

## Oxidative stress Malondialdehyde (MDA) levels in serum after ZNO-Nano and Vitamin C Supplementation treatment in X-ray-Irradiated Female Rats

Received th 1 July 2026,  
Accepted th 15 July 2026

Cite this: NJBM. 2026, 2(3), 22-25

**Keywords:** MDA, Female rats,  
ZNO-Nano, Vitamin C, X-ray.

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### ABSTRACT

In this research, ZNO-Nano and Vitamin C Supplementation administered for female rats after exposure to X-ray radiation to evaluate their mitigating effects on specific biological parameters expressed by MDA level of female rat serum. Five groups of female rats were investigated: non-radiation control group G1, n = 6, animals received 4Gy does irradiation G2 n=6, animals received 6Gy does whole body irradiation G3 n=6, animals exposed to radiation of 4Gy and received orally daily dose of ZNO-Nano and Vitamin C Supplementation (1L) for two weeks G4 n=6, and animals exposed to radiation 6Gy and received orally daily dose of ZNO-Nano and Vitamin C Supplementation (1L) for two weeks G5 n=6. The entire body of each rat that was not a control was irradiated with 4Gy and 6Gy of radiation using a LINAC system using 6 MV X-rays and the levels of malondialdehyde (MDA), which is an indicator of levels of lipid peroxidation will be taken 24 hours post-irradiation of G2 and G3, and after 24 hours post-irradiation and treatment with ZNO-Nano and Vitamin C Supplementation which continued for the two weeks of G4 and G5. No obvious side effects were found in rats treated with ZNO-Nano and vitamin C supplementation orally in a single dose for two weeks. Based on the findings, ZnO-Nano and vitamin C supplementation can be used as therapeutic drugs were introduced to reduce oxidative stress, toxicity, and loss by X-ray during radiotherapy. There was a decrease in MDA levels after radiation exposure, However, MDA levels have not returned to normal.

## 1. INTRODUCTION

X-ray offers promising outcome in diagnosis and treatment tasks. Eighty percentage water is contained in the body habitat. Water and the water response is the most important effect of radiation. Water molecules that are "excited" and leave over loose particles as x-rays pass through them have many consequences. These include peroxidation of membrane lipids, inactivation of enzymes, dimerization of polysaccharides, and degradation of nucleic acids [1]. These products can cause irreversible damage to the cell membrane.

An antioxidant is any substance that counteracts oxidation or prevents the reaction of oxygen. Within the metabolic system, antioxidant systems defend the mobile membranes against injury by free radicals. Such antioxidants have enzymatic (glutathione peroxidase, superoxide dismutase, catalase) and non-enzymatic (vitamins, glutathione, ceruloplasmin) systems [3, 4].

Zinc oxide is one of the most significant metal oxide nanoparticles (NPs). One of the used NPs is preferred due to their superior biocompatibility, low cost, and coffee oxidation. Corporate products like cosmetics, coatings, rubbers, and paints are using increasing amounts of zinc oxide [5]. Zinc is also believed to help stabilize the structure of insulin. The ZNO-Nano, therefore, is developed for the treatment of diabetes.

Proven as an effective remedy. Moreover, ZNO-Nano exhibits high-quality photo luminescent properties, making it one of the best competitors in bio imaging [6]. Zinc is an essential trace element that is also incorporated into a variety of enzyme structures. It may be essential for nucleic, protein, and neurogenesis; the substances of the framework, including muscles, bones, pores, skin and brain are known to contain high levels of Zinc are supplemented in framework metabolism [7]. Herbal antioxidants containing vitamin C after exposure of X rays had a protective effect against chromosomal damage by reactive species generated by irradiation [9].

## 2. Materials and Methods

### 2.1. Animals.

We acquired female rats weighing between 150 to 157 grams and aged 8 weeks from the Faculty of Science in Kufa, Najaf, Iraq. Animals are left in the animal room for four weeks to acclimatize to conditions and maintained on a general pellet weight-reduction schedule and water lighting preferences minimum 6 animals were used per facility. The Laboratory Animal Care throughout the experiment was in line with the Research Ethics Committee (REC) for experimental study (human and animal subjects) at the University of Kufa (uokufa.edu.iq), Najaf, Iraq. The study's serial number is noted as 17566.

