2], Susan Cheraghi (PhD)[1]

[1]Radiation Biology Research Center, Iran University of Medical Science (IUMS), Tehran, Iran
[2]Radiation Sciences Research Center (ARSRC), Aja University of Medical Sciences, Tehran, Iran

## ABSTRACT

**Background:** Radiotherapy, a highly effective method of radiation-based treating cancers, can reduce the size of tumors and affect healthy tissues. Radiation-induced lymphopenia as a side effect of radiation therapy can reduce the effectiveness of the treatment.

**Objective:** This study aimed to examine how taurine can protect peripheral blood lymphocytes from radiation-based apoptosis.

**Material and Methods:** In this experimental study, the effects of the taurine on lymphocytes were studied, and blood samples were divided into three groups: a negative control group that was not treated, a positive control group that was treated with cysteine (100 μg/ml), and a group that was treated with taurine (100 μg. mL$^{-1}$) in three different doses (4, 8 & 12 Gy) before irradiation. The percentage of apoptotic and necrotic lymphocytes was measured using flow cytometry 48 and 72 hours after the irradiation, respectively.

**Results:** According to the groups treated with taurine, the number of lymphocytes undergoing apoptosis was lower and higher compared to the negative and positive control groups, respectively. The decrease in this value was more pronounced 48 hours after radiation compared to 72 hours. Furthermore, there was a slight increase in the number of apoptotic lymphocytes with increasing radiation dose.

**Conclusion:** Taurine effectively protects human peripheral blood lymphocytes from radiation-based apoptosis.

*Corresponding author:
Hamed Bagheri
Radiation Sciences Research Center (ARSRC), Aja University of Medical Sciences, Tehran, Iran
E-mail: hamed_parto@yahoo.com

## Introduction

High-energy photon beams can indirectly cause damage to living cells through various quantum phenomena, including the photoelectric effect, Compton scattering, and Auger effect. As the human body consists of over 60% water, the free radicals, produced by the breakdown of water molecules during radiation exposure, can

interact with vital cellular components, leading to significant disruption of cell function [1]. On the other hand, radiotherapy remains a powerful treatment option for tumors, with advantages over chemotherapy or surgery. However, radiotherapy can negatively impact neighboring healthy tissues, leading to the necessitation of dose limitation to minimize harm. Accordingly, the utilization of radiation sensitizers, mitigators, or protectors with biological properties can overcome this limitation and reduce the adverse effects of radiation on healthy tissues while maintaining therapeutic effectiveness against tumors [2, 3]. Lymphocytopenia, a common side effect of radiotherapy in cancer patients, can be misleading when evaluating the response to cancer treatment as an unfavorable prognostic marker [4]. Radiation has a range of biological effects on cells, and one of the most significant outcomes is cell death, resulting in the loss of reproductive ability. Additionally, highly differentiated cells like neurons and myocytes can experience a decrease in metabolic activity following radiation exposure. Radiation-induced cell death can occur through two primary mechanisms: (A) mitotic catastrophe, the most common mechanism in most cell types when exposed to radiation, and (B) apoptosis, in specific types of irradiated cells, such as hematopoietic and lymphoid cells. Apoptosis refers to programmed cell death, where cells undergo a controlled and regulated self-destruction process in response to various signals, including radiation-induced damage [5]. Lymphocytes are the most radiation-sensitive and rapidly decline in number due to apoptosis [6]. Many factors can intensify or attenuate the Ionizing Radiation (IR) effects on cell damage [1]. Radioprotective agents are administered before irradiation to minimize the harmful effects of radiation on cells and are also structurally diverse with different mechanisms to neutralize radiation-induced cell injuries [7]. Antioxidants, one of the most commonly reported agents, can be considered radioprotection due

to interrupting free radical chain reactions [8-11]. Taurine is a natural amino acid in many tissues with cytoprotection against oxidative stress by attenuating apoptosis [12]. Taurine's antioxidant effect [13-19] and its radioprotective activity have been investigated in several studies [20-23]. While taurine does not directly scavenge Reactive Oxygen Species (ROS), it exerts a significant inhibitory effect on ROS generation, effectively reducing their levels in biological systems [24]. In the present study, the influence of taurine is assessed on cell death induced by radiation in human peripheral blood lymphocytes with the Flow Cytometry (FCM) method for analysis.

In summary, Taurine is widely recognized as a potent radioprotective agent due to the reduction of radiation-induced apoptosis at a concentration of 100 μg/ml. Additionally, taurine demonstrates significant efficacy in scavenging ROS at physiological concentrations and also a protective effect on lymphocytes by regulating the production of pro-inflammatory cytokines. Moreover, it is important to note that taurine does not exhibit any detrimental effects on lymphocyte cells, highlighting its favorable safety profile.

# Material and Methods

### Chemical Reagents

In this experimental study, 2-aminoethanesulfonic acid (Taurine), L-cysteine, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, trypan blue dye, and Dimethyl Sulfoxide (DMSO) were obtained from Sigma-Aldrich or Merck. Roswell Park Memorial Institute medium (RPMI-1640), which contains Fetal Bovine Serum (FBS), phosphate-buffered saline (PBS), and penicillin-streptomycin, was prepared from Biowest (France). Ficoll-Hypaque solution and Red Blood Cell (RBC) lysis buffer (1X) were purchased from DNAbiotrch and Kiazist companies (Iran), respectively. The

staining kit, including Fluorescein Isothiocyanate (FITC) and annexin V/Propidium Iodide (PI), was provided by Hoffmann-La Roche Ltd. (Switzerland).

## Blood Sampling

Blood samples were collected from volunteers who provided informed consent. The samples were collected in heparinized tubes to prevent clotting. The volunteers were carefully selected and met specific criteria: they were non-smokers, non-alcoholic, not currently taking any medication or supplements, free from any known diseases, and had no history of recent exposure to IR within the last month. The age range of the volunteers was between 20 and 25 years old.

