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Research Article

Effect of ZNO and Vitamin C Supplementation on IFN-a Serum Levels in X-ray-Irradiated Female Rats: A Dose-Response Study

Doaa Falah Razzaq [1*]

[1] *Department of Physics, College of Science, University of Kufa, Najaf, Iraq.*
[*] *Corresponding author: doaaf.aldabbag@uokufa.edu.iq*

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Abstract

Ionizing radiation, consisting of X-rays, interacts with the immune system in complicated ways, impacting numerous cells through a mechanism related to oxidative stress. The purpose of the current study was to examine the potential therapeutic impact of ZnO-nano and vitamin C supplementation on immunity-toxicity caused by X-ray irradiation in female rats. The experiment was conducted using five groups, each consisting of 6 female rats. Group 1: X-ray non-irradiated control female rats (G_1), while groups 2 and 3 received a single dose of 4 Gy (G_2, G_4) and 6 Gy (G_3, G_5) X-ray radiation using a linear accelerator. The animals in groups 4 and 5 (G_4, G_5) were treated with ZnO-nano and vitamin C supplementation at 0.01 mg/kg/day (5 mg) of ZnO-nano and 0.4 mg/kg/day (180 mg) of vitamin C for 14 consecutive days, starting the day after irradiation. The results revealed that exposing rats to X-ray irradiation caused a decrease in interferon-alpha (IFN-a) levels in rats exposed to 4 Gy (G_2) and 6 Gy (G_3) of radiation. It also shows that both zinc oxide nanomaterials (ZnO-nano) and Vitamin C supplementation can provide radioprotective effects, and they work synergistically. Vitamin c Supplementation has been proven to protect against radiation-induced harm, while ZnO-nano can reduce oxidative stress and inflammation related to radiation. Combining dietary ZnO-nano with Vitamin C supplementation should undoubtedly enhance these protective effects, such as regulating IFN-a, which is involved in the immune response radiation. It can be concluded that ZnO-nano and Vitamin C supplementation should undoubtedly enhance protective effects, such as regulating IFN-a, which is involved in the immune response to radiation by combating free radical generation and boosting antioxidant defenses mechanisms.

1. Introduction

Numerous functions of cytokines are especially pertinent to research on radiation. Proinflammatory cytokines are the main components of immediate early gene responses and can be rapidly activated following tissue irradiation. They both produce loose radicals, such as reactive oxygen and nitrogen species (ROS/RNS), which is how they converge with the effects of ionizing radiation. "Self" molecules secreted or released from cells after irradiation feed the identical paradigm by way of signaling for ROS and cytokine production. As a result, multilayered feedback manage circuits can be generated that perpetuate the radiation tissue damage reaction. The seasoned-inflammatory phase lasts until situations that are thought to be demanding on host integrity are eliminated. Antioxidant, anti-inflammatory cytokines then act to repair homeostasis [1].

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A glycoprotein called IFN-a is produced by peripheral blood B-lymphocytes. It contains antiviral and antiproliferative properties and, for this reason anti-tumor effect. It has modulating results on the immunological system [2, 3]. IFN is usually well tolerated and results in long-lasting complete cytogenetic responses as a treatment for hematological relapse following BMT [4].

Nanotechnology has been advanced in lots of areas over the past a long time, one of the maximum important regions of this technology is nanomaterial area, which performs a vital position in biomedical applications. Zinc oxide nanoparticles (ZnO-nano) are highly utilized materials in biomedical applications due to their ability to penetrate barriers and target cells effectively in various dis Zinc oxide nanoparticles (ZnO-nano) are highly utilized materials in biomedical applications due to their ability to penetrate barriers and target cells effectively in various diseases [5].

Vitamin C Supplementation is a water-soluble compound used as widely as possible to consist of beauty, pharm cortical, and agricultural fields because of its biological antioxidant properties [6].

Early biochemical modifications, which occur during radiation risk or shortly after, had been notion to be answerable for most of the results of ionizing radiation in mammalian cells. The present work was carried out and devoted for studying the Zinc oxide nanoparticles (ZnO-nano) are highly utilized materials in biomedical applications due to their ability to penetrate barriers and target cells effectively in various diseases. ZnO-nano's effectiveness in reversing irradiation-induced changes in IFN-a levels. Furthermore, ZnO nanoparticles were prepared at the nanoscale for oral administration to female rats [7].