## 2.2. Irradiation

X-ray irradiation was performed using a linear accelerator using energy 6 MV (LINAC, Elekta Model Versa HD, and Canada) at Amal Alhayat Hospital in Najaf, Iraq. The irradiation dose rate was 3Gy/min for the 4Gy of 12 female rats and 5Gy/min for the 6Gy of 12 female rats. Female rats were exposed to 1.25 min of irradiation, as shown in Fig (1).

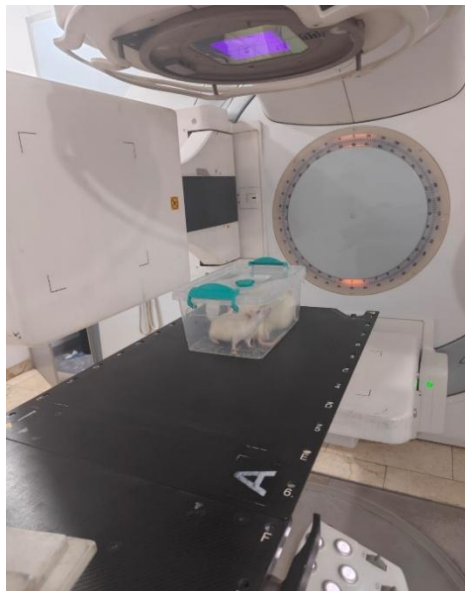


**Figure (1).** Show female rats that had been irradiated with dose of 4Gy and 6Gy for whole body irradiated the use of X-rays from a linear accelerator (LINAC).

## 2.3. Experimental Design

Group I: non-irradiated control group,  $n = 6$ . Group II: whole body irradiated group; animals received 4Gy irradiation  $G_2$   $n=6$ . The 3rd group: Whole body irradiated group; Animals received 6Gy whole body irradiation  $G_3$   $n=6$ .

Animals that underwent radiation exposure with 4Gy and received a daily oral dose of ZNO-Nano and Vitamin C Supplementation (1L) for two weeks. The fifth group comprised of animals that received an oral supplement of ZNO-Nano and Vitamin C together with exposure to radiation equal to 6Gy for two weeks  $G_5$   $n=6$ .

## 2.4. Animal treatment with ZNO-Nano and Vitamin C Supplementation

Female rats (6 rats per group  $G_4$  and  $G_5$ ) were administered for two weeks 0.01 mg/kg/day orally daily dose of ZNO-Nano Nano-powder; Product Properties: PurityEight99%, white nano-powder, D50 10–30 nm, SSA 20–30 m<sup>2</sup>/g, Product #: 8410DL) bought from Sky Spring Nanotechnology, Texas, USA) and 0.4 mg/kg/day of Vitamin C Supplementation purchased from Mer Avit; Merpharmagmbh, Germany. An aggregate of ZNO-Nano and Vitamin C Supplementation dissolved in deionized water of 1L, thatmixedusing a Magnetic Stirrer (Hotplate Stirrer, Lab Tec, and Italy) for 20mins at a stirring speed of 8rpm.

## 2.4. Preparation of Samples

Blood samples were collected after 24h exposure to 4Gy and 6Gy of X-ray irradiation for  $G_2$ ,  $G_3$  and after two weeks for  $G_4$ ,

$G_5$  by using a glass capillary tube. Before sampling, all female rats in the experimental corporations have been injected with ketamine and xylazine at doses of 90 mg/kg and 10 mg/kg, respectively, as recommended. The serum samples were stored in a freezer at -80°C [10,9].

## 2.5. Rat Malondialdehyde, MDA, ELISA Kit

MDAELISA Kit, Catalogue Number: SL0475Ra, Sun Long Biotech Co., LTD, China was utilized in this study.

## 2.6. Statistical Analysis

To investigate for differences, use the ANOVA test to see if there were statistically significant differences between the means of five groups. The results comparisons were made utilizing SPSS program followed by conducting hoc compare with Tukey's multiple compare test. The significances values are supplied as mean  $\pm$ SD. The P values were  $P < 0.05$ ,  $< 0.01$ .

