Original article

Induction of Radio-resistance (Adaptive Response) in Mice using Low Doses of Gamma Rays

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Abstract

Background: The present study has been conducted in the Laboratory of Secondary Standard Calibration, Suba, Khartoum, Sudan, in collaboration with the Faculty of Science and Technology, Al-Neelain University, Khartoum, Sudan, to evaluate the adaptive response and protective effects induced on biological systems due to their exposure to small gamma-radiation doses.
Method: Mice were categorized into 5 groups according to the amount of irradiation they have received, each group has been received specific small priming gamma radiation dose for one month with interval of 3 days followed by a single challenging dose; those groups were as follows; control, 5 mGy+7 Gy, 6 mGy+7 Gy, 7 mGy+7 Gy, and 7 Gy. A day after the last dose (challenging dose), blood samples were collected from all of the groups and were analyzed, for the effects of these irradiation treatments, using a machine called Complete Blood Count (CBC).
Results: The analysis has shown that mice received the smallest priming dose of gamma-radiation (5 mGy) were not able to develop radio-resistance against the challenging dose (7 Gy) similar to those mice which have not received priming doses at all. When compared to the other groups, which exposed to higher priming doses (6 mGy and 7 mGy), we have noticed some resistance has been developed, and to some extent, it was dose dependent. The survival curve has also shown clearly that there is a significant dose-dependent adaptive response when compared the different group members for the number and time of death.

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Introduction

After it has been discovered in beginning of the 15th century by ancient Egypt and Rome, ionizing radiation was involved in the process of treating cancer. One of the most important concepts to do so is the adaptive response, in which the cell receives direct exposure from low "priming" dose of ionizing radiation prior to being exposed to much higher dose ionizing radiation. Due to this hypothesis, the radio adaptive response may be induced in irradiated cells that occur in presence of transmissible factor (s) in their growth medium (Iyer *et al*., 2002). Several studies suggested the linear no threshold hypothesis, in which, some adaptive responses can be observed when exposing cells to low priming doses prior to their exposure to much higher challenging dose. This also may induce bystander signaling pathways. However, the interactions between two processes appear to be pertinent. Nevertheless, the response of low dose radiation is simply a stress mechanism of biological systems that might induce modulation of expression gene (Han *et al*., 2009).

Ionizing radiation can be broadly categorized into electromagnetic or particulate radiations (Lehnert, 2007). X-

and γ-rays are two forms of electromagnetic radiation, which are commonly employed in medical and biological applications. Particulate Radiations are another type of ionizing radiations that encountered in our nature, and used experimentally or clinically are particles such as electrons, protons, γ-particles, heavy charged ions and neutrons (Lehnert, 2007).

The absorption of photon depends on the photon energy, as well as the chemical composition of absorbing material. Therefore, high energy photon is used in radiotherapy; this energy deposition by Compton Effect, part of the energy is given to electrons as a kinetic energy and the remaining energy of photon would be used to deflect from original path, however, low energy of photon is used in diagnostic radiobiology. The process occurs due to both Compton Effect and photoelectric effect. In photoelectric effect the photon gives all energy to an abound electron; part of this energy is used to overcome the binding energy of electron while remaining energy is given to electron as a kinetic energy (Han *et al*., 2009).

Ionizing radiation directly absorbed in biological material has damaging effect due to either direct action or indirect action (Martin *et al*., 2013). In the direct action the high LET radiation interacts with biological material. The target itself may be ionized or excited through coulomb interactions, leading that to the chain of physical and chemical events to produce biological damage (Martin *et al*., 2013).

Low LET of radiation causes indirect action due to the interactions of radiation with water produced water ion (H_2O+) and hydroxyl radical ($O\dot{H}$), these free radicals can break chemical bonds and produce chemical changes that lead to biological damage. Concluding from that the photon interactions produce high energy electron, it moves through tissue to produce free radicals in water, which may produce changes in DNA. These changes could be resulted in major biological effects (Martin *et al*., 2013).

