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Watermelon Radioprotection against Radiation Stress in Mice

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ABSTRACT

Ionising radiation (IR) has been extensively used as therapy and diagnostic modality to detect abnormalities inside a human body. Interaction between IR and cells can lead to production of free radicals. This study aims to evaluate radioprotective properties of 50% watermelon juice against low dose ionising radiation (LDIR)-induced stress in mice lung and liver tissues following 14 days of juice supplementation. Eighteen (18) ICR mice were randomly divided into three groups**,** negative control (Cx), radiation (Rx) and treatment group (Tx). The Cx group was treated with normal diet and filtered water while the Rx group was given a normal diet, filtered water and irradiated with 100 µGy x-ray; Tx group was fed a normal diet, 50% watermelon juice and irradiated with 100 µGy x-ray. After 14 days, level of superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) in lung and liver tissues were evaluated. The SOD inhibition activity revealed a significant decrease in Rx and Tx compared with Cx (p<0.001). The Rx and Tx showed significant reduction in GSH level compared with Cx (p<0.001) respectively. The MDA levels for lung tissues revealed a statistically significant value between Tx and Cx (p<0.05). The results suggest that 14 days supplementation of 50% watermelon juice was insufficient to foster radioprotective properties against LDIR-induced stress.

Keywords: Glutathione (GSH), low dose ionizing radiation (LDIR), malondialdehyde (MDA), oxidative stress, superoxide dismutase (SOD)

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INTRODUCTION

Ionising radiation (IR) is a radiation energy that is capable of removing tightly bound electrons from the atomic orbital during an interaction, thus ionising the atom. According to a study. The IR exhibits wide beneficial applications in medicine involving the

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management of a wide range of tumours, along with chemotherapy and surgery (Mohammad, Mohamed, Zakaria, Abdul Razak, & Saad, 2014). Despite its efficacy in killing tumour cells, radiation suffers from several major drawbacks like damage to surrounding healthy tissue and deoxyribonucleic acid (DNA) to patients and radiotherapy workers (Mansour, 2013). In a low dose radiation exposure, oxidative stress may induce modification to redox balance after a certain exposure period. Studies showed that acute exposure to IR may contribute to water radiolysis, thereby generating reactive oxygen species (ROS) (Devasagayam et al., 2004; Meydan, Gursel, Bilgici, Can, & Ozbek, 2011).

The ROS including superoxide anion (O_2-), hydroxyl radical (OH−) and hydrogen peroxide (H_2O_2) are produced by aerobic organism (Stice et al., 2015). Overproduction of ROS may cause oxidative damage to cellular macromolecules such as DNA, lipids, and proteins, but they are also beneficial in regulating homeostasis at cellular level in normal healthy tissues (Eltahawy, Abunour, & Elsonbaty, 2012; Mansour, 2013; Jagetia & Ravikiran, 2014; Saada, Rezk, Eltahawy, & Wiley, 2010; Stice et al., 2015). In a normal physiological condition, antioxidant will counterbalance the excessive amount of ROS via neutralisation. Imbalance between antioxidant and ROS would lead to oxidative stress and several biological consequences (Freitinger, Zölzer, Beránek, & Racek, 2012).

An antioxidant is a molecule that has the ability to scavenge free radicals and protect body from oxidative damage. Antioxidant system can be classified to enzymatic antioxidant (e.g. superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (e.g. glutathione, ascorbic acid, tocopherol and carotene). Those biosubstances may be assessed as markers for oxidative stress due to their participation in cellular defence system. According to a study, fruits and vegetables can provide the best antioxidant properties against the development of chronic disease (Asita & Molise, 2011).

However, there is lack of information about the radioprotective properties of watermelon as antioxidant against radiation-induced oxidative stress. Hence, the properties of watermelon juice against low dose radiation-induced oxidative stress are yet to be fully characterised. The maximum likelihood method proposed by Shang, may not be suitable for this study (Shang, 2011). Thus, this study was designed to evaluate radioprotective properties of local watermelon juice against low-dose ionising radiation (LDIR)-induced oxidative stress in lung and liver tissues of mice.