## Isolation of Peripheral Blood Mononuclear Cell (PBMC) from Whole Blood

Peripheral Blood Mononuclear Cells (PBMCs) were insulated by Ficoll-Hypaque gradient centrifugation—field [25], which Arne Boyum first described in 1968 [26]. The collected blood containing anticoagulant was diluted with equal PBS and layered carefully over Ficoll-Hypaque solution without intermixing. The upper layer was composed and discarded after centrifugation (3000 rpm with monitoring room temperature for 30 min). The interface layer was contained in a new centrifuge tube, washed twice with PBS, and centrifuged (1500 rpm, 10 min) once for further purification. The trypan blue dye exclusion test examined the viability of cell suspension, and a hemocytometer counted the number of viable cells.

## DPPH Radical Scavenging Assay

The DPPH free radical assay assessed the free radical-scavenging capacity of the Taurine & cysteine, following the method reported by Br &-Williams et al. [27]. Accordingly, the different concentrations of each substance (100, 200, 300, 400, and 500 µg. mL$^{-1}$) were

prepared in 100 µL DMSO. Then, 1900 µL of DPPH reagent was added to each sample. The mixtures were incubated at room temperature in darkness for 20 min. Then, all models were centrifuged (12,000 rpm, 2 min), and their related absorbance was measured at 517 nm by a spectrophotometer. The control samples included 100 µL DMSO and 1900 µL DPPH reagent. Cysteine was also used as a standard antioxidant agent [28].

The percentage of free Radical-Scavenging Activity (RSA) was calculated as follows:

$$RSA\% = [(\bar{A}_{control} - A_{sample})/\bar{A}_{control}] \times 100$$

where, $\bar{A}_{control}$ was the average of control absorbance, and A sample was the absorbance of the test sample.

## Cell Viability Assay

The MTT study investigated the effect of cytotoxicity of Taurine on lymphocytes [29]. PBMCs were seeded at 10,000 cells per well in 96-well plates. After 24 hours of incubation, different concentrations of Taurine (25, 50, 100, 200, 300, 400, 500, and 1000 mM) were added to the plates with eight replicates each. The plates were then incubated at 37 °C in 5% $CO_2$ for 24, 48, and 72 hours. After each incubation, the culture medium was removed, and the cells were washed twice with 1 mL of PBS. 100 µL of MTT stock solution (0.5 mg/mL, in PBS) was added to each well, and the plates were incubated for 4 hours under the previous conditions. The plates were centrifuged (3000 rpm for 5 min), and the supernatant was removed. Next, 100 µL of DMSO was added to each well, and the plates were gently agitated to dissolve the formed formazan crystals. Finally, the absorbance of the plates was measured at a wavelength of 570 nm. Taurin solvent was the only control substance. As per the following calculation, the percentage of relative cell survival was calculated:

Relative cell survival (%)=$(\bar{A}_{sample} - \bar{A}_{control}) \times 100$

where, $\bar{A}_{sample}$ was the average test optical density, and $\bar{A}_{control}$ was the control optical density at 570 nm.

The controls contained only Taurine solvent.

## Irradiation conditions

To investigate the cytoprotective effect of Taurine against IR, a medical linear accelerator (Elekta Compact model, Sweden) was used to irradiate both the control and treated blood samples, using 6 MV X-rays at a dose rate of 2 Gy/min. The Source-to-Surface Distance (SSD) was set at 100 cm, and the Monitor Unit (MU) was adjusted to deliver a dose of 100 cGy. The samples were irradiated at room temperature with doses of 4, 8, and 12 Gy.

## FCM is used to evaluate Apoptotic Cells using FITC-Annexin V & PI Staining

The blood samples were divided into different groups to investigate the radioprotective effect of Taurine on radiation-induced apoptosis in human peripheral blood lymphocytes, as follows:

**Group I.** treated with Taurine solvent without irradiation and incubated for 48 hours, **Group II.** treated with Taurine solvent, irradiated at 4 Gy and set for 48 hours, **Group III.** treated with Taurine (100 µg. mL$^{-1}$), irradiated at 4 Gy, and incubated for 48 hours, **Group IV.** treated with cysteine (100 µg. mL$^{-1}$), irradiated at 4 Gy, and incubated for 48 hours, **Group V.** treated with Taurine solvent, irradiated at 8 Gy, and set for 48 hours, **Group VI.** treated with Taurine (100 µg. mL$^{-1}$), irradiated at 8 Gy, and incubated for 48 hours, **Group VII.** treated with cysteine (100 µg. mL$^{-1}$), irradiated at 8 Gy, and incubated for 48 hours, **Group VIII.** treated with Taurine solvent, irradiated at 12 Gy, and set for 48 hours, **Group IX.** treated with Taurine (100 µg. mL$^{-1}$), irradiated at 12 Gy, and incubated for 48 hours, **Group X.** treated with cysteine (100 µg. mL$^{-1}$), irradiated at a dose of 12 Gy, and incubated for 48 hours, **Group XI.** treated with Taurine solvent and set for 72 hours without irradiation, **Group XII.** treated with Taurine solvent, irradiated at 4 Gy, and arranged for 72 hours, **Group XIII.** treated with Taurine (100 µg. mL$^{-1}$), irradiated at 4 Gy, and incubated for 72 hours, **Group XIV.** treated with cysteine (100 µg. mL$^{-1}$), irradiated at 4 Gy, and incubated for 72 hours, **Group XV.** treated with Taurine solvent, irradiated at 8 Gy, and set for 72 hours, **Group XVI.** treated with Taurine (100 µg. mL$^{-1}$), irradiated at 8 Gy, and incubated for 72 hours, **Group XVII.** treated with cysteine (100 µg. mL$^{-1}$), irradiated at 8 Gy, and incubated for 72 hours, **Group XVIII.** treated with Taurine solvent, irradiated at 12 Gy, and set for 72 hours, **Group XIX.** treated with Taurine (100 µg. mL$^{-1}$), irradiated at 12 Gy, and incubated for 72 hours, and **Group XX.** treated with cysteine (100 µg. mL$^{-1}$), irradiated at 12 Gy, and incubated for 72 hours.