The response of total body to acute exposure is affected by accumulated responses to radiation of all organs in the body; for example, the actual total body dose above 1Gy that the response as specific radiation syndrome. In other word, the response of tissue to radiation damage either as acute effects or late (chronic) effects; the acute effects soon occur after exposure to radiation in target tissue. Studies were conducted on the amount of radiation required to kill half the animals in 30 days that depend on the dose rate (Martin *et al*., 2013).

These effects of radiation can be classified into two categories. A stochastic effect is defined as the probability of occurrence increases with the dose, and deterministic effect which is defined as the severity increase with increasing the dose (Martin *et al*., 2013). The biological effects of exposing to radiation were observed to become a directly proportional to the radiation dose (Martin *et al*., 2013).

Low dose of ionizing radiation (IR) is harmful. In this study suggested the dose response curve the low dose region brought the validity of predict cancer risk in this region; estimating the risk of low dose exposure this is adaptive response. Suzuki observed the cancer risk increased with low dose radiation exposure, increasing the size of cancer risk is measuring by excess relative risk (Benner *et al*., 2003). The protective responses may be observing reduction spontaneously in incidence of cancer, that also through action of bystander effect. At low dose of low LET irradiation delay the appearance of spontaneous lymphoma and spinal osteosarcoma late in life, the low LET irradiation shows the existence of threshold dose. The incidence of cancer deducted below single low dose irradiation. With Increasing in the radiation absorbing increase the incidence of DNA damage and genomic instability. The cellular response appears to low values of absorbed dose of ionizing radiation. In the penetrating of radiation to particle tracks arise stochastically through exposed the tissue with low density at low doses. These tracks generate unevenly distributed ionization and excitation of molecules along the track of path (Du, 2005).

The evidence of role for DNA repair in the HRS/IRR response, DNA repair stems from dependence of HRS on LE (Martin *et al*., 2013). At low dose of LET irradiation the cell culture

increases to 4 hours after irradiation and lasted to 3 days. In this process can stimulate detoxification of ROS at 4 h after irradiation; this response accompanied a change in enzyme activities. At low doses energy deposition occurred in about 40% of cells and the bystander effect can cause the adaptive response (Fleck *et al*., 1999). However, single dose of 20 mGy of gamma radiation can cause more than 100 gene expression within 2 h (Du, 2005).

Some radio-protective mechanisms are produced when exposed to small doses of low LET of ionizing radiation (Han *et al*., 2009). These mechanisms are induced by two different ways. The first one is that small doses below 30 cGy protect against exposure to radiation substantially larger than that doses which are called the adaptive response. The second one; response to single dose depends on dose itself; so that, exposure to low dose of LET of ionizing radiation is more effective than larger dose above the threshold which induces radioprotection. Low dose hypersensitivity (HRS) induced radio resistance (IRR) with increase the dose (Han *et al*., 2009). Nevertheless, cells may expose to low dose of ionizing radiation as "priming dose" to protect them against the effects of larger dose "challenging dose" that are given several hours later (Pollycove, 2007). In human, animals and other mammalian cells, it has been observed that adaptive response increases the rate of DNA repair (Azzam *et al*., 1996) and reduce the spontaneous induction of neoplastic transformation (Martin *et al*., 2013; Du, 2007).

Ionizing radiation is encountered in our natural environment and is also generated and used by mankind, e.g., for medical uses. A better understanding of the biological effects of ionizing radiation will lead to better usage of and better protection. The main objectives of the current study are; to verify the adaptive response induction in biological systems due to low doses of γ-radiation, to determine the effective priming doses to be applied in such experiments, and to examine the range biological indicators for the adaptive response.

Materials and Methods

Study Design

The present study has been conducted in the Laboratory of Secondary Standard Calibration, Soba, Khartoum, Sudan, to evaluate the adaptive response and protective effects generated on biological systems due to their exposure to small gamma-radiation doses. Mice were categorized into 5 groups according to the amount of irradiation they have received, each group has been received specific small priming gamma radiation dose for one month with interval of 3 days followed by a single challenging dose; those groups were as follows; control, 5 mGy+7 Gy, 6 mGy+7 Gy, 7 mGy+7 Gy, and 7 Gy. One day after the last dose (challenging dose), blood samples have been collected from all of the groups and have been analyzed for the effects of these irradiation treatments. To do so, we have used a machine called Complete Blood Count (CBC) to read all blood parameters, and also, survival curve has been established. Data obtained from this experiment were analyzed using the Statistical Package for Social Sciences (SPSS).