MATERIALS AND METHODS

Chemicals

OxiSelect ™ Superoxide Dismutase Activity Assay kit, OxiSelect ™ Glutathione (GSSG/GSH) Assay kit and OxiSelect ™ TBARS Assay kit (MDA Quantification) were purchased from Cell Biolabs, Inc. Other chemicals used throughout the study were purchased from Sigma-Alderich.

50% Watermelon Juice Preparation

Locally harvested watermelon (Citrullus Lanatus (Thunb) Matsum and Nakai) were purchased from the Selangor Fruit Valley's dealer in Saujana Utama, Selangor. The fruits were washed and peeled and the flesh cut into smaller pieces and blended using fruit juice extractor (Pensonic Classic Series Juice Extractor: PJ-67S). The pure fruit juice was then diluted with filtered tap water in ratio 1:1 (v/v) to obtain a 50% watermelon juice. The juice was freshly prepared twice a day for the mice's consumption.

Ethics Approval

Animal study was approved by the UiTM Committee of Animal Research and Ethics (UiTM CARE), UiTM, Puncak Alam, Selangor, with strict compliance to the guidelines. All procedures were performed under diethyl ether anaesthesia.

Animal Handling and Study Design

Eighteen (18) four-week-old male ICR mice weighing about 30g each, were obtained from Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, UiTM Puncak Alam, Selangor. The mice were placed in cages with corncob bedding in Animal Holding Room (FSK 1,5 at 6^{th} floor) equipped with ventilation system, temperature of 25 + 2^{o}C and 70 air changes per hour (ACH) pressure. The mice underwent 2 weeks acclimatisation period and fed normal pellet diet with filtered tap water ad libitum. For study design, the mice were randomly placed in three groups that consisted of six mice per group, and divided into negative control group (Cx), radiation group (Rx) and treatment group (Tx). Animals from Rx and Tx were given filtered tap water and 50% watermelon juice for 14 days respectively, and exposed to 100µGy on day 15. All animals were euthanised by cervical dislocation and their liver and lung tissues were immediately harvested and kept at -80^{o}C for further analyses.

Superoxide Dismutase (SOD) Inhibition Activity Assay

The tissue sample was homogenised in 5 ml cold 1X Lysis Buffer per gram tissue using mortar and pestle. The sample was centrifuged at 12,000 rpm for 10 minutes. The tissue lysate supernatant was collected and assayed directly for SOD activity assay. Absorbance was read at 490 nm using POLARstar Omega Microplate Reader. The SOD inhibition activity assay was determined by inhibition percentage of the SOD activity.

Total Glutathione (GSH) Assay

One-gram tissue sample was washed with isotonic saline solution of 1X PBS with 0.16 mg/ml heparin to prevent coagulation. The tissue was blot-dried and weighed before 5% ice-cold MPA was added and homogenised using mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 15 minutes at 4^{o}C and supernatant was collected and assayed directly for the

GSSG level. The POLARstar Omega Microplate Reader was set at 405 nm and the absorbance reading was set at one-minute interval for 10 minutes. The concentration of total GSH in samples was demonstrated by comparison to standard curve.

Lipid Peroxidation Product, MDA Assay

One-gram tissue was resuspended in 50 to 100 mg/ml PBS containing 1X butylated hydroxytoluene (BHT) and homogenised on ice using mortar and pestle. The lysate was spun at 10,000 rpm for five minutes. The supernatant was collected and assayed directly to determine TBARS level. The interaction of MDA in samples with thiobarbituric acid (TBA) at 95^{o}C were measured spectrophotometrically at 532 nm with POLARstar Omega Microplate Reader and determined by MDA standard curve.

