Radioprotective Effect of Watermelon Juice Against Low Dose Ionizing Radiation-Induced Inflammatory Response in Mice

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Abstract: Exposure to ionizing radiation (IR) may increase tumor formation risk and has been linked to inflammatory response. It is known that IR can cause direct tissue damage, with activation of pro-inflammatory mediators released by macrophages, epithelial cells and fibroblasts. Production of pro-inflammatory cytokines induced by radiation may be overcome by lycopene, a naturally occurring antioxidant in watermelon. Watermelon is known to have about 40% higher lycopene than raw tomatoes. Lycopene has the ability in down regulation of inflammatory response that includes inhibiting the pro-inflammatory cytokines. The present study was designed to evaluate the radioprotective effect of watermelon juice [Citrullus lanatus (Thunb.) Matsum. and Nakai] on low dose ionizing radiation induced inflammation in mice. Fifteen mice were divided randomly into 3 groups: negative control (normal diet), positive control (normal diet + low dose IR) and supplementation (50% watermelon juice + low dose IR). Supplementation group was given 50% watermelon juice (v/v) for 28 consecutive days ad libitum and low dose IR was given on day 29 with single dose 100µGy. In lung, TNF-α and IL-6 levels showed significant differences (p = 0.05, p = 0.01) respectively between supplementation and positive control groups. There were significant differences in lung IL-6 levels between negative and positive control groups (p = 0.01). Significant differences were also observed in liver TNF-α levels between negative control and supplementation groups (p = 0.02). In conclusion, the study demonstrated that watermelon juice has a protective effect against low dose IR – induced inflammatory response.

Key words: Inflammatory Response • Ionizing Radiation • Tumor Necrosis Factor-Alpha • Interleukin-6 • Watermelon

INTRODUCTION

Inflammation is a complex mechanism [1] related to reactive oxygen species (ROS) when produced redundantly [1-3]. Radiation may cause ROS production to elevate [4]. Radiation induces ROS production and leads to inflammatory response as indicated by the production of inflammatory cytokines. Pro-inflammatory mediators are released through local and systemic reaction simultaneously when triggered by insults [5]. Three major pro-inflammatory cytokines that participate in acute phase response are Interleukin 1 (IL-1), Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) [6].

Ionizing radiation (IR) exerts its biological effects when ROS is yielded [4]. Since cell is constituted mainly by water (~80%), the energy from IR is absorbed largely by water that in turn leads to production of free-radicals when the cell is excited and ionized [7]. High ionizing
Watermelon has lycopene with year-round mean of 4868µg/100g whereas tomato has 3025 µg/100g lycopene concentration [17]. The intake of watermelon as daily supplement can prevent certain types of cancers [17]. Therefore, watermelon’s potential benefit against inflammation is highly attentive. The present study was designed to measure inflammatory response in low dose ionizing radiation-induced mice following supplementation with 50% watermelon juice.

**MATERIALS AND METHODS**

*Study Design and Animals Handling:* Fifteen (n=15), four-week-old male ICR mice, weighed around 25 grams (g) were used as models. Mice were obtained from Laboratory Animals and Facility (LAFAM), Faculty of Pharmacy of UiTM Puncak Alam. Mice were allowed to acclimatize to constant room temperature (RT) of 22 ± 2°C during adjustment period, exposed to 12-hours (h) light and 12-h dark cycle and kept at mean relative humidity in the animal house of Faculty of Health Science (FSK) at FSK1, 5. Each mouse was weighed using PW124 analytical balance (Adam Equipment Co. Ltd., Kingston, Milton Keynes, UK) and given identification number prior to supplementation. The body weights were measured once a week during supplementation period. Five mice (n=5) from supplement group were housed in separate cages. Mice were grouped into three experimental groups with different criteria (Table 1). The experiments were approved and conducted in compliance with the rules of Animal Research and Ethics Committee of UiTM Puncak Alam.

*Animals Diet:* All mice had free access to food pellet and filter-tap water throughout three weeks adjustment period. During supplementation period, five mice of the supplement group were given watermelon juice for 28 consecutive days while negative and positive control groups were given normal diet *ad libitum* (Table 1).