2. Methods

2.1. Animals

Inbred of female rats (1–2 weeks old, one 150 –157 g) procured from the animal breeding colony of the Faculty of Science – University of Kufa, Najaf, Iraq were acclimatized beneath preferred laboratory conditions for 1 week prior to the experiment. Animals were housed in polycarbonic ate cages and maintained at room temperature below stand arid laboratory situations. Animals have been stored on everyday weight loss program and water advert libitum. This take a look at turned into completed after the approval of Institutional Ethics Committee (approval no. 17569).

2.2. Irradiation of female rats

Female rats have been housed in Perspex containers for the irradiation procedure. A total of 30 rats had been exposed to complete-frame irradiation using X-rays. Of these, 12 female rats obtained an excessive dose of four Gy, whilst another 12 female rats obtained a dose of 6 Gy. The tactics took place at Amal Alhayat Hospital in Najaf and Alamal National Hospital for Cancer Management in Baghdad, the use of a linear accelerator (LINAC). The 4 Gy organization's irradiation dose fee was three Gy/min, while the 6 Gy group's was five Gy/min. Each female rats become uncovered to irradiation for 1.2mins.

2.3. ZnO-nano and Vitamin C Supplementation Treatment

Sky Spring Nanotechnology in Texas, USA, supplied the ZnO nano (white nanopowder, product properties: D50 10-30 nm, SSA 20-30 m^2/g, product number: 8410DL, product properties: purity 99.8%). Vitamin C supplement (meals supplement with sweeteners, lemon-flavored, 180 mg per tablet) was changed to be purchased from Mer Avit; Merpharmagmbh Germany. An aggregate of five mg of ZnO-nano debris and one hundred eighty mg of Vitamin C supplementation was dissolved in deionized water at a volume of 1 L, and mixed using a magnetic stirrer (Hotplate Stirrer, Lab Tec, Italy) for 15 minutes at a stirring speed of 7 rpm. The rats have been administered this combination each day through oral gavage at doses of 0.01 mg/kg/day (5 mg) of ZnO-nano and 0.4 mg/kg/day (one hundred eighty mg) of Vitamin C Supplementation for 14 consecutive days. The doses of ZnO-nano and Vitamin C supplementation were administered to the irradiated rats 24 hours after irradiation and were below the median lethal dose ($LD50 > 5\times10^3$ mg/kg), with no signs or symptoms of toxicity observed [8].

2.4. Experimental Design

(1L) for 14 days. Group three (G_3, n All female rats were divided into five groups (n = 5). Group 1 (G_1, n = 6) served as the control group. Group 2 (G_2, n = 6) consisted of female rats that were irradiated with a high dose of 4 Gy for whole-body irradiation and received daily supplementation of ZnO-nano and Vitamin C. 6) Additionally, this group consisted of female rats that were irradiated with a single dose of four Gy and received the same daily doses of ZnO-nano and Vitamin C for 14 days. Group four (G_4, n = 6) included protected female rats that were irradiated with a high single dose of 6 Gy for whole-body irradiation. Finally, Group five (G_5, n = 6) was produced from female rats that had been irradiated with a single excessive dose of 6 Gy and received daily doses of ZnO-nano and Vitamin C supplementation for 14 days, as shown in Figure 1.

Figure 1 Show female rats that had been irradiated with a high unmarried dose stage of four Gy and 6 Gy for complete-body publicity the use of X-rays from a linear accelerator (LINAC).

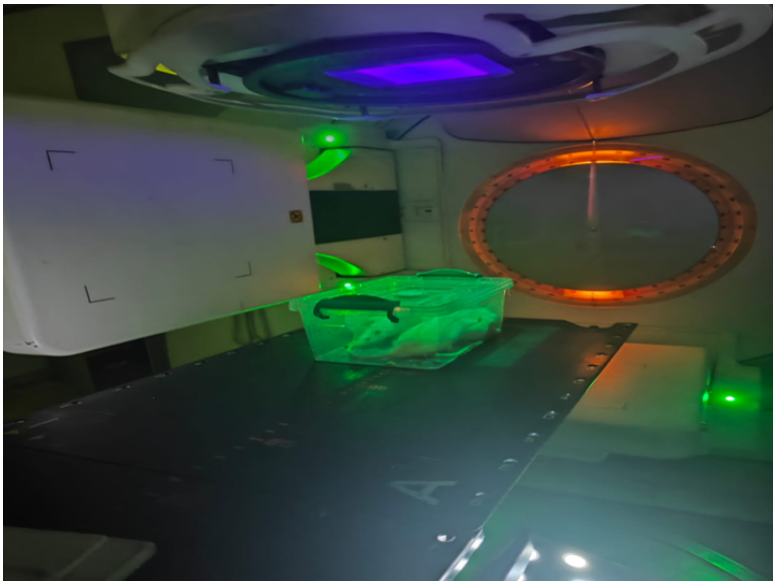

Figure 1: Female rats that had been irradiated with a high unmarried dose stage of four Gy and 6 Gy for complete-body publicity the use of X-rays from a linear accelerator (LINAC)

