Effect of Gamma Ray on Reactive Oxygen Species at Experimental Animals

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Abstract
The present study was designed to investigate the oxidative stress, which is due to the effect of low doses of gamma irradiation. Animals were divided into 6 groups, where the first group kept as control group, while 2nd, 3rd, 4th, 5th and 6th groups were exposed to gamma ray at 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy once weekly for one month respectively. Rats were subjected to gamma radiation and nitric oxides (NO), superoxide dismutase (SOD), malondialdehyde, alanine aminotransferase (ALT), aspartate amino transferase (AST) and white blood cells (WBCs) were measured. SOD recorded highly significant decrease with percent change -22.7%, -25.01%, -55.01% -55.4% and -56.38% respectively in groups which were exposed to 1.5, 2, 2.5, 3 and 3.5 Gy. MDA serum level have a significant increase in groups which were exposed to successive doses of 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively with percent change 25.7%, 41.8% 59.1% 65.6% 75.8% consequently as compared to control group. Nitric oxide serum level is markedly showed highly significant increase in rats exposed to successive dose of 1.5, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively with percent change 59.6%, 74.17%, 87.1%, 120.1% and 130.6% consequently as compared to control group. ALT and AST recorded highly significant increase in rats exposure to successive doses of 1.5, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively. On the other hand WBCs recorded highly significant decreased in rats exposed to successive dose of 1.5, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively. WBCs recorded percent change -26.11%, -32.3%, -47.8%, -50.1% and -53.6% respectively as compared to control group.

Keywords: Reactive oxygen species; Nitric oxides; Superoxide dismutase; Gamma ray; Malondialdehyde

Introduction
Exposure to ionizing radiation initiates a cascade of events including oxidative damage that leads to alteration of tissue physiological function [1]. Lipid peroxidation is considered to be a critical event of ionizing radiation effect. Most of the toxic effects of ionizing radiation are due to generation of reactive oxygen species (ROS) by radiolysis of water which triggers formation of several reactive intermediates [2].

Exposure to gamma irradiation induces significant alterations in both physiological and metabolic processes as well as disorders in organs functions and blood biochemical level [3]. Radiation produced highly reactive and dangerous molecular species called free radicals in cells and tissues, which have high energies and can break chemical bonds. Free radicals may be formed within cells as well as in the extra cellular medium and can interact with membrane lipids, nucleic acid, carbohydrate and protein [4]. Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction [5].

The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis [6]. ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy, experimentation, or even space flight [7]. Against oxidative stress, cells are equipped with several natural enzymatic and non-enzymatic antioxidant defenses [8]. The exposure to ionizing radiation leads to depletion of these endogenous antioxidants and ultimately to the development of systemic disease. Irradiation up to 4 Gy caused marked decrease in serum enzymes and its pineal biosynthesis 3 and 5 days after radiation exposure [9]. In addition, total antioxidant capacity of plasma was reduced in patients exposed to whole body irradiation for the purpose of reducing tumor growth. Consequently, the cellular antioxidant capacity is decreased and the organs become more susceptible to the deleterious effects of ROS [10].

Radiation damage, to a large extend is caused by the overproduction of ROS, including superoxide anion (O₂•⁻), hydroxyl radical (•OH), and hydrogen peroxide (H₂O₂), that decrease the levels of antioxidants, resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polysaturated fatty acids found in cellular membranes, and sulphydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur [11].

Oxidative stress is the inappropriate exposure to ROS and results from the imbalance between prooxidants and antioxidants leading to cell damage (damage of lipids, proteins, carbohydrates and nucleic acids) and tissue injury [12]. This imbalance may be due to an excess of prooxidant agents, a deficiency of antioxidant agents or both factors simultaneously. Radiation injury to living cells is, to large extent, due to oxidative stress. Evidence suggests that a cell’s oxidative state not only plays a role at the time of radiation exposure, but also has effects long after exposure. As the result of irradiation, cells can produce ROS for several minutes or even hours after being exposed. In addition to ROS production, cells are stimulated to increase their expression of antioxidants [11].