Experimental Mice

Mice aged 5 weeks old purchased from the Veterinary Research Center, Soba, (Khartoum, Sudan) were maintained in a conventional animal facility under a 12 h light-12 h dark photoperiod (lights on from 7:00 a.m. to 7:00 p.m.). Animals housed in alcohol-cleaned cages, were allowed free access to standard laboratory chow and water. Animals were acclimatized to the laboratory conditions for 1 week before use. To avoid possible effects from the developmental condition of the animals, any mouse of 6 weeks old with a significantly higher or lower body weight (approximately) was omitted from this study. In the present study at least 5 mice were used in each experimental group. All experimental protocols involving mice were reviewed and approved by The National Institute of Nuclear Application in biological Sciences, and were performed in strict accordance with the Institute's Guidelines for the Care and Use of Laboratory Animals.

Source of gamma ray

The source of gamma ray used in the present study is the gamma calibrator OB-85; it is a circular-shaped. The diameter of the OB-85 is 50 cm located at a distance of one

meter away from the experimental mice. There are three sources contained in the gamma calibrator OB-85 such as cobalt-60, cesium-137 and external Am-241 with activity 37 GBq, 740 GBq and 7.4 GBq respectively. Source irradiator was supplied with a lead attenuator with thickness of 1.8 cm that was placed at the exit window of the irradiator to vary the air kerma that is required to cover the instrument scales at particular calibration distance. The laser was used for the selection of the irradiator field in the experiment by the junction of the two beams. The calibrator control room was located outside the gamma-ray source's room, from where timer adjustment and irradiation can be accomplished.

Irradiation of mice

Priming Dose

Mice were categorized into five groups according to the amount of irradiation they have received, each group has been received specific small priming gamma radiation dose for one month with interval of 3 days directly on a one meter distance from the beam of source cs-137 at energy 667 KeV; those groups were as shown in table 1.

Table 1: Priming Doses and Exposure Time

Group	Priming Dose	Exposure Time
A	5 mGy	9 min 11 sec
B	6 mGy	11 min 36 sec
C	7 mGy	15 min 33 sec
D	0	0
Control	0	0

Challenging Dose

By the end of the priming doses, mice in group A,B,C and D were irradiated with 7 Gy challenging dose for 17 seconds exposure time using the gamma cell irradiator (Table 2). These mice were observed for the radio-adaptive response.

Collection of Blood Samples

One day after the last dose (challenging dose) 1 ml blood samplesfrom all the mice in each group were collected in EDTA tube andanalyzed using the complete blood count (CBC) machine at the Institute of Medicinal Plants.

Table 2: Challenging Dose and Exposure Time

Group	Challenging Dose	Exposure Time
A	7 Gy	17 sec
B	7 Gy	17 sec
C	7 Gy	17 sec
D	7 Gy	17 sec
Control	0	0

Data Analysis

Data obtained from observing mice after each treatment and data from blood parameters were analyzed using the Statistical Package for Social Sciences (SPSS).

Results and Discussion

Effects of Gamma Radiation on Blood

After a whole month of priming radiation doses followed by single killing dose, data of blood parameters has been obtained using Complete Blood Count (CBC) device, which is able to read the most important parameters of the blood, such as cellular components and hematological indicators.

Effects on Blood Cell Count

The different doses of gamma irradiation have shown different effects on the count of most blood cells, especially white and red blood cells, which have generally shown a great decrease on their counts when received 5 mGy+7 Gy and 7 Gy doses, with increased cell numbers when treated with higher priming doses (6 mGy and 7 mGy) (Fig. 1 and 2) respectively.