2.5. Preparation of Samples

All female rats in the experimental groups have been injected with ketamine and xylazine at doses of 90 mg/kg and 10 mg/kg, respectively, as recommended in the study by Kumar and Clover on the intraperitoneal co-management of low-dose urethane with xylazine and ketamine for prolonged surgical anesthesia in female rats [9].These marketers have been mixed with low-dose urethane to facilitate prolonged surgical anesthesia throughout the test. After collecting the blood, allow it to clot undisturbed at room temperature for about 15 minutes. Remove the clot using a centrifuge set at 2,000 rpm for 15 minutes. If any precipitates appear during storage, the sample must be centrifuged again.

Immunological Investigation of Interferon-alpha (INF-α): TNF-α levels in serum from female rats were measured using the Rat Interferon Alpha (INF-α) ELISA Kit from SunLong Biotech Co., LTD [10].

2.6. Statistical Analysis

Statistical comparisons of the data were performed using the SPSS software. The results were analyzed with ANOVA followed by post hoc comparisons using Tukey's multiple comparisons test. The statistical values are presented as mean $\pm$ SD. Adjusted P values were set at $P < .05$.

3. Results & Discussion

3.1. Results

Reduction in cytokine INF-alpha levels due to radiation exposure was observed in full-body female rats (G_2, G_3, and G_5) irradiated with X-ray at 4 Gy and 6 Gy, with significant decreases in INF-alpha levels in G_5 compared to controls Table 1 & 2. Oral supplementation with ZnO-nano and Vitamin C improved inflammatory factors considerably compared to the irradiated group, but their activity and levels still differed significantly from the control, as shown in Table 2 and Figure 2.

Presents the adjusted significant P value of INF-a levels in serum female rats set at (P= 0.0347$<$ .05*) for (G_4) (Dose 4 Gy + ZnO-NPs and Vit.C) compared to the control (G_1), and also shows that the adjusted P values are (ns) for INF-a levels in serum female rats groups (G_2, G_3, & G_5) compared to the control (G_1), as shown in Table 2 and Figure 2.

Table 1: Mean $\pm$ SD are shown for Control (G_1), Dose 4 Gy (G_2), Dose 6 Gy (G_3), Dose 4 Gy + Nano-ZnO and Vit-C (G_4), and Dose 6 Gy + Nano-ZnO and Vit-C (G_5)

Groups	Mean $\pm$ SD
Control (G_1)	7.79 $\pm$ 4.98
Dose 4 Gy (G_2)	6.33 $\pm$ 2.49
Dose 6 Gy (G_3)	3.66 $\pm$ 0.9
Dose 4 Gy + Nano Zn and Vit C (G_4)	2.85 $\pm$ 1.01
Dose 6 Gy + Nano Zn and Vit C (G_5)	5.05 $\pm$ 2.22

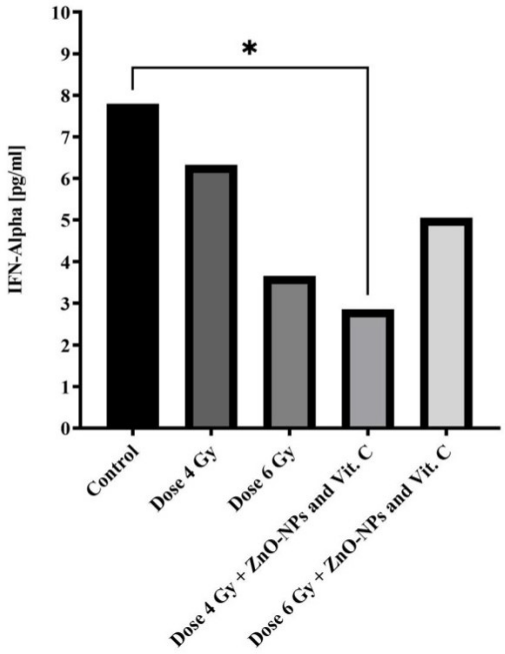

Figure 2: Shows the effect of ZnO-nano and Vitamin C supplementation on IFN-α levels in serum of female rats across various groups. Results are expressed as Mean $\pm$ SD (n = 5). P = 0.0347$<$ .05*for (G_4) (Dose 4 Gy + ZnO-NPs and Vit.C) compared to control (G_1). The adjusted P values are non-significant (ns) for INF-α levels in serum across groups (G_2, G_3, G_5) compared to control (G_1)