In a 1.5 ml microtube, a total of 12 µl of freshly collected blood was mixed with RPMI-1640 medium supplemented with 10% FBS and 1-5% penicillin-streptomycin. The culture medium containing 100 µg of Taurine or cysteine was a positive control, and negative controls only had a complete cultural medium. All prepared samples were left in the 5% CO$_2$ incubator at 37 °C for 1 hour before irradiation. One of the control groups was not irradiated to determine the effect of IR on lymphocytes. The samples and controls were incubated for 48 and 72 hours after irradiation. Using RBC lysis buffer and centrifugation, erythrocytes were lit before FCM analysis. Then, the sediment cells are suspended in a 100 µL staining buffer. Annexin V-FITC and PI (5 and 10 µL) were added to each cell suspension. Finally, differential cell counting and viability analysis were performed by an FCM instrument. Three control groups were prepared for the flow cytometry analysis: unstained cells, Annexin V-only stained cells, and PI-only stained cells. Based on the analysis, the quadrants were identified as follows: 1) lower left quadrant, with positive cells for both Annexin V and PI staining, 2) lower right quadrant with positive cells for Annexin V staining but negative for PI staining; this quadrant represents

early-apoptotic lymphocytes, showing cells with externalized phosphatidylserine and intact cell membranes, 3) upper right quadrant, with positive cells for both Annexin V and PI staining with late-apoptotic lymphocytes, indicating cells with further progression in the apoptotic process and compromised cell membranes, and 4) upper left quadrant, with negative cells for Annexin V staining but positive for PI staining. Necrotic lymphocytes are typically in this quadrant, indicating cells with compromised membrane integrity.

Flow cytometry analysis can identify different cell populations, such as live, early-apoptotic, late-apoptotic, and necrotic lymphocytes, by analyzing the staining patterns and their location in the quadrants of Annexin V and PI. This technique can lead to the precise characterization of cell states based on their specific staining characteristics in flow cytometry analysis.

## Statistical Analysis

Unpaired two-tailed t-tests were conducted using Microsoft Excel software (version 19) to compare the means of two samples. A significance level of $P<0.005$ was used to determine statistical significance in this analysis.

# Results

## Evaluation of *In vitro* Antioxidant Activity

The change in the RSA percentage was plotted against concentrations 100-500 µg. mL$^{-1}$ for both Taurine and cysteine. The graphs indicated a linear relationship with a mild slope between the RSA percentage and the changes in concentration of both Taurine and cysteine (Figure 1). The best-fit linear equations and coefficients of determination resulted as follows:

RSA(%)=-0.02 C(µg. mL$^{-1}$)+23(R$^2$=0.9912)     Eq(1)
RSA(%)=0.04 C(µg. mL$^{-1}$)+62.5(R$^2$=0.9943)     Eq(2)

As shown in the diagrams, unlike cysteine, Taurine's DPPH radical scavenging capacity decreases with increasing concentration. Also, the values of the RSA percentage for taurine are higher than for cysteine at all concentration points.
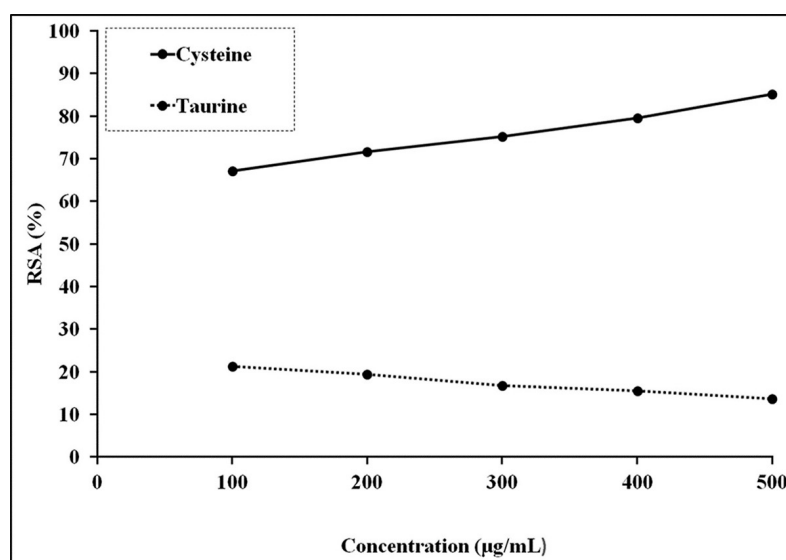


**Figure 1:** The scavenging effect of different concentrations of Taurine (...) & cysteine (---) on DPPH radical at 517 nm. The number of examined samples in each engagement is equal to 3. Dots have covered the standard deviations due to their small magnitude. (DPPH: 2-diphenyl-1-picrylhydrazyl, RSA: free radical-scavenging activity)

## Cytotoxicity-MTT Assay

Figure 2 presents the percentage of lymphocyte viability at different time points (24, 48, and 72 hours) following treatment with varying concentrations of Taurine, up to 1 M (mol). The findings indicate that increasing the concentration of Taurine beyond 100 µM can lead to decreasing lymphocyte viability of the experimental group at both 48 and 72 hours. However, viability statistically significantly reduced at concentrations of 1000 mM after 24 hours and above 300 mM between 48 and 72 hours. Nevertheless, it is worth noting that the viability of lymphocytes remains above 85%, indicating that Taurine exhibits low toxicity towards human blood lymphocytes.

## *Ex-vivo* Quantification of Apoptotic Cells Using FCM

Figures 3 and 4 display the percentages of apoptotic lymphocytes in non-irradiated samples at 48 and 72 hours post-irradiation, and the percentages of apoptotic lymphocytes are relatively low in the non-irradiated samples, with average values of 3.68% at 48 hours and 5.19% at 72 hours.

