

## Induction of Oxidative Stress Following Low Dose Ionizing Radiation in ICR Mice

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**Abstract:** Oxidative stress may be triggered by a various sources and one of the exogenous factors that may trigger oxidative stress is ionizing radiation. Nowadays, the ionizing radiation has been used as a diagnostic and therapeutic tools regardless the side effect that follows. This animal modeling study aimed to evaluate the induction of oxidative stress following the low dose ionizing radiation exposure. Twelve adult male ICR mice were randomly divided into two groups (negative control and radiation group) which consisted of six mice per group. Both groups received filtered tap water and normal pellet diet for fourteen days of acclimatization period prior to total body exposure of 100 $\mu$ Gy x-ray on day fifteen. The activities of superoxide dismutase (SOD), glutathione (GSH) level and malondialdehyde (MDA) level were determined in brains and lungs. Brains GSH level and MDA level decreased significantly in negative group compared to radiation group with  $p=0.006$  and  $p=0.03$ , respectively. The brain's SOD activities also decreased significantly in radiation group ( $p<0.05$ ). In lung tissues the GSH level and MDA level were significantly increased in radiation group compared to negative group ( $p=0.01$ ,  $p=0.001$ ). Meanwhile, lung's SOD activities decreased significantly in radiation group when compared to negative group ( $p<0.05$ ). The findings of this study may postulate that ionizing radiation could induce the oxidative stress in mice.

**Key words:** Low Dose Ionizing Radiation • Oxidative Stress • Glutathione • Superoxide Dismutase  
• Malondialdehyde

### INTRODUCTION

In respiring cells, a small amount of oxygen consumed is reduced and this can lead to a production of highly chemical entities, called reactive oxygen species (ROS) [1] that has been reported to have dual roles which bring beneficial and deleterious effects. Marian Valko *et al.* [2] and Valko *et al.* [3] stated that in biological systems, the cellular functions focus on redox reaction which consist of oxidation or reduction of prooxidants and antioxidants. Oxygen-derived free radicals are the most reactive ROS which are electrochemically imbalance

and easily react with cell. If the balance of redox reaction was distorted, oxidative stress may occur and lead to various pathological conditions [1].

Radiation plays an important part in human's life which both important and dangerous as it is commonly used as therapy against certain diseases and a diagnostic tool for detecting abnormalities present inside human body. Ionizing radiation (IR) specifically has become a necessary part in modern medicine [4]. A recent study by Georgieva *et al.* [5] revealed that IR can lead to ROS formation and results in damage either directly on the cells or indirectly affecting cellular DNA.

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According to the article by Puthran *et al.* [6] x-ray belongs to indirect ionizing electromagnetic group of radiation which have a high penetrating power due to its linear energy transfer (LET). The term of radiation indirect effect may be referred to an ability of IR in producing free radicals which causing oxidative stress through water radiolysis mechanism [7]. The lethal effect could be worsened when coupled with oxygen effects, where the tissue injury through lipid peroxidation will be enhanced [6]. Azab *et al.* [7] found that LET radiation may cause the generation of ROS that interacts with biological molecules and producing toxic free radicals. The final product of peroxidation is malondialdehyde (MDA), major aldehyde product that is mutagenic in cells and could be assessed to evaluate tissue injury [8, 9]. Radiation-induced oxidative stress may increase MDA and subsequently being an interest for researchers.

GSH, a well known antioxidant provides major a function in protecting oxidative injury by participating in the cellular system of defense against oxidative damage. It was reported that GSH tissue levels can be significantly reduced due to oxidative stress, permitting enhanced free radical-induced tissue damage [10] and its intracellular concentration may be assessed as an indicator of oxidative stress.

Defense mechanisms such as superoxide dismutase (SOD) which is responsible for reduction of  $H_2O_2$  also contributes in catalyzing the breakdown of superoxide anion into oxygen. These mechanisms are very important in overcoming oxidative damage caused by ROS [11]. Beside functioning as a protection and defense mechanism for tissue and cell aging, SOD can be quantified in order to assess the level of cell protection [12]. In antioxidant response, SOD is necessary for biological defense in human body against superoxide anion Nandi *et al.* [13].

Concerning the importance of assessing the induction of oxidative stress following IR, the further research should be done in depth. Low dose ionizing radiation (LDIR) is one of the interesting parts where it may also induce oxidative damage to the cells. Thus the present study was conducted to investigate the occurrence of oxidative stress following to low dose ionizing radiation (LDIR) exposure.