## 3. Results

From our results, irradiated female rats with 4Gy ( $G_2$ ) and 6Gy ( $G_3$ ) had an effect on the oxidative strain in the serum, manifested by means of awesome lower ( $P \leq 0.01$ ) in the level of MDA, as compared with the corresponding values within the manage group ( $G_1$ ) (Tables 1 and 2, and Figure 2). Decrease MDA levels with ( $P \leq 0.05$ ) in female rat's serum of exposed to radiation 4Gy and received orally daily dose of ZNO-Nano and Vitamin C Supplementation ( $G_4$ ) In comparison with the corresponding values in the ( $G_1$ ) (Tables 1 and 2, Figure 2). As seen in (Tables 1 and 2, Fig2), comparing group ( $G_5$ ) and control group ( $G_1$ ) in term of MDA level, no significant difference was seen.

On the other hand, 0.01 mg/kg/day orally daily dose of ZNO-Nano and 0.4 mg/kg/day of Vitamin C Supplementation administration for two weeks after irradiation with 4Gy for female rats groups ( $G_4$ ), discovered a mild modulation advantageous in the level of oxidative stress denoted by a decreased ( $P \leq 0.05$ ) of MDA as compared with the corresponding values in the rats ( $G_1$ ) in (Tables 1 and 2, Fig 2). No effect was seen on the MDA level in rats serum with administration of dose of 0.01 mg/kg/day orally daily of ZNO-Nano and 0.4 mg/kg/day of Vitamin C Supplementation (no significant) for two weeks after irradiation with 6Gy for female rats groups ( $G_5$ ), as compared the comparing value in the female rats ( $G_1$ ) in (Tables 1 and 2, Fig 2).

**Table 1:** Mean values  $\pm$  SD are shown of MDA levels in female rat's serum for Control ( $G_1$ ), Dose 4Gy ( $G_2$ ), Dose 6Gy ( $G_3$ ), Dose 4Gy + Nano-ZnO and Vit-C( $G_4$ ), and Dose 6Gy + Nano-ZnO and Vit-C ( $G_5$ ).

| Groups    | Mean $\pm$ SD     |
|-----------|-------------------|
| <b>G1</b> | 27.77 $\pm$ 14.19 |
| <b>G2</b> | 9.69 $\pm$ 2.75   |
| <b>G3</b> | 8.86 $\pm$ 1.22   |
| <b>G4</b> | 15.30 $\pm$ 4.42  |
| <b>G5</b> | 18.37 $\pm$ 6.34  |

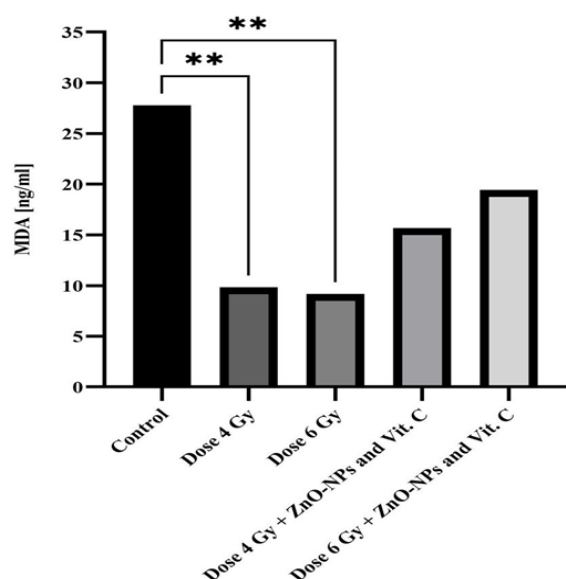


**Figure 2:** Effect of ZNO-Nano and Vitamin C Supplementation on MDA levels female rat's serum in different groups of rats. Results are expressed as mean  $\pm$  SD (n = 5). Statistical significance (highly significant) is indicated: G<sub>1</sub> vs. G<sub>2</sub> at P  $\leq$  .01, G<sub>1</sub> vs. G<sub>3</sub> at P  $\leq$  .01 and G<sub>1</sub> vs. G<sub>4</sub> at P  $\leq$  .05.