As the radiation dose escalates from 4 to 12 Gy, the percentages of apoptotic lymphocytes gradually increase in the irradiated samples at both time points. At 48 hours post-irradiation, the percentages of lymphocyte viability were 38.54%, 40.49%, and 44.09% for doses of 4, 8, and 12 Gy, respectively. Similarly, at 72 hours post-irradiation, the percentages were 40.61%, 42.91%, and 45.62% for doses of 4, 8, and 12 Gy, respectively.

Treatment of the samples with Taurine leads to a decrease in the percentage of apoptotic lymphocytes at both time points, which is more pronounced at 48 hours post-irradiation compared to 72 hours for all three doses. At 48 hours post-irradiation, the percentages are reduced to 13.15%, 15.96%, and 18.76% for doses of 4, 8, and 12 Gy, respectively. At 72 hours post-irradiation, the percentages are reduced to 22.93%, 25.87%, and 27.31% for doses of 4, 8, and 12 Gy, respectively. The decline in the percentages of apoptotic lymphocytes was also more significant for cysteine-treated samples than for Taurine -treated (6.69, 6.91, and 7.87 for 48 hours and 8.18, 9.31, and 12.24 for 72 hours post-irradiation
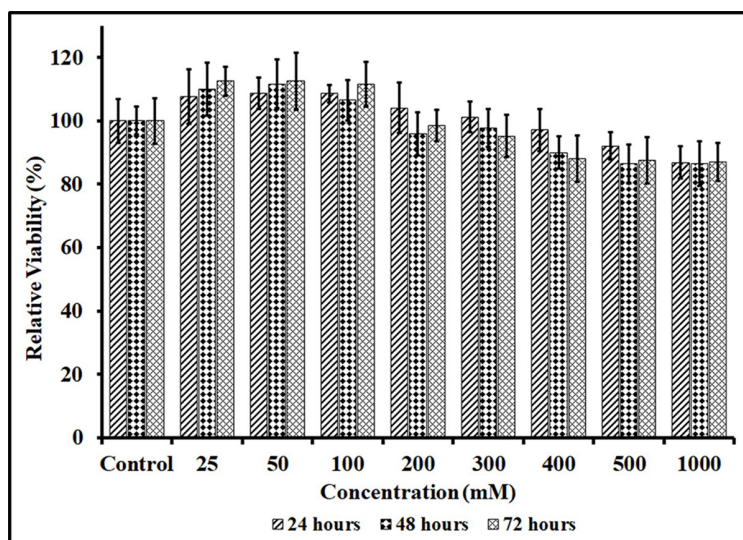


**Figure 2:** Different Taurine concentrations (25-1000 mM) affect lymphocyte viability after 24, 48, & 72 hours of incubation using MTT assay. Data are expressed as the means of eight replicate experiments with error bars. *$P \leq 0.005$ compared to controls (untreated groups) (MMT: 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide)

at 4, 8, and 12 Gy, respectively). The percentage of apoptotic lymphocytes as a function of radiation dose (both times in the presence and absence of Taurine and cysteine) has been separately plotted in Figure 4. The background values (non-irradiated and untreated samples) have been subtracted from the data shown in the graph Figures 5-7.

## Discussion

Developing radioprotective agents is an ongoing and important topic in radiation protection. Individuals can be exposed to IR in various circumstances, such as patients
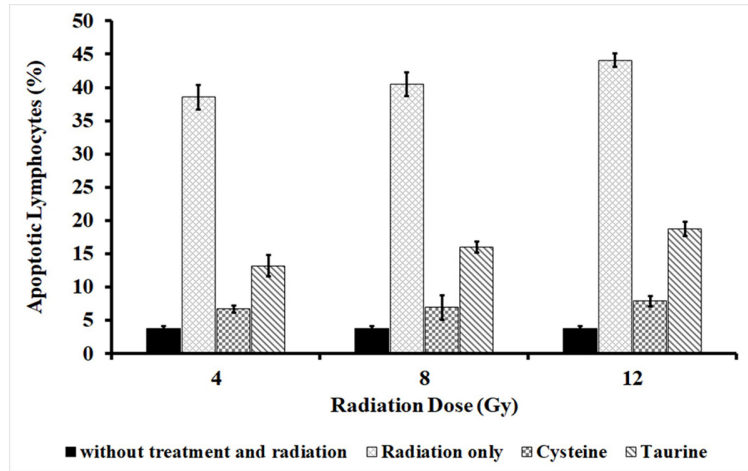


**Figure 3:** Effect of Taurine on radiation-induced apoptosis in lymphocytes & comparison with controls after 48 hours post-radiation. The percentages of apoptotic lymphocytes are depicted as a histogram with standard deviations at three doses of 4, 8, & 12 Gy for four sample groups: non-irradiated & untreated, untreated with irradiation (negative control), cysteine-treated with irradiation (100 µg. mL$^{-1}$; positive control), & Taurine -treated with irradiation (100 µg. mL$^{-1}$)
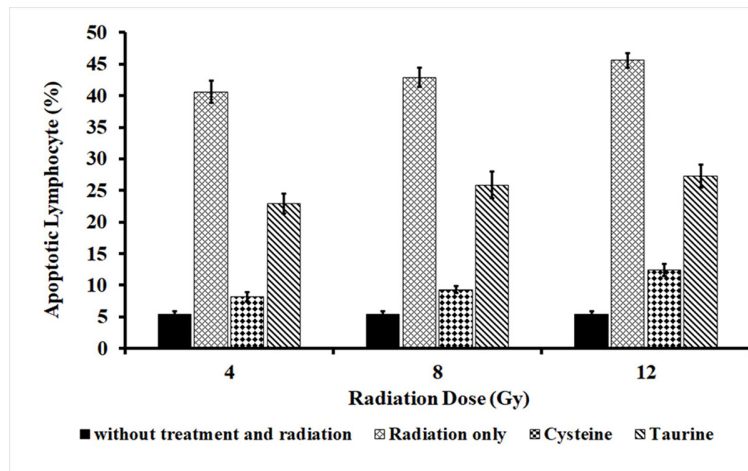