## MATERIALS AND METHODS

**Chemicals, Consumables and Materials:** Oxiselect™ Total Glutathione Assay kit, Oxiselect™ TBARS Assay

Kit (MDA Quantification), Oxiselect™ Superoxide Dismutase Activity Assay, 1X phosphate buffer solution (PBS), ethanol, N-butanol were purchased from Sigma-Aldrich Co. Heparin concentrated of 2mg/ml obtained from Merck Co. while the corncob bedding and mice pellet were supplied by A Sapphire Co. Personal protective equipments had been provided by Department of Medical Laboratory Technology, UiTM Puncak Alam.

**Animal Handling:** All animal studies were conducted in accordance with the criteria of the investigations and Universiti Teknologi MARA Committee of Animal Research and Ethics (UiTM CARE) guidelines concerning to the use of experimental animals. Twelve healthy four weeks old male ICR mice weight of 30 grams were obtained from Laboratory Animal Facility and Management (LAFAM), UiTM Puncak Alam campus and placed separately in cages with corncob bedding. Mice were kept in humidity (70 Air Change per Hour pressure) and temperature controlled ( $21 \pm 2$  °C) environment in ventilated room. Mice underwent acclimatization period for fourteen days and normal pellet diet along with filtered tap water were given *Ad libitum*.

**Study Design:** The study involved two groups of 7 weeks old male ICR mice which consisted of a negative control (Neg) and radiation group. A total of twelve mice were divided randomly to these two groups. Mice from radiation group were exposed with single fractionated of 100  $\mu$ Gy x-ray for total body irradiation on the day fifteenth.

**Irradiation:** The irradiation consisted of low dose ionizing radiation by using x-ray were performed under the guidelines provided by Department of Medical Imaging, UiTM Puncak Alam Campus and lead by nuclear medicine expertise. Mice were placed in cages under Philips Bucky DIAGNOST x-ray machine and exposed to single fractionated of 100  $\mu$ Gy x-ray for total body irradiation.

**Animal Cervical Dislocation and Tissues Preparation:** All mice from the experimental groups were sacrificed by cervical dislocation. The brain, lung and liver tissues were excised immediately. Tissues were washed with cold isotonic PBS containing 0.16 % heparin to prevent blood clot that may interfere in the test. The tissue samples were weighted and placed on ice to maintain the integrity. Further preparation was done according to the particular test.

**Glutathione (GSH) Antioxidant Assay:** Tissues were blot dried and weighted. Ice-cold 5% Metaphosphoric acid (MPA) was added and homogenized then the homogenate were centrifuged 12,000 rpm for 15 min at 4°C. Supernatant were collected and the level of GSH was measured kinetically with a spectrophotometric kit (Oxiselect™ Total Glutathione Assay, Cat. No. STA-312; Cell Biolabs, Inc, San Diego, USA), using a method of simple enzymatic recycling reaction for quantification. The principle is based on glutathione reductase reduces oxidized glutathione (GSSG) to reduced form (GSH) with the presence of NADPH. The chromogen reacts with thiol group of GSH to produce colored compound detectable at 405nm with POLARstar Omega reader. The content in samples determined by comparison with glutathione standard curve. Rate of chromophore production is proportional to the concentration of glutathione within sample.

**Lipid Peroxidation Product, Malondialdehyde (MDA)**

**Assay:** Tissues were resuspended at 100 mg/mL in PBS containing 1X BHT. Five grams of the tissue samples were homogenized on ice, spinned at 10,000 g for 5 min and the supernatant collected thus assayed directly for its TBARS level. The MDA level was measured with end point spectrophotometric kit (Oxiselect™ TBARS Assay Kit MDA Quantification, Cat. No. STA-330; Cell Biolabs, Inc, San Diego, USA), using a direct quantitative measurement of MDA. MDA in samples and standards reacted with TBA (Thiobarbituric Acid) at 95°C and incubated then read spectrophotometrically at 532nm with POLARstar Omega reader. MDA level was determined by comparison with predetermined MDA standard curve.

**Superoxide Dismutase Activity Assay:** The activity of SOD was determined by using Oxiselect™ Superoxide Dismutase Activity Assay (Cat.No.STA-340; Cell Biolabs, Inc, Sn Diego). Tissues were homogenized on ice in 7 mL of cold 1X Lysis Buffer per gram tissue followed by centrifugation at 12000 x g for 10 minutes. The supernatant of tissue lysate was collected and proceed to further analysis. Superoxide anions generated by Xanthine/Xanthine Oxidase system was detected with a Chromagen Solution by measuring the absorbance reading at 490nm using POLARstar Omega reader. The activity of SOD was determined as the inhibition percentage of chromogen reduction.

**Statistical Analysis:** The mean  $\pm$  SEM (Standard Error of Mean) were calculated and statistical analysis were done using SPSS statistical package version 18.0 (SPSS Inc.

Chicago, IL, USA). Data were analyzed by one way analysis of variance (ANOVA) and followed by Tukey test for multiple comparison of mean. Significant of the differences was considered when  $p$  value is less than 0.05 ( $p < 0.05$ ).

