ORIGINAL ARTICLE

Papaya (*Carica papaya* L.) juice changes hepatic TNF-α and antioxidant status induced by low dose ionizing radiation in mice

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Abstract:

Ionizing radiation has the ability to induce oxidative stress through free radicals formation. Hepatic display multiple functional alterations after ionizing radiations exposure. Papaya (*Carica papaya* L.) is widely cultivated globally with high antioxidant and anti stimulant activity against oxidative stress. The study was aims to assess the radioprotective effect of 50% papaya juice (Carica papaya L.) in alleviating low dose ionizing radiation-induced oxidative stress in mice hepatic tissue. Eighteen male Balb/c mice were assigned into three equal groups. Negative control group received filtered tap water and normal diet *ad libitum*, radiation group given filtered tap water and normal diet *ad libitium* and supplementation group was supplemented with 50% papaya (*Carica papaya* L.) juice for 28 days. Both radiation and supplementation groups were exposed to 100 µGy gamma radiation on day 29. The activities of superoxide dismutase (SOD), total glutathione (GSH) and TNF-α levels were determined in hepatic tissues. SOD inhibition activity in supplementation group showed highly significant increment in comparison to radiation group (p=0.001), total GSH levels of liver tissue in supplementation group showed significant increment compared to radiation group (p=0.015) and TNF-α levels showed significant differences between positive and supplementation groups (p=0.000). In conclusion, these results postulates that there are significant effect of low-dose ionizing radiation against oxidative stress and inflammation, indicating the protective effects of 50% of papaya (*Carica papaya* L.) against low dose ionizing radiation induced oxidative stress in mice hepatic tissues.

Keywords: GSH, hepatic, ionizing radiation, papaya, SOD, TNF-α

1. INTRODUCTION

Broad research has been done to reveal on oxidative stress mechanism that can cause variety of diseases such as cancer and diabetes [1]. There are many sources of free radicals that may be produced within the body; such as exposure to ionizing radiation (IR). IR has brought both benefit and harm to human, as patient get benefit from treatment (chemotherapy) and unfavourable situations for workers who deal with radiation daily.

According to World Health Organization, there are several individuals that are involved in radiation exposure in their daily work activity, which include Radiation Oncologist (RO), Radiation Therapist (RT) and Medical Physicist (MP). Radiation can easily penetrate deep inside human bodies and able to damage some of the biological cells. Human bodies possess natural antioxidant defence mechanism which prevents free radicals from damaging cells. Research in managing implication of radiation is important in increasing awareness among radiotherapist commonly, about the harm they might experience throughout their career [2].

Liver is sensitive to radiation and also produce abundant enzymatic and non-enzymatic antioxidant agent (naturally produced antioxidant) including superoxide dismutase (SOD) and glutathione (GSH) which will fight against reactive oxygen species (ROS) generated [3] from IR exposure. When the body's antioxidant agent is no longer able to bear the increasing level of free radicals, diet contains high antioxidants is needed. Fruits like watermelons, berries and papaya are important in preventing oxidative stress from free radicals. *Carica papaya* Linn. known as papaya has various benefits in human health. Even though there is lack of evidence that papaya is able to reduce oxidative damage by scavenging free radicals, the extensive use of papaya in medicinal field as proposed [4] proves that papaya is beneficial to health. Papayas are with rich phytochemicals with antioxidant benefits such as carotenes, vitamin C and flavonoids (vitamin B, folate and panthotenic acid), minerals and fibre. All of these phytochemicals able to provide barriers to colon cancer and improve cardiovascular strength [4].

The purpose of this study is to investigate the radioprotective effect of prepared 50% papaya (*Carica papaya* L.) juice on the status of oxidative stress in mice liver tissue which is exposed to low dose ionizing radiation (LDIR) by measuring superoxide dismutase (SOD) and total glutathione (GSH) levels.

2. MATERIALS AND METHODS

2.1.1 Animal handling

Eighteen (n=18) Balb/c male mice, age about 4-6 weeks and weight 20 ± 5 gram. All of the mice were placed in four different cages for negative and positive control groups. The other mice were caged in five individually cages for supplement group. They were allowed to acclimatize to constant room temperature of 22 ± 2°C for one week prior to the initiation of the experiment to permit environment adaptation. The experiments were conducted in approval from UiTM Care (38/2014).