**Figure 4:** Effect of Taurine on radiation-induced apoptosis in lymphocytes & comparison with controls after 72 hours post-radiation. The percentages of apoptotic lymphocytes are depicted as a histogram with standard deviations at three doses of 4, 8, & 12 Gy for four sample groups: non-irradiated & untreated, untreated with irradiation (negative control), cysteine-treated with irradiation (100 µg. mL$^{-1}$; positive control), & Taurine -treated with irradiation (100 µg. mL$^{-1}$)

undergoing radiotherapy or nuclear imaging for diagnostic or therapeutic purposes, or those who receive high doses of IR in nuclear incidents [30]. The radioprotective agents are needed to be developed from the diverse situations, in which individuals may encounter IR, emphasizing the ongoing relevance and significance of this field in radiation protection [7]. Taurine is a natural compound with broad protective and therapeutic actions, which is suitable for human applications, unlike most synthetic radioprotectors, with high toxicity [7, 31]. The evaluation of Taurine's ability to scavenge DPPH free radicals, compared to cysteine, revealed that Taurine is not as potent as cysteine in terms of radical scavenging.
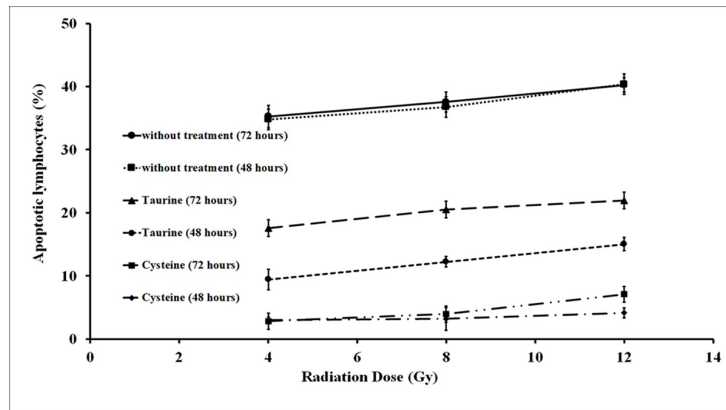


**Figure 5:** The percentage of apoptotic lymphocytes as a function of dose & comparison of the presence or absence of Taurine (100 µg. mL$^{-1}$) & cysteine (100 µg. mL$^{-1}$) on the frequency of apoptosis induced by various doses of X-rays after 48 & 72 hours post-irradiation. Data are presented as mean values with standard errors. Background values (non-irradiated & untreated samples) are subtracted from each point shown on the graph.



**Figure 6:** Effect of Taurine & cysteine on radiation-induced apoptosis in lymphocytes as cytograms at three doses of 4, 8, & 12 Gy after 48 hours post-irradiation. Non-irradiated & untreated (**A**), untreated with irradiation (**B, E & H**), Taurine-treated with irradiation (**C, F & I**), & cysteine-treated with irradiation (**D, G & J**).
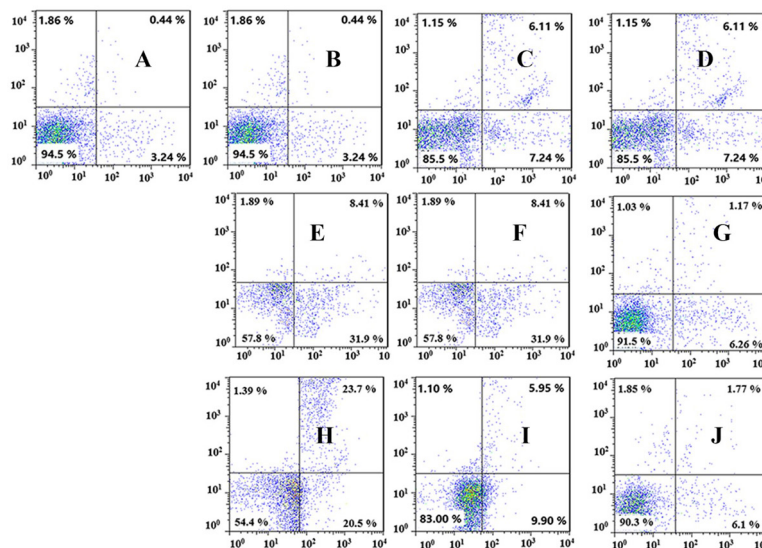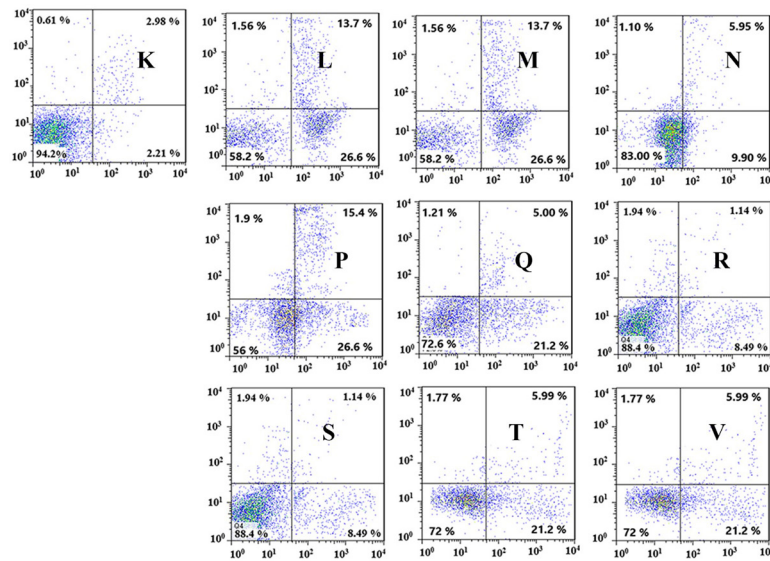
**Figure 7:** Effect of Taurine & cysteine on radiation-induced apoptosis in lymphocytes as cytograms at three doses of 4, 8, & 12 Gy after 72 hours post-irradiation. Non-irradiated & untreated (**K**), untreated with irradiation (**L, P & S**), Taurine-treated with irradiation (**M, Q & T**), & cysteine-treated with irradiation (**N, R & V**).