## RESULTS

After acute irradiation of low dose x-ray, none of the mice showed any physical sign of radiation effects.

**Gsh Antioxidant Assay in Mice Brain's and Lung's Tissues:** Figure 1 showed the levels of GSH contained in mice's brain and lung tissues. GSH levels were measured in negative and radiation group of both tissues. Brain's GSH levels in radiation group was significantly higher than negative control group ( $p=0.006$ ) (Fig. 1). Meanwhile, GSH levels in lung's tissues of negative control group showed a significant increased compared to radiation group ( $p=0.01$ ).

**Lipid Peroxidation Product, Mda Assay in Mice Brain's and Lung's Tissues:** The results of MDA levels in brain's and lung's tissues are presented in Figure 2. MDA levels in radiation group of brain's tissues showed a significant decreased in mean value compared to negative group ( $p=0.03$ ). Lung's MDA levels in radiation group was measured as  $30.05 \pm 0.94 \mu\text{M}$ , significantly higher than those of negative group which is  $22.45 \pm 1.01 \mu\text{M}$  ( $p=0.001$ ) (Fig. 2).

**Superoxide Dismutase Activity Assay in Mice Brain's and Lung's Tissues:** Figure 3 presented the percentage of SOD inhibition activities in brain's and lung's tissues. There was a significant difference in SOD inhibition activities between radiation and negative groups for brain's tissues ( $p=0.00$ ). The percentage of SOD inhibition activities of radiation group in lung's tissues reported significant difference from negative control group ( $p=0.001$ ). Lung's SODs inhibition activities in radiation group decreases about 11.32% compared to negative group.

## DISCUSSION

IR is classified as a potent carcinogen which causes several effects depending on exposure and absorption dose, duration of exposure and interval after exposure and susceptibility of tissues to IR [14]. Based on the findings of this study, GSH levels in lungs of irradiated mice were significantly decreased compared to GSH levels in

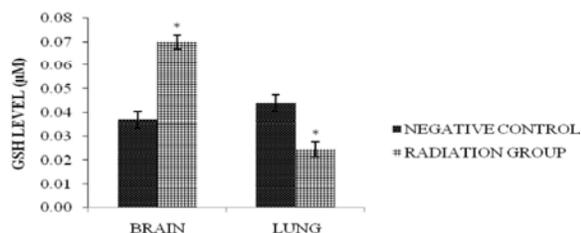


Fig. 1: GSH levels in brain's and lung's tissues. The bar chart shows GSH levels in brain's and lung's tissues in negative control and radiation groups. Values were expressed as mean  $\pm$  S.E.M (n = 6), \*Indicated significant difference when compared to negative control group (p < 0.05).

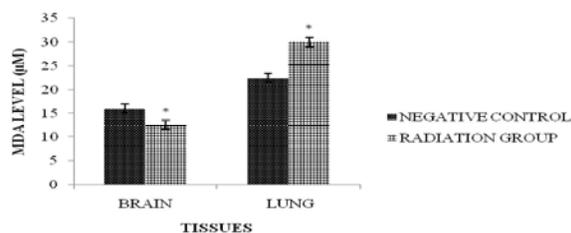


Fig. 2: MDA levels in mice's brain and lung tissues. The bar chart shows levels of MDA in brain's and lung's tissues for negative control and radiation groups. Values were expressed as mean  $\pm$  S.E.M (n = 6), \*Indicated significant difference when compared to negative control group (p < 0.05).

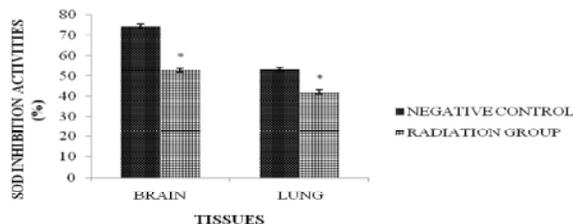


Fig. 3: Superoxide dismutase (SOD) activity (percentage of inhibition) in brain's and lung's tissues. The bar chart shows the percentage SOD inhibition activity in mice's brain and lung tissues of negative control and radiation group. Values were expressed as mean  $\pm$  S.E.M (n = 6), \*Indicated significant difference when compared to negative control group (p < 0.05).

negative group. This may possibly due to low dose radiation leads to oxidative stress that has been triggered in the lung tissues. This outcome is in line with Meydan *et al.*[11], who suggested that ROS causes negative

impacts in antioxidant defense mechanism by depleting the GSH intracellular concentration. According to a study, lung tissues are sensitive to radiation that may promote production of ROS [15] and lead to depleting of GSH levels in radiated tissues [11].