<table>
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<tr>
<th>Table 1: The experimental groups and their respective criteria</th>
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<td><strong>Group</strong></td>
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<tr>
<td>Negative Control (n=5)</td>
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<td>Supplement Group (n=5)</td>
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Mice were divided into three groups (n=5). For 28 consecutive days, supplement group was given watermelon juice while negative and positive control groups were given normal diet *ad libitum*. On day 29th, positive control and supplement groups were given x-ray exposure of 100 µGy.
Supplementation with 50% Watermelon Juice: Watermelon was bought from local Federal Agriculture Marketing Authority (FAMA) stall that was directly supplied with watermelon from Selangor Fruit Valley, Rawang. Watermelon juice was prepared freshly twice a day. Watermelon juice was prepared 12 hourly at 8 AM and 8 PM. Watermelon was peeled and the seeds were removed. Watermelon flesh was chopped into small pieces. Five pieces were blended smoothly into juice by using PJ-67S Juicer Extractor (Pensonic Holdings Bhd., Perai, Pulau Pinang, MY). Water was added to prepare watermelon juice with 50% concentration juice at 1:1 (v/v). 100mL 50% watermelon juice was placed in the water-bottle inside the cages. After 12h, the remaining volume of watermelon juice was measured. Measurement of the remaining watermelon juice was taken in every preparation of fresh watermelon juice.

Radiation Exposure of Animal; Mice were weighed before and after radiation exposure. The procedure of radiation exposure was done at Department of Medical Imaging, FSK, UiTM Puncak Alam. Ten mice were exposed to full-body x-ray radiation with the dose of 100 µGy using Bucky Diagnost x-ray generator (Philips Co. andover, MA, USA) (Table 1). Mice were restrained in well-ventilated transparent boxes during radiation exposure.

Euthanization of Animal and Sampling: Mice were euthanized by cervical dislocation technique within 8h post- radiation exposure and lungs and livers were collected in sterile containers. The lung tissues extraction method was conducted according to manufacturer’s instruction of Cell Lysis Reagent for Mammalian Tissue (Sigma-Aldrich Co. LLC, St. Louis, MO, USA). A ratio of tissue to cell lysis reagent of 1:20 (1 g of tissue/20 mL of reagent) was based according to manufacturer’s instruction. Tissue was weighed using analytical balance. Each tissue was disrupted in Cell Lysis Reagent for Mammalian Tissue (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) and homogenized using mortar and pestle. Sample was centrifuged at 15,000 x g for 10 minutes (min) using K24IR refrigerated centrifuge (Centurion Scientific Ltd., Chichester, West Sussex, UK). Supernatants were removed into pre-chilled tube and kept at -70°C until ELISA testing.

Measurement of TNF-α and IL-6: The TNF-α and IL-6 concentrations were determined using commercial ELISA kits (Thermo Scientific-Pierce Biotechnolgy, Rockford, IL, USA). Supernatants from homogenized lung tissues and liver tissues were applied onto 96-well plates pre-coated with antibody specific for mouse TNF-α and IL-6 followed by serial incubation and wash according to manufacturer’s instructions. Absorbance was measured within 30 minutes at 450 minus 550nm using POLARstar Omega micro plate reader (BMG LABTECH GmbH, Ortenberg, Germany). All samples were measured in duplicates. The concentration of TNF-α and IL-6 level in each sample was calculated based on a standard curve.

Statistical Analysis: Statistical analysis was performed using SPSS Version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Tukey test was used to compare the mean between controls and supplement groups. Values were considered significantly different when \( p<0.05 \).

RESULTS

TNF-α Level in Lungs: Fig. 1 demonstrates the level of TNF-α in low-dose ionizing radiation-induced mice following supplementation with 50% watermelon juice. Values were expressed as mean ± standard errors of the mean (SEM). Based on the result, there were no significant differences \( (p=0.12) \) in level of TNF-α between negative and positive groups. The level of TNF-α for negative group was 716.53 ± 91.60 pg/mL and level of TNF-α for positive group was 913.87 ± 86.28 pg/mL. The positive group showed elevated level of TNF-α compared to negative group by 52.73 pg/mL (Fig. 1).