Statistical Analysis

Data obtained was analysed by analysis of variance (ANOVA) test, followed by *post hoc* Tukey test using social science statistical package (SPSS) version 21.0. The differences were considered significant when p value was less than 0.05 ($p < 0.05$). An effective computer technology like 3D gait signatures computed from 3D data could not be implied due to several limitations such as cost, facilities and lack of expertise.

RESULTS

General Examination

After irradiation with total body x-ray, the animals were observed for behavioural and physical changes. However, no significant physical changes were observed. Gross examination for liver and lung tissues revealed no pathological defects.

SOD Inhibition Activity in Lung and Liver Tissues

Figure 1 shows SOD inhibition activity in lung and liver tissues of mice. In lung tissues, there was no statistical difference observed in pair wise comparisons. However, there was an increasing trend of SOD inhibition activity between groups. The lung Tx showed highest inhibition percentage of SOD activity (54.0064 ± 2.6362 %) compared with Rx (51.1560 ± 1.5651 %) and Cx (48.4603 ± 1.2417 %). In liver tissues, results revealed that SOD inhibition activity was significantly decreased in Rx and Tx when compared with Cx (p<0.001). The mean percentage of SOD inhibition activity in liver Cx was 48.29 ± 2.74 %, Rx was 24.03 ± 3.64 % and Tx was 31.53 ± 2.97 %. There was an increasing trend of SOD inhibition activity in liver Tx compared with Rx; however, there was no statistical difference observed in the pair wise comparison.

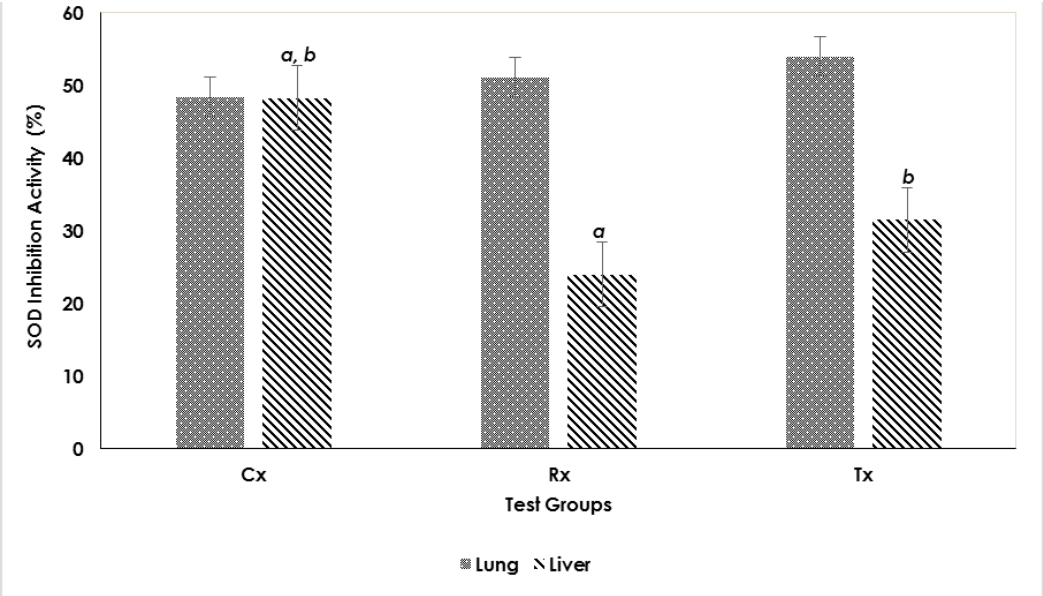


Figure 1. Determination of SOD inhibition activity in lung and liver tissues. The result represents the SOD inhibition activity (%) of Cx, Rx and Tx test groups in lung and liver tissues. Values were expressed as mean ± SEM (n=6)

[a] *Indicates significant differences between Rx and Cx of liver tissues (*$p<0.001$)
[b] *Indicates significant differences between Tx and Cx of liver tissues (*$p<0.001$)