Meanwhile, GSH levels in irradiated brain tissues showed a significant increased compared to negative group. The result was contradicted with previous study which showed a decreased levels of GSH in brain irradiated tissues. This phenomenon may be explained by the nature of GSH, that intracellular content of GSH is responsive to environmental factors and functioned in balancing between usage and synthesization [3]. This indicated that an exposure to ROS can increases content of GSH by increasing GSH synthesis rate. The outcome of this study are in agreement with Valko *et al.* (2006) [3], which suggested that any exposure to high ROS production or any compound-induced ROS production would affect the level of GSH thus recommends that higher GSH synthesis will lead to increases in tissue's GSH level.

On the other side, exposure to 4-hydroxy-2-nonenal (HNE) which is a side product of lipid peroxidation other than MDA also may increase the GSH content in tissues by elevating GSH synthesization because HNE is removed from many cells through GSH activities [3]. High HNE production due to high lipid peroxidation also means that high MDA production will lead to high GSH level and this may correlate between GSH and MDA level. Referring to the current results (Figure 2), the irradiated brain's MDA levels was significantly reduced compared to the negative group. The possible explanation from the current findings might be that, LDIR may contribute to adaptive responses and antioxidant production specifically GSH in overcoming ROS generated by LDIR. Nomura and Yamaoka [16] in their study reported that female C57BL/6 mice irradiated with  $\gamma$ -rays from  $^{137}\text{Cs}$  source at 50 cGy showed small production of free radical that could trigger the production of antioxidant enzymes including SOD and catalase. This finding is in agreement with Yamaoka *et al.* [17] study which showed decreased lipid peroxide levels and elevated SODs activities after rats induced by low dose x-ray in brain tissues. According to the American Society for Nondestructive Testing (NDT) Resource Center [18], brain was classified as lower radiosensitive organ which shows lowest relative susceptibility towards the injurious action of radiation. This may give another explanation for the significant decreased in MDA levels of radiation group is that; brain is not really a radiosensitive organ.

Meanwhile for the lung tissues, the level of MDA in radiation group treated with 100  $\mu$ Gy x-ray showed significant increment compared with negative group and this may indicate the occurrence of oxidative damage induced by LDIR. The LDIR has destructed lipid portion and leads to high MDA concentration in irradiated mice. This is in agreement with Devasagayam *et al.* [19] study which stated that lipid peroxidation may disrupt biological membranes and damaged membrane's structure. A study by Van Ginkel and Sevanian [20] stated that MDA itself has shown to cause extreme alterations in structural and functional of membranes.

In order to reduce the effect from LDIR and prevent further lipid peroxidation, an enzymatic antioxidant defense system such as SOD is also involved. SOD is the only enzymatic system quenching  $O_2^-$  to oxygen and  $H_2O_2$  and play a significant role against oxidative stress, especially in lung. Decrease percentage of SOD inhibition activities in both lung and brain tissues of radiation group compared to negative group may be associated with overproduction of free radical produced after mice exposed to total body radiation of 100  $\mu$ Gy x-ray (Figure 3). The production of free radical out weight the level of antioxidant in lung causes an insufficient dismutation of  $O_2^-$  by SOD. The results were in line with the finding of a previous work by Saada *et al.* [21] which found that SOD activities decreased significantly in small intestine of male albino rats after total body irradiation of 6 Gy gamma ray. The decrease in SODs inhibition activities may explain that excess ROS interacts with enzyme molecules and may lead to partial inactivation and denaturation [22].

SOD activities in brain tissues for radiation group show significant decreased compared to negative group. This is in agreement with the study by Kesari *et al.* [23] which showed that radiation promotes oxidative damage to tissues and lead to depletion of SOD activities in order to counteract the production of free radical. For brain tissues, the results of negative group indicated a significant increased compared to radiation group. A study showed that SOD activities in Wistar rat brain's tissue exposed to low intensity microwave showed a decreased in SOD activities compared to control group [23]. Another study showed that rat brain's tissues exposed to radiation can lead to inhibition of hippocampal neurogenesis which related to radiation forming oxidative stress to the tissue [17] and lead in increasing of free radicals of superoxide due to the damage triggered by radiation [11].

In conclusion, the outcomes of this study revealed that exposure to LDIR may trigger oxidative stress then promoting tissue injury. It is advisable that further study on any radiation suppressing agent should be conducted.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Faculty of Health Sciences, Universiti Teknologi MARA Puncak Alam and UiTM Research Management Institute (RIF: 600-RMI/DANA 5/3/00/RIF 400/2012) for providing financial funds. Authors also acknowledge the Department of Medical Laboratory Technology, Department of Medical Imaging and Postgraduate Department, Pharmacogenomics Centre (PROMISE) in Faculty of Pharmacy UiTM and FRIM for providing facilities throughout this study. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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