2.1.2 Preparation of papaya juice as supplement

Fresh matured papaya was obtained from local stall and authentication of the papaya breed has been done at Forest Research Malaysia (FRIM), Selangor, Malaysia. Papaya was cleaned and skin was peeled before cut and seeds were being discarded. The pulp was chopped into small cubes by using knife and inserted into Panasonic juice extractor (model PJ-67S) to extract the juice. After that, papaya juice (100% concentration) was filtered by using coffee strainer and remnants were discarded. Distilled water was added to filtered papaya juice to prepared 50% concentration of papaya juice 1: 1 (v/v). 50 ml of 50% papaya juice was placed in the drinking water bottle for mice and placed in cages. The papaya juice was prepared and given to supplement group twice daily at 8.30 am and 8.30 pm as exchange for filtered tap water for 28 days.

2.1.3 Low dose ionizing radiation (LDIR)

Ten mice from positive control and supplement groups were exposed to whole body radiation with a single dose of 100µGy.This procedure was done on day 29 of experiment at 8.30 am at the Medical Imaging Department, Faculty of Health Sciences, UiTM Puncak Alam by a qualified radiographer.

2.2 Liver superoxide dismutase (SOD)

2.2.1 Sample preparation

Potassium phosphate dibasic (KH_2PO_4), potassium phosphate monobasic (K_2HPO_4), sodium chloride (NaCl), L-methionine, niro blue tetrazolium (NBT) and riboflavin were purchased from sigma Aldrich Co., Triton X-100 was purchased from Amresco. After dissection process, hepatic tissues were rinsed in ice cold 1.15% NaCl, pH 7.2 [8] to remove any residual blood. About 1g of hepatic tissues was cut into small pieces and homogenized with ice-chilled mortar and pestle in 2 ml ice-cold appropriated buffer (Tris buffered saline, pH7.4). Then, tissues homogenate was centrifuged at 9000x g for 15 minutes at 4°C [5].

2.2.2 Assay principle

After collecting supernatant, estimation procedure of SOD is conducted according to [6] adapted from [7]. A total of 27 ml 50 mM potassium phosphate solution pH 7.8, 1.5 ml L-methione, 1 ml of nitro blue tetrazolium and 0.75 ml Triton–X 100 were mixed thoroughly and used later as master mixture. After that, 1.0 mL of the mixture was transferred to cuvette, followed by 20 µl sample supernatant and then 10 µl of riboflavin. For blank, supernatant was replaced with 20 µl of distilled water, while maintaining other mixture in cuvette.

Cuvettes containing samples and standard were covered with aluminium foil and place in dark that contain two 20-watt Syvania Grolux fluorescent lamps for 7 mins. Then, cuvettes were removed from the box and assayed spectrophotometrically. The absorbance was read at 560 nm by measuring the colour changes. A single unit of SOD enzyme represent the amount of enzyme required to inhibit the rate if NBT oxidation by 50% at 25°C.

Calculation:

$$\% \text{ SOD inhibition} = \frac{\text{Absorbance (control-sample) x } 100}{\text{Control}}$$

2.3 Determination of total glutathione

Total glutathione was measured by using commercially available kit (Cayman). Materials and reagents used in measuring GSH are GSH MES buffer (2x) metaphosphoric acid (Sigma-Aldrich) glutathione disulphide standard, GSH co-factor mixture, reconstitute enzyme mixture and reconstitute dithionitroben zoic acid (DTNB). Before dissected, liver was rinsed with PBS (pH 7.4) and then was homogenizedon ice using mortar and pestle in 5-10 ml cold 50 mM GSH MES buffer per gram of tissue. After that, prepared homogenate is centrifuged at 10,000x for 15 minutes at 4°C. The supernatant of the homogenate tissue was collected and deproteinized before assaying (Figure 1). After that, aliquot GSSG standard and MES buffer pepared. For every mole of GSSG is equivalent to 2 moles of GSH. 50 µL standard is transferred to 7 wells and followed by 50 µL of sample in other sample wells. The assay cocktail is prepared in 20 ml vial that contains 11.26 mL MES buffer, 0.45 mL reconstituted DTNB. 150 µL freshly prepared Assay cocktail transferred to each standard and samples wells, giving total volume of 200 µL in each well. Then, the plate cover is replaced and plate in dark on orbital shanker. The absorbance was read at 405 nm for 30 minutes.