IL-6 Level in Lungs: Fig. 2 demonstrates the level of IL-6 in low-dose ionizing radiation-induced mice following supplementation with 50% watermelon juice. Based on the result, there were significant differences \( (p=0.01) \) in level of IL-6 between positive and negative groups. The level of IL-6 for negative group was not expressed. The level of IL-6 for positive group was 24.00 ± 6.60 pg/mL. There were also significant differences \( (p=0.01) \) in IL-6 level between positive and supplement groups. The level of IL-6 for supplement group was 2.80 ± 2.40 pg/mL.
Fig. 1: Level of TNF-\(\alpha\) in lung tissue of low-dose ionizing radiation-induced mice following supplementation with 50% watermelon juice. Values are expressed as mean ± SEM (n=5).
*Significantly different from positive group (\(p=0.05\)).

Fig. 2: Level of IL-6 in lung tissue of low-dose ionizing radiation-induced mice following supplementation with 50% watermelon juice. Values are expressed as mean ± SEM (n=5).
**Significantly different from negative group (\(p=0.01\)).
*Significantly different from positive group (\(p=0.01\)).

The positive group showed elevated level of IL-6 compared to supplement group by 21.20 pg/mL (Fig. 2). However, there were no significant differences (\(p=0.87\)) in level of IL-6 between negative and supplement groups.

**TNF-\(\alpha\) Level in Liver:** Fig. 3 demonstrates the level of TNF-\(\alpha\) in low-dose ionizing radiation-induced mice following supplementation with 50% watermelon juice. Based on the result, there were no significant differences (\(p=0.11\)) in level of TNF-\(\alpha\) between negative and positive groups. The level of TNF-\(\alpha\) for negative group was 4162.87 ± 178.89 pg/mL and level of TNF-\(\alpha\) for positive group was 3804.20 ± 113.93 pg/mL. The negative group showed elevated level of TNF-\(\alpha\) compared to positive group by 358.67 pg/mL (Fig. 3). Also, there were no significant differences (\(p=0.51\)) in TNF-\(\alpha\) level between positive and supplement groups. The level of TNF-\(\alpha\) for supplement group was 3663.53 ± 108.78 pg/mL. The positive group showed decreased level of TNF-\(\alpha\) compared to supplement group by 140.67 pg/mL (Fig. 3). However, there were significant differences (\(p=0.02\)) in level of TNF-\(\alpha\) between negative and supplement groups. Supplementation group showed decreased level of TNF-\(\alpha\) compared to negative group by 499.34 pg/mL (Fig. 3).
IL-6 Level in Liver: Fig. 4 demonstrates the level of IL-6 in low-dose ionizing radiation-induced mice following supplementation with 50% watermelon juice. Based on the result, there were no significant differences ($p=0.11$) in level of IL-6 between negative and positive groups. The level of IL-6 for negative group was $359.40 \pm 39.03$ pg/mL and level of IL-6 for positive group was $489.80 \pm 57.79$ pg/mL. The positive group showed elevated level of IL-6 compared to negative and supplement group by $130.40$ pg/mL and $73.20$ pg/mL respectively (Fig. 4). Based on the result, there were no significant differences ($p=0.45$) in level of IL-6 between positive and supplement groups. There were also no significant differences ($p=0.60$) in level of IL-6 between negative and supplement groups. The level of IL-6 for supplement group was $416.60 \pm 15.68$ pg/mL. Supplement group showed elevated level of IL-6 compared to negative group by $57.2$ pg/mL (Fig. 4).

DISCUSSION

For more than a century until recently, the biological effects of low dose IR has been debated and investigated [8, 11]. While the risk of low dose IR remains unclear, high dose IR clearly produce deleterious outcome in humans including generation of cancer [8]. Studies on low dose IR is essential concerning its societal importance on varied issues including screening tests for cancer, future of nuclear power, frequent-flyer risks, occupational radiation exposure, manned space exploration and radiological terrorism [8]. Among these issues, studies on low dose IR have become necessarily important in diagnostic setting. To date, IR is assumed carcinogenic even at low doses when radiation protection is applied in health line including industry and public health [18].