Aim of This Study
This study was designed to realize the hazardous effects of low successive doses during exposure for gamma irradiation. Many workers in medical and industrial and petroleum fields may be exposed...
Materials and Methods

Animals and experimental design

Sixty male albino rats weighing between 180-200 g were used in this study. All animals were maintained under standard laboratory conditions housed 5 per cage (60 cm x 30 cm x 20 cm). Rats were kept in cages under hygienic conditions, fed on standard rodents chow and were allowed free access to food and water at the National Centre for Radiation Research and Technology, Atomic Energy Authority (NCCRRT), Cairo, Egypt. Animals were divided into 6 groups, where the first group kept as control group, while 2nd, 3rd, 4th, 5th and 6th groups were exposed to gamma ray at 1.5, 2, 2.5, 3 and 3.5 Gy delivered at a dose rate of 0.65 Gy min⁻¹.

Irradiation facilities

Whole body gamma- irradiation was performed at the National Centre for Radiation Research and Technology, Atomic Energy Authority (NCCRRT), Cairo, Egypt, using caesium-137 in a Gamma cell-40 Irradiator (Atomic Energy of Canada Limited, Canada). Animal groups were irradiated at successive doses levels of 1.5, 2, 2.5 , 3 and 3.5 Gy delivered at a dose rate of 0.65 Gy min⁻¹.

Methods

Measurements of superoxide dismutase (SOD)

Measurement of the activity of superoxide dismutase (SOD) in the liver and skeletal muscle tissues was done based on the method of Minami and Yoshikawa [13]. The assay relies on the ability of the enzyme to inhibit the Phenazine Methosulphate (PMS) mediated reduction of Nitroblue Tetrazolium (NBT) dye. The increase in absorbance at 560nm due to the formation of reduced NBT was recorded in a spectrophotometer (laboratory of biochemical Theodor Research Institute Cairo, Egypt). The absorbance at 550 nm was measured in a microplate reader with a correction wavelength of 650 nm. The sensitivity of this assay is <0.5 mM.

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Measurements of malondialdehyde (MDA)

Malondialdehyde (MDA) determination was according to the method adopted by Draper and Hardley [14]. The method implies the measurement of MDA as one of the main products of lipid peroxidation by the thiobarbituric acid method. The principle of the method is based on the reaction of MDA with thiobarbituric acid (TBA) with the resulting pink colored tri-methyl complex with a maximum absorption at 530-532 nm. The samples were analyzed by spectrophotometer, (laboratory of biochemical Theodor Research Institute Cairo, Egypt) (Milton Roy spectronic 3000 ARRAY double beam spectrometer).

Measurement of nitric oxide

Nitric oxide in serum was measured by technique according to Nussler et al. [15]. A standardized colorimetric assay for nitrite (NO⁻²) was applied as an indirect index of NO production using the Greiss reagent. Briefly, 100 ml of sample was mixed with an equal volume of Greiss reagent (1% sulfanilamide, 0.1% naphthylene diamine dihydrochloride, 2.5% phosphoric acid) and incubated at room temperature for 10 min. Sodium nitrite (NO⁻³) (Sigma) was used as the standard in range of 0.156 mM – 20 mM concentration at laboratory of Biochemical Theodor Research Institute Cairo, Egypt. The absorbance at 540 nm was measured in an Eppendorf reader with a correction wavelength of 550 nm. The sensitivity of this assay is <0.5 mM.

Leucocytic count (WBCs)

The hemocytometer with Neubauer ruling was mounted with whole blood to which EDTA was added as an anticoagulant in the test tube. Whole blood was mixed with weak acid solution (2% acetic acid and 5% gentian violet to dilute the blood and hemolyze the red blood cells [16].

AST and ALT determination was according to the method adopted by Henry and Frankle [17].

Statistical Analysis

The results obtained in the present study were expressed as mean ± SEM. The statistical difference between various groups were analysed by the Student’s t-test and the significance was observed at *** Very highly significant P<0.001, **highly significant P<0.01, *Significant P<0.05

Results and Discussion

As shown in Table 1 the results obtained from rats exposed to four successive doses during one onth for whole body irradiation (1.5 Gy) are characterized by significant decrease (P<0.05) in blood SOD content in compared to control group. On the other hand the groups were exposed to 2, 2.5 , 3 and 3.5 Gy recorded highly significant decrease with percent change -25.01% , -55.01% , -55.4% respectively in compared to control group. In agreement with these results [18] and Mansour and Afez [19] recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides after whole body gamma-irradiation. The significant decrease (P<0.05) in the activity of SOD and CAT might also be attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation [20]. Our results are in accordance with those of Gutterige [21] who observed a significant decrease in SOD and catalase activity after exposure to irradiation due to the excess production of hydroxyl radicals. The most potent oxidant stimulates the lipid peroxidation process and other reactive oxygen species. SOD is an important endogenous antioxidant enzyme which acts as the first line defense system against ROS and converts the superoxide radicals to H₂O₂. Glutathione peroxidase present in the