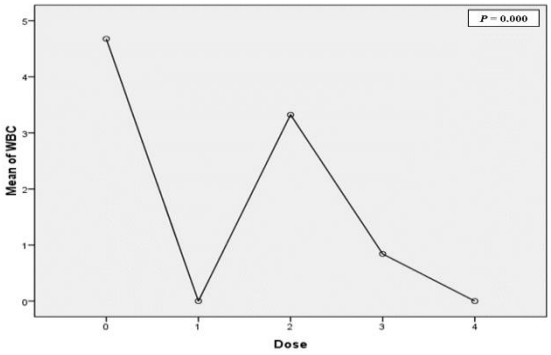

Fig. (1). White blood cells ($X10^0/L$)

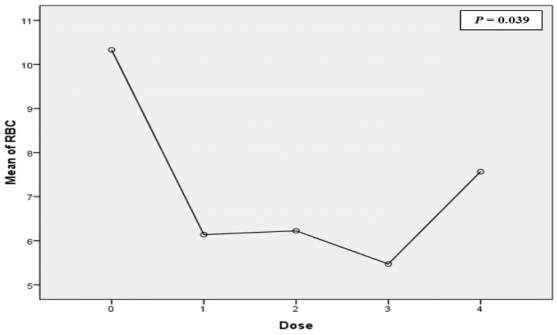

Fig. 2: Red Blood Cells (x10^{12}/L)

While lymphocytes, monocytes and granulocytes have shown greater decline on their counts compared to WBCs and RBCs when received 5 mGy+7 Gy and 7 Gy doses; higher priming doses (6 mGy and 7 mGy) have shown clear increase in the count of these cell types.

The cell in hyper-radio-sensitivity (HRS) can excess 20 of times more sensitive than higher doses of about 1 Gy.

On the other hand, the priming doses have led to higher cell count even than control group, this result has agreed with Joiner MC *et. al.* (1996) who has described that in human lymphocytes, the adaptive response to small doses of ionizing radiation induces resistance (Fig. 3 -5).

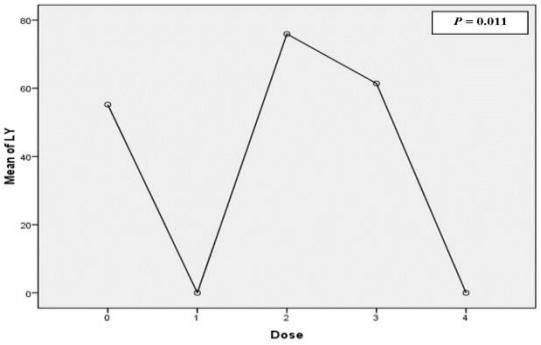

Fig. (3). Lymphocytes (x10^{9}/L)

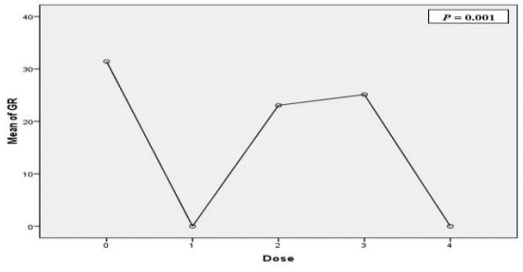

Fig. (4). Granulocytes (x10^{9}/L)

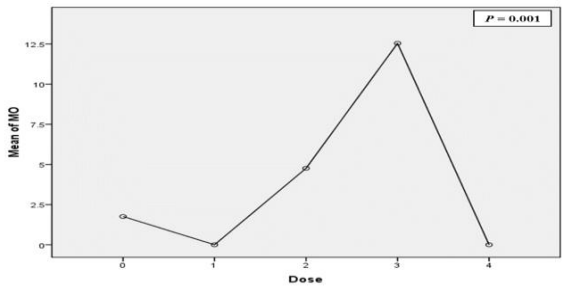

Fig. (5). Monocytes (x10^{9}/L)

Platelets have also shown a declining in their count when received 5 mGy+7 Gy and 7 Gy doses; while higher priming doses (6 mGy and 7 mGy), followed by the killing dose (7 Gy); have shown much decrease in their count (Fig. .6).