Exposure to IR may lead to disturbance of normal tissue function through modification that finally causes tissue death [19]. TNF-$\alpha$ is one of the pro-inflammatory cytokines that promotes inflammation [20]. Based on present findings, significant difference in TNF-$\alpha$ level in lung of supplement group was observed when compared to positive group. The results demonstrated that supplementation of 50% watermelon juice with its natural source of lycopene and other antioxidants such as flavonoid and phenolic may help in down regulation of TNF-$\alpha$ level in lung. Ma and Kinneer [21] reported that phenolic has the ability to inhibit signal-induced TNF transcription, thus controlling cytokine induction through its properties as anti-inflammatory. Adding to that a study also found flavonoids may act as preventive agents in reducing lung injury induced by radiation [22].

Study findings showed that lung exposed to low dose x-radiation on positive group demonstrates a significant increase in IL-6 level compared to negative group. The outcome provides evidence that low-dose IR may induce inflammatory response by the production of IL-6 in lung. Under normal circumstances, cytokines are not detectable in body fluids or tissues [22]. This statement highly agrees with the present findings that the negative group demonstrate no expression of IL-6. IL-6 secreted by alveolar macrophages, type II pneumocytes and T-lymphocytes and are involved in immunologic responses and inflammation [1, 23]. Increased IL-6 level in lung from positive group may be caused by whole body irradiation-induced lung injury. This study outcome was supported by Ao et al. [23] study indicating significant increase in mice lung IL-6 level when induced by 12 Gy x-radiation. During acute lung injury, alveolar macrophages secrete pro-inflammatory cytokines IL-1, IL-6 and TNF-$\alpha$ that act locally to activate neutrophils and stimulate chemotaxis [24]. Influxes of macrophages and inflammatory cells into alveoli resulted from induction of pro-inflammatory cytokines IL-1, IL-6 and TNF-$\alpha$ [25] and elevated level of IL-6 in lung after radiation exposure is possibly due to increase macrophages production into alveolar.

Based on the findings, a significant decrease in lung IL-6 level from supplement group was observed when compared to positive group. The present study showed that inflammatory response was down regulated by 50% watermelon juice and possibly by the act of the watermelon lycopene contents that demonstrates radio protective effect against IR. The findings highly agrees with a study conducted by Yaping et al. [1] that demonstrated significant reduction in ear inflammation when mice are supplemented with lycopene at lowest dose of 0.1 g/kg body weight. Also, decrease levels of IL-6 were observed in mice with adipose tissue inflammation when treated with lycopene [26].

Relative to the type of tissue involved, lycopene shows a prominent effect in decreasing cancer risk in lung, prostate and stomach cancers [27]. A review study by Palozza et al. [28] suggested increasing evidence advocating the preventive effect of lycopene in the formation and development of lung cancer. Mechanism of action of lycopene against tumor development is related to modulation of cytokine expression [28]. Radio protective effect of watermelon is achieved when lycopene exerts its effect by scavenging singlet oxygen due to strong affinity towards free radicals as well as preventing cells from lipid peroxidation [4].
The current study also demonstrated significant differences in liver TNF-α level between negative and supplement group. Under normal circumstances, cytokines are produced by liver cells at low levels [29]. This explains the increase level of TNF-α in negative group. Excessive amount of TNF-α level in liver may result in liver injury [29], therefore lowering the production of TNF-α induced by IR will protect liver against damage. A study conducted by Agarwal and Rao [30] indicated that lycopene is the major source of carotenoid and concentrated in adrenal gland, testes, prostate gland and mainly in liver [28, 30]. Once the liver’s immune cells (kupffer cells) become activated, the production of cytokines may increase dramatically [29].

CONCLUSION

Overall, 50% watermelon juice may counterbalance the ROS production induced by low dose IR. From the study outcomes, the study proposed that supplementation of 50% watermelon juice may improve inflammatory response by decreasing the production of TNF-α and IL-6 levels following lycopene action on scavenge the singlet oxygen that caused the response.

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REFERENCES