Total Glutathione (GSH) in Lung and Liver Tissues

Figure 2 refers to the mean value of total GSH level in lung and liver tissues measured in micromolar (μM). In lung tissues, exposure to LDIR in Rx (0.5286 ± 0.0046 μM) significantly decreased the total GSH level when compared with Cx (3.7106 ± 0.0847 μM) with $p < 0.001$. Similar trend was observed in lung Tx (0.4482 ± 0.8149 μM) when compared with Cx ($p < 0.001$). GSH level in lung Tx was depleted compared with Rx; however, no significant differences were observed. In liver tissues, total GSH level showed a significant reduction of total GSH level in Rx (0.70 ± 0.04 μM) and Tx (0.73 ± 0.04 μM) when compared with control group (4.62 ± 0.29 μM) with $p<0.001$. The GSH level in liver Tx was higher compared with Rx; however, no significant differences were observed.

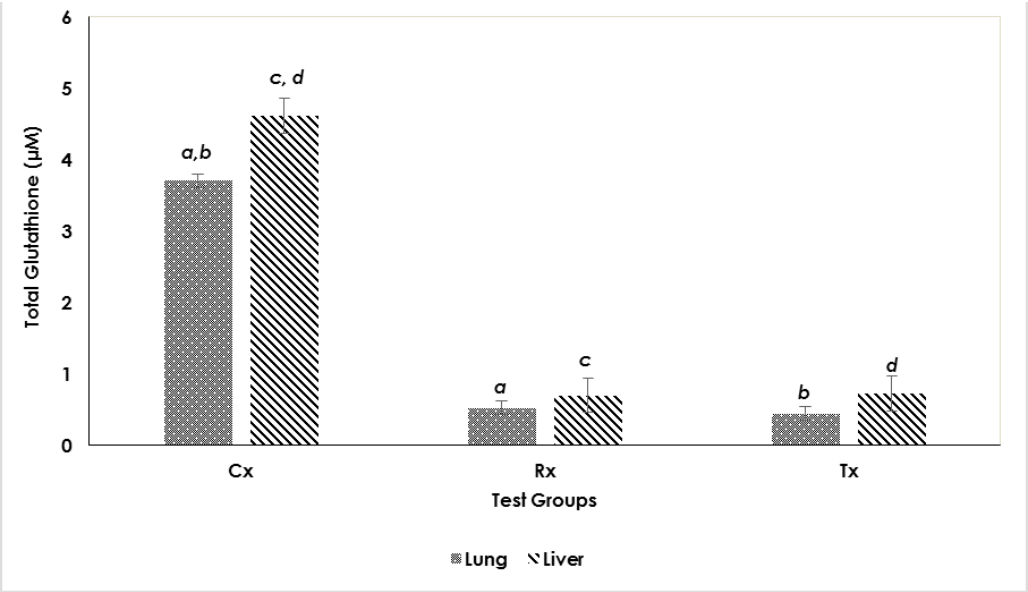


Figure 2. Determination of total glutathione (GSH) in lung and liver tissues. The result represents the total glutathione (GSH) of Cx, Rx and Tx test groups in lung and liver tissues. Values were expressed as mean ± SEM (n=6)

[a] *Indicates significant differences between Rx and Cx of lung tissues (p< 0.001)*
[b] *Indicates significant differences between Tx and Cx of lung tissues (p< 0.001)*
[c] *Indicates significant differences between Rx and Cx of liver tissues(p<0.001)*
[d] *Indicates significant differences between Tx and Cx of liver tissues (p<0.001)*

Lipid Peroxidation Product, MDA in Lung and Liver Tissues

Determination of MDA level in tissues could reflect the lipid peroxidation occurrence because it is the main product for oxidation process. Figure 3 shows that the level of MDA in lung Tx (23.6853 ± 0.4387 µM) was significantly increased compared with Cx (16.7081 ± 1.4351 µM) with $p<0.05$. There was an increasing trend of MDA level in lung Tx compared with Rx, and between Rx and Cx. However, there was no statistical difference observed in the pair wise comparisons. In liver tissues, there was no statistical difference observed in the pair wise comparisons. However, there was an increasing trend of MDA level in Tx (16.58 ± 1.00 µM) and Rx (17.38 ± 0.97 µM) when compared with Cx (16.13 ± 1.58 µM).