**Table 2:** presents the significance of MDA levels in female rat's serum set at P < .05\* and P < .01\*\*, for groups G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub>.

| Tukey's multiple comparisons test of MDA levels in female rat's serum | Summary | Adjusted Value | P |
|-----------------------------------------------------------------------|---------|----------------|---|
| G <sub>1</sub> vs. G <sub>2</sub>                                     | **      | 0.0020         |   |
| G <sub>1</sub> vs. G <sub>3</sub>                                     | **      | 0.0012         |   |
| G <sub>1</sub> vs. G <sub>4</sub>                                     | *       | 0.0470         |   |
| G <sub>1</sub> vs. G <sub>5</sub>                                     | ns      | 0.1984         |   |
| G <sub>2</sub> vs. G <sub>3</sub>                                     | ns      | 0.9996         |   |
| G <sub>2</sub> vs. G <sub>4</sub>                                     | ns      | 0.6316         |   |
| G <sub>2</sub> vs. G <sub>5</sub>                                     | ns      | 0.2246         |   |
| G <sub>3</sub> vs. G <sub>4</sub>                                     | ns      | 0.5057         |   |
| G <sub>3</sub> vs. G <sub>5</sub>                                     | ns      | 0.1560         |   |
| G <sub>4</sub> vs. G <sub>5</sub>                                     | ns      | 0.9374         |   |

## 4. Discussion

This study aims to investigate the possible ameliorating effect of ZNO-Nano and Vitamin C Supplementation on some biochemical markers expressed by the degree of MDA of female rat serum induced by X-ray radiation.

Radiation-induced overproduction of ROS and RNS can lead to oxidative modifications of cellular molecules [11].

Intra-cell Signalling Via ROS and RNS

ROS and RNS after publicity to radiation can start the intracellular signalling cascades that might the seasoned-inflammatory protein expressions. Inflammatory cells secrete

various reactive species in the area of burning thus causing amplified oxidative damage [12]. The results showed that MDA levels in female rats' serum of whole body irradiated group 4Gy and 6Gy in this study were decreases compared to control-group, and these results are consistent with what the Emy Khoironi et al., MDA levels decreased extensively from the third to the seventh day after X-ray radiation exposure. However, MDA levels have not return back to ordinary levels [13]. There is research that indicating increased MDA levels in serum of the rats after X-ray irradiation which are a result of oxidative pressure, where the radiation damage cells and cause increase in lipid peroxidation levels and leads to a tumor. The MDA is a marker for this harm, and its higher concentration suggests that the frame's herbal antioxidant defences are being beaten with the aid of the unfastened radicals produced by the radiation. This tumour may be dose-dependent, which means higher radiation doses result in greater enormous spikes in MDA stages [14-16].

A low MDA levels in irradiated animals often signifies that the protecting agent is running with the aid of mitigating the oxidative damage as a result of radiation, decrease over time indicates that the organism's own protection mechanisms are actively working to restore the redox stability and A large drop in MDA degrees demonstrates a reversal of the oxidative damage that could be a hallmark of radiation exposure[17, 18]. Vitamin C can mitigate the effects of radiation on rats with the aid of reducing malondialdehyde (MDA) levels, a marker of oxidative strain. After radiation exposure. This shows vitamin C facilitates by using scavenging unfastened radicals and enhancing the body's antioxidant defines systems[19][20].

The impact of ZNO-Nano on MDA tiers after radiation exposure is complicated and depends on the dose. Low doses of ZNO-Nano can act as radio protective retailers, mitigating the increase in MDA stages caused by radiation-brought on oxidative stress. However, excessive doses of ZNO-Nano can independently growth MDA levels, mainly while combined with radiation, indicating a dose-based and probably nephrotoxic or cardiovascular toxic impact[21, 22].

## 5. Conclusions

No obvious side effects found in rats suffers that were treated with ZNO-Nano and vitamin C supplementation were given orally with a single dose for two weeks.

ZNO-Nano's lethal dose for rat, 50% (LD50) is >5g/kgb.w. Simple to consume orally. As previously planned, the designation of the dosage is 0.01 mg/kg. All rats survived the test period without showing any abnormal pathology induced by the test substance. This paper was designed to evaluate the feasibility and in vivo activity of the extracts of ZnO-Nano and vitamin C supplementation as a functional dietary supplement to reduce X-ray irradiation related Oxidative damage and poisonous symptoms. According to the results, ZnO-Nano and vitamin C supplementation can reduce effects of oxidative stress, toxicity, and damage of X-ray radiation during radiotherapy, thus can be used as therapeutic drugs.

**CONFLICT OF INTEREST**

The authors claim no conflict of interest.

**ETHICAL APPROVAL.** Not applicable.

**FUNDING.** Not applicable.

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