Cysteine, containing a thiol group, exhibited greater strength in this regard. However, the difference in scavenging ability between Taurine and cysteine was minimal at a concentration of 100 µg/mL, showing a weaker interaction between Taurine and DPPH radicals compared to cysteine and DPPH radicals due to the indirect antioxidant effect of Taurine within the concentration range of 0.8-4 mM. Nevertheless in vitro studies have demonstrated that Taurine effectively scavenges ROS at physiological concentrations [32]. The Taurine toxicity in lymphocytes was examined by MTT assay up to the attention of 1 M of Taurine at 24, 48, and 72 hours after incubation. No significant difference was observed between the control and Taurine-treated groups at a concentration of 100 µg/mL across all three time points, presenting that Taurine does not have a toxic effect on lymphocyte viability at this concentration. It is worth noting that the current study was conducted within the safe concentration range of Taurine in human plasma [33]. Various methods have been used to detect apoptosis based on morphological changes or dysfunction of different cell parts [34]. FCM is a rapid and dependable technique for counting and sorting cells or particles based on their specific physical and biochemical characteristics, leading to the quantitative analysis of different cell populations, for the distinction between viable, apoptotic, and necrotic cells [35]. FCM also provides valuable insights by quantitative measurements and the identification of specific cell populations based on their unique features [36]. Whole blood samples in three groups were irradiated at doses: 4, 8, and 12 Gy using a linear accelerator as an X-ray source. In the current study, the impact of Taurine on radiation-induced apoptosis was examined at 48 and 72 hours following irradiation. Apoptosis was not assessed within the initial 24 hours since a significant frequency of apoptotic events was not observed during that time frame. Consequently, the investigation's focus was shifted toward later time points to capture the potential effects of Taurine on radiation-induced apoptosis during those specific periods, resulting in a more thorough examination of the impact of Taurine on apoptosis over an

extended duration following irradiation [37]. Taurine could lead to a remarkable reduction of radiation-induced apoptosis at a concentration of 100 µg/mL, showing the radioprotective potency of Taurine that decreased with increasing radiation dose. The percentages of apoptosis were higher at 48 hours compared to 72 hours post-irradiation (71.96, 66.77, and 62.73% for 48 hours, and 50.69, 42.92, and 45.85% for 72 hours post-irradiation at 4, 8, & 12 Gy, respectively). The cysteine potency in radioprotection also was more than Taurine (92, 89.76, and 89.65% for 48 hours post-irradiation, and 90.3, 87.93, & 81.93% for 72 hours at 4, 8, and 12 Gy, respectively). The observed differences in results between Taurine and cysteine can be attributed to variations in their antioxidant mechanisms. Cysteine is considered a conventional antioxidant that has the ability to directly neutralize free radicals through its thiol group. In contrast, Taurine's action in combating free radicals is indirect and likely involves different approaches to counteract oxidants. The antioxidant activity of Taurine is believed to be mediated through various mechanisms, although the exact mechanisms are still under investigation. These proposed mechanisms include scavenging of ROS, modulation of intracellular antioxidant enzymes, and preservation of cellular redox balance. The diverse antioxidant mechanisms of Taurine contribute to its effectiveness in protecting against oxidative stress and its potential as a radioprotective agent [38]. The protective effect of Taurine on lymphocytes may be associated with its ability to regulate the production of pro-inflammatory cytokines by these cells [38].

Accordingly, Taurine, as a radioprotective agent, can reduce radiation-induced apoptosis at a concentration of 100 µg/ml with significant efficacy in scavenging ROS at physiological concentrations. Moreover, it protectively affects lymphocytes by regulating the production of pro-inflammatory cytokines without any detrimental impacts on lymphocyte cells.

## Conclusion

Taurine has shown a protective effect on healthy cells by reducing free radicals and cellular damage, as well as modulating radiation-induced apoptosis in human lymphocytes. Due to its potential to mitigate radiation-induced lymphopenia with lower toxicity, Taurine is considered a valuable candidate for pre-treatment in radiation therapy. Given its favorable safety profile, Taurine can be extensively used as a radioprotector, effectively minimizing the side effects associated with exposure to IR.

## Authors' Contribution

M. Rajaeinejad, H. Bagheri, M. Afshar Ardalan contributed to the conception & design. Sh. Faraji, M. Rajaeinejad, SS. Mirshafieyan: Contributed to all experimental work, data & statistical analysis, & interpretation of data. H. Bagheri: We are responsible for overall supervision. H. Moutabian, F. Ehsani, & M. Pourarjm drafted the manuscript, which F. Alazamani & S. Cheraghi revised. All authors read & approve the final manuscript.

## Ethical Approval

This research was approved by the AJA University of Medical Sciences (Ethic cod: IR.AJAUMS.REC.1397.077).

## Informed Consent

The research participants provided verbal consent to receive blood samples.

## Conflict of Interest

None

## References

1. Liu L, Liang Z, Ma S, Li L, Liu X. Radioprotective countermeasures for radiation injury (Review). *Mol Med Rep*. 2023;**27**(3):66. doi: 10.3892/mmr.2023.12953. PubMed PMID: 36799170. PubMed PMCID: PMC9926870.

2. El Naqa I, Johansson A, Owen D, Cuneo K, Cao Y, Matuszak M, et al. Modeling of Normal Tissue Complications Using Imaging and Biomarkers After Radiation Therapy for Hepatocellular Carcinoma.

*Int J Radiat Oncol Biol Phys.* 2018;**100**(2):335-43. doi: 10.1016/j.ijrobp.2017.10.005. PubMed PMID: 29353652. PubMed PMCID: PMC5779633.

3. Ziyadi S, Iddar A, Errafiy N, Ridaoui K, Kabine M, El Mzibri M, Moutaouakkil A. Protective Effect of Some Essential Oils Against Gamma-Radiation Damages in Tetrahymena pyriformis Exposed to Cobalt-60 Source. *Curr Microbiol.* 2022;**79**(9):279. doi: 10.1007/s00284-022-02924-3. PubMed PMID: 35920924.