Table 2: Presents the adjusted P value of INF-α levels in serum of female rats set at (P $<$ .05*), for groups G_1, G_2, G_3, G_4, and G_5. The results show significance for the following comparisons: G_1 vs G$-$2 (ns), G_1 vs G_3 (ns), G_1 vs G_5 (ns), and a highly significant difference between G_1 and G_4 at (P $\leq$ .05)

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. Dose 4 Gy	ns	0.8851
Control vs. Dose 6 Gy	ns	0.1006
Control vs. Dose 4 Gy + ZnO-NPs and Vit. C	*	0.0347
Control vs. Dose 6 Gy + ZnO-NPs and Vit. C	ns	0.4382
Dose 4 Gy vs. Dose 6 Gy	ns	0.4151
Dose 4 Gy vs. Dose 4 Gy + ZnO-NPs and Vit. C	ns	0.1803
Dose 4 Gy vs. Dose 6 Gy + ZnO-NPs and Vit. C	ns	0.9150
Dose 6 Gy vs. Dose 4 Gy + ZnO-NPs and Vit. C	ns	0.9832
Dose 6 Gy vs. Dose 6 Gy + ZnO-NPs and Vit. C	ns	0.8843
Dose 4 Gy + ZnO-NPs and Vit. C vs. Dose 6 Gy + ZnO-NPs and Vit. C	ns	0.5990

3.2. Discussion

Radiation can affect a wide range of tissues, especially those with high rates of cell division, leading to infection and necrosis as key factors in the damage caused by high doses of radiation [11]. Several physiological processes may also cause cell damage under irradiation, but the formation of oxygen free radicals following lipid peroxidation is likely a major part of this cascade of events. The reactive oxygen species (ROS) interact with cellular molecules, including DNA, lipids, and proteins.

The current paper clarifies that exposure to X-rays can result in decreased interferon-alpha (IFN-α) levels in rats exposed to 4 Gy (G_2) and 6 Gy (G_3) of radiation, as well as changes in the physiological response volume to oral gavage of ZnO-nano and vitamin C supplementation in groups G_4 (4 Gy) and G_5 (6 Gy), probably due to radiation-induced immune system alterations. This decrease may be part of a broader spectrum of immune system changes, where high doses of ionizing radiation may lead to immunosuppressive outcomes. Ionizing radiation, including X-rays, interacts with the immune system in complex ways, impacting various cells and soluble factors [12].

IFN-α is a type of cytokine known for its role in modulating the immune system and inducing antiviral innate immune responses. It also plays a role in antitumor activity, as well as in inflammation that can disrupt the normal functioning of the immune system, potentially leading to a decrease in IFN-α production [13]. Low doses of ionizing radiation may cause subtle changes in immune function, while higher doses are more likely to be immunosuppressive [14].

The effect of mixed ZnO-nano and Vitamin C supplementation on interferon-alpha (IFN-a) levels in female rats exposed to X-ray radiation has not been immediately studied. However, this research shows that both ZnO-nano and Vitamin C supplementation can provide radioprotective effects, and they work synergistically. Vitamin C has been proven to protect against radiation-induced harm, while ZnO-nano can reduce oxidative stress and inflammation associated with radiation. Co-administration of dietary ZnO-nano and Vitamin C

supplementation should likely enhance these protective effects, such as the regulation of IFN-a, which is involved in the immune response to radiation [7].

4. Conclusion

From the results obtained, high-dose X-ray exposure can cause cell damage and infection, which could disrupt the regular functioning of the immune system, doubtlessly leading to a decrease in IFN-α production. Although there is no direct examination of diet ZnO-nano and Vitamin C supplementation on IFN-α levels after X-ray radiation, this paper suggests that their combined use may offer significant radioprotective benefits. This is likely due to the individual antioxidant and anti-inflammatory properties of both substances, and possibly through synergistic interactions that impact the immune response, including IFN-α levels.

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Ethical Approval: The study was conducted after obtaining approval from the Institutional Ethics Committee (Approval No. 17569).

Informed Consent: Not applicable. This study was conducted exclusively on experimental animals and did not involve human participants.

Conflict of Interest: The author declares no competing interests.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Clinical Trial Registration: Not applicable. This study was an experimental animal study and was not a clinical trial.

Reporting Guidelines Statement: ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines.

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc).

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