![Table 1: Level of SOD in rats exposed to different doses of gamma ray.](image-url)
cytoplasm of the cells removes H₂O₂ by coupling its reduction to H₂O with oxidation of GSH. Glutathione reductase regenerates GSH from oxidized glutathione in the presence of NADPH. GSH is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular non protein thiol compound [21].

The data presented in Table 2 show that MDA serum level have a significant increase in groups of rats exposed to successive dose of 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively showed percent change 25.7%, 41.8%, 59.1%, 65.6%, 75.8% consequently as compared to control group. MDA elevation take place as a result of histopathological changes in the liver included dilatation of blood vessels congestion in the lobules, enlargement of portal areas, and infiltration of mixed inflammatory cells around the necrotic hepatocytes and the portal area. These results was found to be in agreement with that observed by Burlakova [22] who reported that exposure to γ-irradiation induced liver lesion was associated with massive elevation in liver MDA level. Also the MDA elevation has been well accepted as a reliable marker of lipid peroxidation. Exposure to γ-irradiation decreased the activities of these antioxidant enzymes in the tissues, indicative of oxidative stress in the liver. The decline in these enzymes in the present study could be explained by the fact that excess superoxide radicals may inactivate H₂O₂ scavengers, thus resulting in inactivation of superoxide dismutase [23]. From the previous reports of Tyurina et al. [24] the increase in MDA could be explained on the basis that ionizing radiation induces lipid peroxidation through the radiolysis of water in the aqueous media of the cells which leads to production of hydroxyl radicals (•OH). Hydroxyl radicals interact with the polyunsaturated fatty acids in the lipid portion of biological membranes initiating the lipid peroxidation and finally damage the cell membranes [25]. Also, the exposure to γ-radiation produces a decrease of membrane fluidity of the erythrocytes, membranes which was suggested to be resulted from lipid peroxidation of polyunsaturated fatty acids in such membranes induced by g-irradiation [26]. Some studies have reported that irradiation increases MDA formation as an end product of lipid peroxidation.

The data presented in Table 3 show that nitric oxide serum level is markedly highly significant increase in rats exposure to successive dose of 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively with percent change 59.69%, 74.17%, 87.1%, 120.1% and 130.6% consequently as compared to control group. In the present study, irradiation of rats induced lipid peroxidation significantly and protein oxidation and increased NO levels and reduced antioxidant defense indicating increased oxidative stress. The increased levels of TBARS (an index of lipid peroxidation) in NO of gamma-irradiated rats, may be due to the attack of free radical on cell membrane phospholipids and circulating lipids and, thus, TBARS acts as a sensitive biomarker for oxidative stress that occurs as part of the pathogenesis of various diseases [27,28]. These results are in agreement with Ou et al. [29], which postulated that free radicals caused lipid peroxidation in the irradiated tissue. Moreover, Ibuki et al. [30] examined a wide variety effects for ionizing radiation at doses 3 Gy and 4 Gy in nitric oxide , which indicated an increased in NO in blood level at dose 3Gy and 4Gy. Radiation induced injury on peripheral blood and NO concentration. The increase of NO production in irradiated macrophages contributed to tumoricidal activity, with the activation mechanisms differing between high-dose and low-dose irradiation. Our results also in agreement with Ohata et al. [31] who investigated the role of nitric oxide in relation to radiation damage, by examining changes in mouse serum nitrate concentrations after irradiation. They reported that post-irradiation serum nitrate concentrations increased dose-dependently with radiation dose, and, claim the known physiological functions of nitric oxide imply that it should prevent radiation-induced death. Consistent with previous studies, our results in agreements with that observed by Ibuki et al. [32] which have shown NO in blood and hepatic tissue for gamma -irradiated groups, the NO level exhibited a significant (p<0.001) increase as compared with control. The increased production of NO in the irradiated group is due to induction of NO synthase enzyme, which is considered to be absent under physiological conditions and induced by radiation. Superoxide anion (O₂⁻) can attack NO to form peroxynitrite (ONOO⁻) anion, which may oxidize many biological molecules including sulphydryls (such as glutathione), iron sulfur centers and lipids [33].