4. Cheki M, Mihandoost E, Shirazi A, Mahmoudzadeh A. Prophylactic role of some plants and phytochemicals against radio-genotoxicity in human lymphocytes. *J Cancer Res Ther.* 2016;**12**(4):1234-42. doi: 10.4103/0973-1482.172131. PubMed PMID: 28169233.

5. Hall EJ, Giaccia AJ. Radiobiology for the Radiologist. 8th Edition. Philadelphia: Wolters Kluwer Health; 2019. p. 624.

6. Zheng L, Cao H, Qiu J, Chi C. Inhibitory Effect of FMRFamide on NO Production During Immune Defense in Sepiella japonica. *Front Immunol.* 2022;**13**:825634. doi: 10.3389/fimmu.2022.825634. PubMed PMID: 35572529. PubMed PMCID: PMC9095972.

7. Furukawa S, Nagamatsu A, Nenoi M, Fujimori A, Kakinuma S, Katsube T, et al. Space Radiation Biology for "Living in Space". *Biomed Res Int.* 2020;**2020**:4703286. doi: 10.1155/2020/4703286. PubMed PMID: 32337251. PubMed PMCID: PMC7168699.

8. Wang Q, Xie C, Xi S, Qian F, Peng X, Huang J, Tang F. Radioprotective Effect of Flavonoids on Ionizing Radiation-Induced Brain Damage. *Molecules.* 2020;**25**(23):5719. doi: 10.3390/molecules25235719. PubMed PMID: 33287417. PubMed PMCID: PMC7730479.

9. Laube M, Kniess T, Pietzsch J. Development of Antioxidant COX-2 Inhibitors as Radioprotective Agents for Radiation Therapy-A Hypothesis-Driven Review. *Antioxidants (Basel).* 2016;**5**(2):14. doi: 10.3390/antiox5020014. PubMed PMID: 27104573. PubMed PMCID: PMC4931535.

10. Nukala U, Thakkar S, Krager KJ, Breen PJ, Compadre CM, Aykin-Burns N. Antioxidant Tocols as Radiation Countermeasures (Challenges to be Addressed to Use Tocols as Radiation Countermeasures in Humans). *Antioxidants (Basel).* 2018;**7**(2):33. doi: 10.3390/antiox7020033. PubMed PMID: 29473853. PubMed PMCID: PMC5836023.

11. Szejk M, Kołodziejczyk-Czepas J, Żbikowska HM. Radioprotectors in radiotherapy - advances in the potential application of phytochemicals. *Postepy Hig Med Dosw (Online).* 2016;**70**:722-34. doi: 10.5604/17322693.1208039. PubMed PMID: 27356603.

12. Caine JJ, Geracioti TD. Taurine, energy drinks, and neuroendocrine effects. *Cleve Clin J Med.* 2016;**83**(12):895-904. doi: 10.3949/ccjm.83a.15050. PubMed PMID: 27938518.

13. Kim YS, Kim EK, Jeon NJ, Ryu BI, Hwang JW, Choi EJ, et al. Antioxidant Effect of Taurine-Rich Paroctopus dofleini Extracts Through Inhibiting ROS Production Against LPS-Induced Oxidative Stress In Vitro and In Vivo Model. *Adv Exp Med Biol.* 2017;**975**(Pt 2):1165-77. doi: 10.1007/978-94-024-1079-2_93. PubMed PMID: 28849531.

14. Baliou S, Adamaki M, Ioannou P, Pappa A, Panayiotidis MI, Spandidos DA, et al. Protective role of taurine against oxidative stress (Review). *Mol Med Rep.* 2021;**24**(2):605. doi: 10.3892/mmr.2021.12242. PubMed PMID: 34184084. PubMed PMCID: PMC8240184.

15. Lee CT, Yu LE, Wang JY. Nitroxide antioxidant as a potential strategy to attenuate the oxidative/nitrosative stress induced by hydrogen peroxide plus nitric oxide in cultured neurons. *Nitric Oxide.* 2016;**54**:38-50. doi: 10.1016/j.niox.2016.02.001. PubMed PMID: 26891889.

16. Haider S, Sajid I, Batool Z, Madiha S, Sadir S, Kamil N, et al. Supplementation of Taurine Insulates Against Oxidative Stress, Confers Neuroprotection and Attenuates Memory Impairment in Noise Stress Exposed Male Wistar Rats. *Neurochem Res.* 2020;**45**(11):2762-74. doi: 10.1007/s11064-020-03127-7. PubMed PMID: 32918662.

17. Zeng K, Yang N, Wang D, Li S, Ming J, Wang J, et al. Resveratrol Prevents Retinal Dysfunction by Regulating Glutamate Transporters, Glutamine Synthetase Expression and Activity in Diabetic Retina. *Neurochem Res.* 2016;**41**(5):1050-64. doi: 10.1007/s11064-015-1793-9. PubMed PMID: 26677078.

18. Jong CJ, Sandal P, Schaffer SW. The Role of Taurine in Mitochondria Health: More Than Just an Antioxidant. *Molecules.* 2021;**26**(16):4913. doi: 10.3390/molecules26164913. PubMed PMID: 34443494. PubMed PMCID: PMC8400259.

19. Li Y, Peng Q, Shang J, Dong W, Wu S, Guo X, et al. The role of taurine in male reproduction: Physiology, pathology and toxicology. *Front Endocrinol (Lausanne).* 2023;**14**:1017886. doi: 10.3389/fendo.2023.1017886. PubMed PMID: 36742382. PubMed PMCID: PMC9889556..

20. Gao Y, Li X, Gao J, Zhang Z, Feng Y, Nie

J, et al. Metabolomic Analysis of Radiation-Induced Lung Injury in Rats: The Potential Radioprotective Role of Taurine. *Dose Response.* 2019;**17**(4):1559325819883479. doi: 10.1177/1559325819883479. PubMed PMID: 31700502. PubMed PMCID: PMC6823985.