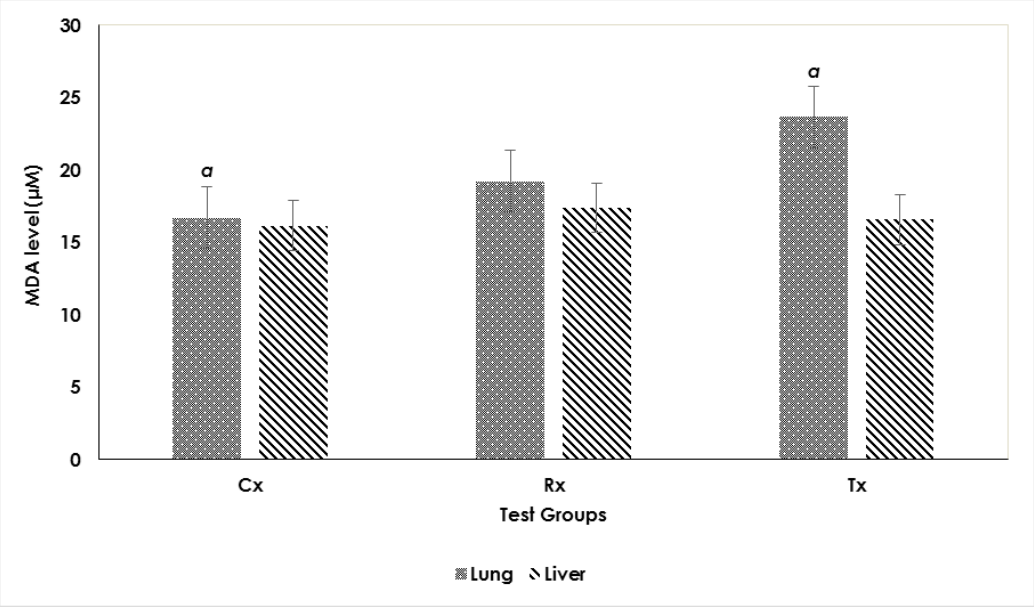


Figure 3. Determination of lipid peroxidation product, MDA in lung and liver tissues. The result represents the MDA level of Cx, Rx and Tx test groups in lung and liver tissues. Values were expressed as mean ± SEM (n=6)

[a] *Indicates significant differences between Tx and Cx of lung tissues ($p < 0.05$)*

DISCUSSION

The SOD as the major antioxidant enzyme, removes superoxide radicals in different cellular compartment by dismutation to oxygen and hydrogen peroxide (Kinnula & Crapo, 2003; Mohammad et al., 2014). Thus, assessment of SOD inhibition activities may reveal oxidative stress in living organisms exposed to LDIR. The determination of SOD inhibition activity in lung and liver tissues had been carried out to evaluate the level of antioxidant enzymes activity (Pan et al., 2012). In lung tissues, an increasing trend observed in SOD inhibition activities of Rx and Tx compared with Cx with no statistical significance. In liver tissues, results revealed that SOD inhibition activity were significantly decreased in Rx and Tx when compared with Cx ($p<0.001$). Results may suggest the overproduction of ROS by the LDIR exposure has taken place in both tissues but liver was more affected. The oxidative stress and redox imbalance caused by 100 µGy radiation in Rx might be well tolerated by the lung tissues of the mice by the regulation of homeostasis. The cells' response and regulation ability towards redox imbalance might vary between different organs. According to Kinula and Crapo, the lungs are directly exposed to higher oxygen concentrations than most other tissues and SOD role in protecting lung tissue has been confirmed by transgenic and knockout animal studies (Kinnula & Crapo, 2003).