As shown in Table 4 ALT and AST highly significant increase in rats exposure to successive dose of 1.5, Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively. The ALT recorded highly significant increase with
percent change 87.9%, 111.1%, 131.2%, 150.5% and 158.3% respectively as compared to control group. On the other hand AST recorded 27.1%, 44.68%, 65.6%, 75.5%, 75.5% and 90.1% in groups which exposed to whole body gamma irradiation at dose level of 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively as compared to control group. The oxidative stress due to free radical formation was greatly augmented during ionizing radiation exposure [34]. It was likely that animal particular antioxidants generally decrease the level of oxidation in such systems by transferring hydrogen atoms to the free radical structure [35]. Gamma irradiation showed an increase in the level of serum AST, ALT and ALP activities indicative to the toxicity induced by radiation exposure (Table 4). These results are in agreement with those previously reported by Fiener et al. [36]. The increase in serum levels of these enzymes may be due to alteration in the dynamic permeability of membranes by ionizing radiation, allowing leakage of biological active material out of the injured cell, which may be associated with cell death or injuries [37]. The peroxidative products affect the cell membrane to become leaky with the consequent release of these enzymes into the serum [38]. Probably, a chemical component in the hawthorn is stabilizing the integrity of the cell membrane, keeping the membrane intact and the enzymes enclosed [39]. Several enzymes of blood are considered as indicators of hepatic dysfunction and damage, and the leakage of hepatic enzymes such as AST, ALT and ALP into blood are routinely used as a reliable biochemical index for hepatocellular damage. It was also found that hepatocellular damage exhibited good correlation with the enzyme leakage to bloodstream [40].

As shown in Table 5 WBCs highly significant decreased in rat’s exposure to successive dose of 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy and 3.5 Gy respectively. WBCs recorded percent change -26.11%, -32.3%, -47.8%, -50.1% and -53.6% respectively in compared to control group. These results confirmed with results of Gridley [41] who observed considerable decrease in the hematological values (WBCs, RBCs, HGB, HCT, PLT) after exposure to 5 Gy of γ-radiation as compared to the control values. Whole-body gamma-irradiation was found to induce the oxidative stress response biology. Cancer Metastasis Rev 23: 311-322.

Conclusion

This study showed that SOD and WBCs significantly decreased after expose to low doses of gamma radiation 1.5 Gy, 2 Gy, 2.5 Gy, 3 Gy, and 3.5 Gy as compared with the control. On the other hand MDA, NO, AST and ALT significantly increased as compared to control group. This result was attributed to the presence of MDA and SOD which are two important compounds in charge of the antioxidant system balance. The increased MDA, NO and decreased SOD levels can be found in injury caused by irradiation. At the same time-point in the irradiated animals, the marked and decrease in SOD activity and rise in MDA and NO content were found in comparison with the controls. In this study we are targeted the low doses of ionizing radiation, and determined which the biochemical parameters have reclaimed to routine biochemical investigation for radiation workers and patients have radiotherapy. Also regarding to radiation safety protection and mitigation of radiation hazards, we should be update the biochemical panels regarding to highly specific sensitive parameters to radiation injury. On the other hand the process of determination for role of SOD and MDA and NO during radiation injury needs to many other physiological and biochemical parameters to make completion evaluation for cell biochemistry and cell reaction with ROS after exposed to low doses of ionizing radiation. This process we can make it as considered of predicting for cells status for long time.

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Table 5: Level of WBCs in rats exposed to different doses of gamma ray.

| Parameters | Control | 1.5 Gy | 2 Gy | 2.5 Gy | 3 Gy | 3.5 Gy |
|------------|---------|--------|------|--------|------|--------|
| WBCs (10³/ul) | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE |
|            | 8.31 ± 0.12 | 6.14 ± 0.11 | 5.62 ± 0.13 | 4.33 ± 0.12 | 4.15 ± 0.09 | 3.85 ± 0.08 |

Table 5: Level of WBCs in rats exposed to different doses of gamma ray.
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