21. Yamashita T, Kato T, Isogai T, Gu Y, Ma N. Protective Effects of Taurine on the Radiation Exposure Induced Cellular Damages in the Mouse Intestine. *Adv Exp Med Biol.* 2019;**1155**:443-50. doi: 10.1007/978-981-13-8023-5_41. PubMed PMID: 31468421.

22. Yang W, Huang J, Xiao B, Liu Y, Zhu Y, Wang F, Sun S. Taurine Protects Mouse Spermatocytes from Ionizing Radiation-Induced Damage Through Activation of Nrf2/HO-1 Signaling. *Cell Physiol Biochem.* 2017;**44**(4):1629-39. doi: 10.1159/000485762. PubMed PMID: 29216642.

23. Christophersen OA. Radiation protection following nuclear power accidents: a survey of putative mechanisms involved in the radioprotective actions of taurine during and after radiation exposure. *Microb Ecol Health Dis.* 2012;**23**(1):14787. doi: 10.3402/mehd.v23i0.14787. PubMed PMID: 23990836. PubMed PMCID: PMC3747764.

24. Baliou S, Adamaki M, Ioannou P, Pappa A, Panayiotidis MI, Christodoulou I, et al. Ameliorative effect of taurine against diabetes and renal-associated disorders (Review). *Med Int (Lond).* 2021;**1**(2):3. doi: 10.3892/mi.2021.3. PubMed PMID: 36699147. PubMed PMCID: PMC9855276.

25. Kundaktepe BP, Sozer V, Papila C, Durmus S, Kocael PC, Simsek G, et al. Associations Between miRNAs and Two Different Cancers: Breast and Colon. *Cancer Manag Res.* 2020;**12**:871-9. doi: 10.2147/CMAR.S227628. PubMed PMID: 32104069. PubMed PMCID: PMC7012229.

26. Grievink HW, Luisman T, Kluft C, Moerland M, Malone KE. Comparison of Three Isolation Techniques for Human Peripheral Blood Mononuclear Cells: Cell Recovery and Viability, Population Composition, and Cell Functionality. *Biopreserv Biobank.* 2016;**14**(5):410-5. doi: 10.1089/bio.2015.0104. PubMed PMID: 27104742.

27. Hamid J, Ahmed D, Waheed A. Evaluation of antioxidative, antimicrobial and anti-diabetic potential of Adiantum venustum and identification of its phytochemicals through GC-MS. *Pak J Pharm Sci.* 2017;**30**(3):705-12. PubMed PMID: 28653913.

28. Kim JH, Jang HJ, Cho WY, Yeon SJ, Lee CH. In vitro antioxidant actions of sulfur-containing amino acids. *Arabian Journal of Chemistry.* 2020;**13**(1):1678-84. doi: 10.1016/j.arab-jc.2017.12.036.

29. Guastaferro M, Reverchon E, Baldino L. Polysaccharide-Based Aerogel Production for Biomedical Applications: A Comparative Review. *Materials (Basel).* 2021;**14**(7):1631. doi: 10.3390/ma14071631. PubMed PMID: 33810582. PubMed PMCID: PMC8037187.

30. Abdullah MAH, Rashid RSM, Amran M, Hejazii F, Azreen NM, Fediuk R, et al. Recent Trends in Advanced Radiation Shielding Concrete for Construction of Facilities: Materials and Properties. *Polymers (Basel).* 2022;**14**(14):2830. doi: 10.3390/polym14142830. PubMed PMID: 35890605. PubMed PMCID: PMC9316934.

31. Hwang D, Ramsey JD, Kabanov AV. Polymeric micelles for the delivery of poorly soluble drugs: From nanoformulation to clinical approval. *Adv Drug Deliv Rev.* 2020;**156**:80-118. doi: 10.1016/j.addr.2020.09.009. PubMed PMID: 32980449. PubMed PMCID: PMC8173698.

32. Duan H, Song W, Guo J, Yan W. Taurine: A source and application for the relief of visual fatigue. *Nutrients.* 2023;**15**(8):1843. doi: 10.3390/nu15081843.

33. Papet I, Rémond D, Dardevet D, Mosoni L, Polakof S, Peyron MA, Savary-Auzeloux I. Sulfur amino acids and skeletal muscle. In Nutrition and skeletal muscle. Elsevier; 2019. p. 335-63.

34. Banfalvi G. Methods to detect apoptotic cell death. *Apoptosis.* 2017;**22**(2):306-23. doi: 10.1007/s10495-016-1333-3. PubMed PMID: 28035493.

35. Sica V, Maiuri MC, Kroemer G, Galluzzi L. Detection of Apoptotic Versus Autophagic Cell Death by Flow Cytometry. *Methods Mol Biol.* 2016;**1419**:1-16. doi: 10.1007/978-1-4939-3581-9_1. PubMed PMID: 27108427.

36. Ward MD, Kaduchak G. Fundamentals of Acoustic Cytometry. *Curr Protoc Cytom.* 2018;**84**(1):e36. doi: 10.1002/cpcy.36. PubMed PMID: 30040220.

37. Nematollahi H, Haddadi Gh, Jorat MV. The Effect of Vitamin C on Apoptosis and Bax/Bcl-2 Proteins Ratio in Peripheral Blood Lymphocytes of Patients during Cardiac Interventional Procedures. *J Biomed Phys Eng.* 2020;**10**(4):421-32. doi: 10.31661/jbpe.v0i0.917. PubMed PMID: 32802790. PubMed PMCID: PMC7416102.

38. Prideaux M, Kitase Y, Kimble M, O'Connell TM, Bonewald LF. Taurine, an osteocyte metabolite, protects against oxidative stress-induced cell death and decreases inhibitors of the Wnt/β-catenin signaling pathway. *Bone.* 2020;**137**:115374. doi: 10.1016/j.bone.2020.115374. PubMed PMID: 32330695. PubMed PMCID: PMC7369146.