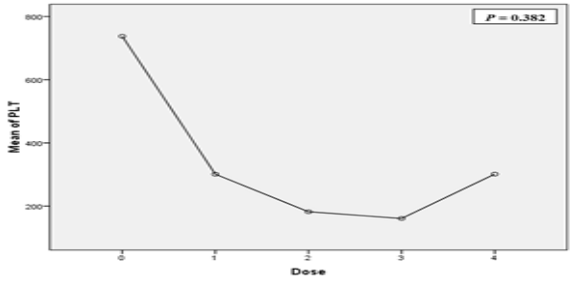

Fig. (6). Platelets(x10^{9}/L)

Effects on Other Blood Parameters

Significant dose-dependent decreases were observed in hemoglobin and hematocrit for the third and fourth groups that received 6 mGy+7 Gy and 7 mGy+7 Gy, respectively; differ from those of 5 mGy and 7 Gy groups which have shown increases in these parameters.

Regarding the mean corpuscular hemoglobin, it has shown a kind of resistance to the high dose of gamma radiation on those mice received priming doses of 6 mGy and 7 mGy (Figs. 7and 8)

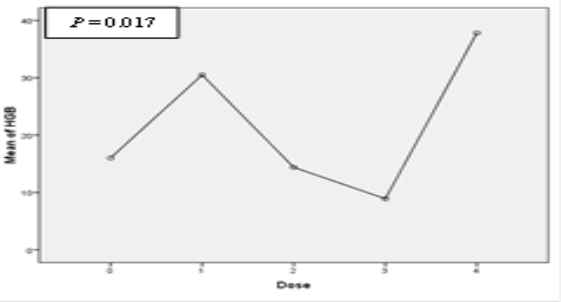

Fig. 7: Hemoglobin (g/dl)

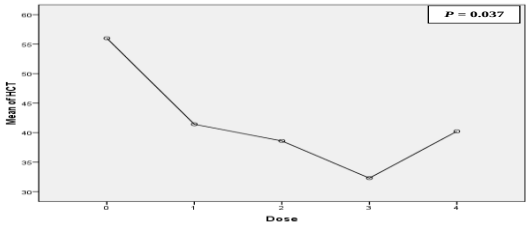

Fig. (8). Hematocrit (%)

Survival Analysis

After thirty days of exposing mice to different low doses of gamma radiation with three days intervals, categorizing them into five groups, 0 mGy, 5 mGy, 6 mGy, 7 mGy, and control group; a single high dose (7 Gy) was applied to all groups except the control one.

Non-significant deaths among control mice have been found in the first week, but when compared to deaths on the other groups it has been clear that the small doses of gamma radiation have a protective role to play on mice received it.

The effect of high dose (7 Gy) of gamma radiation was very killing when it was alone compared to those mice have received priming low doses, which have shown an increased survival (Fig. 9). Collectively, our results indicate that little priming radiation doses can play protective role, similar results have been previously determined by Sawant and his colleagues (2001).

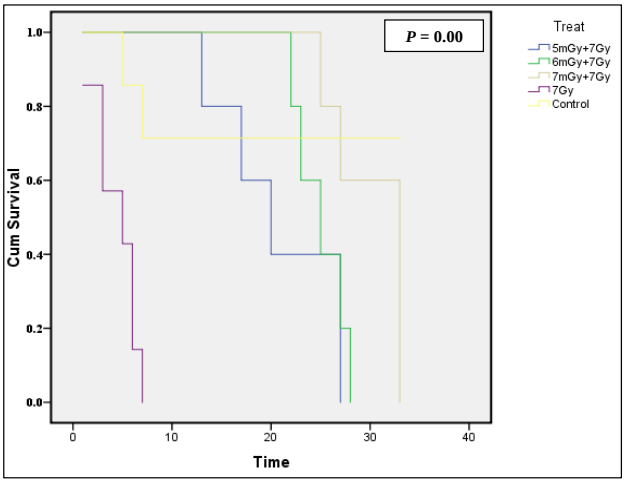

Fig. 9 Survival Analysis Curve

Conclusion and Recommendations

*The present study concluded that adaptive response (radio-resistance) can be developed on biological systems using small radiation doses over a period of time.

* More efforts to decide the types and doses of radiation can be more useful in such type of experiments.

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