Apart from SOD inhibition activities, the assessment of GSH level was one of the most reliable biomarker in evaluating oxidative stress (Sikder et al., 2013). The GSH is non-enzymatic antioxidant which become an indicator of cell health because depletion of GSH level may indicate the susceptibility to oxidant attack (Ran et al., 2014). As stated by Meydan et al., liver has highest concentration of GSH compared with other tissues (Meydan et al., 2011). In this study, exposure to LDIR in both tissues significantly decreased total GSH level in Rx and Tx when compared with Cx (p<0.001). In liver tissues, Tx showed an increasing trend of GSH level against Rx while lung tissue showed a contradictory effect with no statistical difference observed in the pair wise comparisons. This may indicate that exposure to LDIR significantly elevated the oxidative stress in lung and liver tissues as depicted by significant reduction of total GSH level. This was supported by a study by Meydan et al., who conducted a nearly similar study to determine the protective effect of lycopene against radiation-induced hepatic toxicity in mice and discovered that radiation group exposed to single-fraction 8Gy abdominopelvic radiotherapy significantly reduced the GSH level compared with control group (Meydan et al., 2011). In the present study, the observed decrease in GSH level in Rx during radiation exposure was mainly due to high usage of GSH at that particular period of time, to neutralise ROS in the lung and liver tissues. However, 14 days supplementation of 50% watermelon juice was not able to ameliorate the oxidative damage cause by radiation exposure, with no significant difference observed between Tx and Rx for both tissues. This was contrary to the findings of Mohammad et al. that postulated the radioprotective effect of 50% watermelon juice mitigate the GSH level of 28 days treatment group compared with 100 μGy radiation group in mice's brain, liver and lung tissues (Mohammad et al., 2014). The different findings between the two studies were clearly determined by different treatment period of the 50% watermelon juice. This suggested that 14 days pre-treatment of 50% watermelon juice was not enough to achieve a statistical significance in GSH level when compared with the radiation group.

Evaluation of MDA level in lung and liver tissues reflected the occurrence of lipid peroxidation (Saada, Said, Meky, & Azime, 2009). In lung tissues, MDA level in Tx was significantly elevated compared to Cx (p<0.05). There was an increasing trend of MDA level in lung Tx compared with Rx, and between Rx and Cx however, there was no statistical difference observed in the pair wise comparisons. In liver tissues, there was no statistical difference observed within any pair wise comparisons; however, MDA level increased in Tx and Rx compared with Cx. The inconsistent trends of MDA level in both tissues may postulate that the GSH had already quenched most of the ROS in the pathway that contributed to less oxidation of lipid in both tissues. This hypothesis could be supported by significant reduction in GSH level of Tx and Rx when compared with control group for both tissues. The difference between the mean values of Tx compared with Rx, in both lung and liver tissues were not statistically significances, indicating 14 days supplementation of 50% watermelon juice was insufficient to encounter lipid peroxidation. This finding was consistent with that of Saada et al. which revealed an insignificant reduction of MDA level in rat's heart and pancreatic tissues administered with grape seed extract by gavage for 14 days, following 5 Gy whole body gamma radiation (Saada et al., 2009). This may support the finding of present study indicating 14 days supplementation of 50% watermelon juice had insufficient radioprotective effect on MDA level compared with Rx to conclude that natural product pre-treatment should be longer in order to mitigate the MDA levels prior to exposure to radiation.

CONCLUSION

The present study showed that 100 μGy of LDIR are able to cause significant oxidative damage on lung and liver tissues, determined by several biomarkers. The supplementation of 50% watermelon juice for 14 days was insufficient to protect lung and liver from LDIR-induced oxidative stress at cellular level.

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