2.4 Statistical analysis

All data collected were analysed by using Software Package used for Statistical Analysis (SPSS), Predictive Analytics Software version 18. Results of all tissues estimation have been indicated as the mean value ± standard error of the mean (SEM). The level of significance was

calculated using one-way analysis of variance (ANOVA) technique followed by Tukey's Posthoc test multiple comparisons. The minimum level of significance was fixed to value less than 5% ($p<0.05$) which is considered statistically significant.

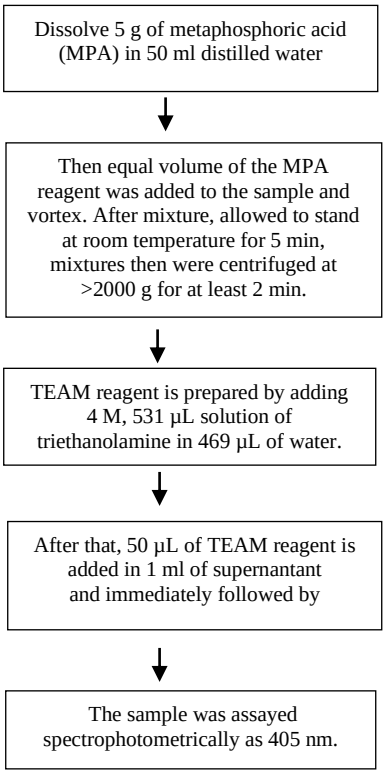

Figure 1: Tissue deproteinization process for GSH assay.

3.0 RESULTS

3.1 Superoxide dismutase inhibition activity in mice's liver tissue

Figure 2 presents data on the percentage of SOD inhibition activity in negative control, radiation and supplementation group in mice's liver tissue. SOD inhibition activity percentage is 71.27 ± 1.82% in negative control group, 32.49 ± 5.04% in radiation group and 77.27 ± 2.99% in supplementation group. 50% papaya (*Carica papaya* L.) juice supplementation showed significant increment of SOD inhibition activity in supplementation group compared to radiation group with p=0.001. There is increment of SOD inhibition activity of about 25% in supplementation group compared to radiation group and increase of about 4% in supplementation group is compared to negative control group.

3.2 Total glutathione level in mice's liver tissue

GSH is tripeptide compound consists of glutamic acid, cysteine and glycine and plays role as coenzyme in oxidation-reduction reactions in cells. Figure 3 shows results on experimental analysis of total GSH in mice's liver tissue. Total GSH level is 460 ± 24.72 µM in negative control group, 332.89 ± 45.93 µM in radiation group and 500 ± 37.16 µM in supplementation group. Supplementation of 50% papaya (*Carica papaya* L.) juice showed significant increment in supplementation group comparing to radiationgroup with p=0.015. Three percent increment of total GSH level was observed in supplementation group comparing to radiation group and there is 6% increment of total GSH level in supplementation group when compared to negative control group.

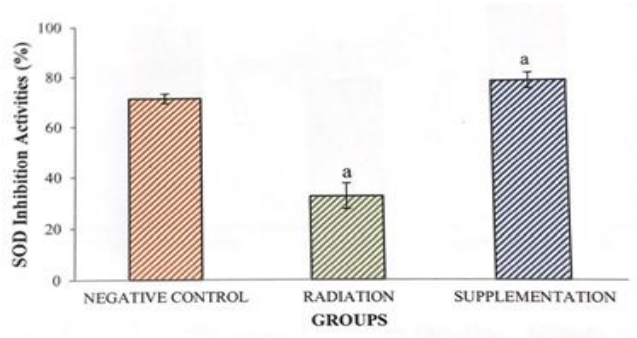

Figure 2: Effect of 50% Carica papaya L. juice on radiation-induced inhibition of superoxide dismutase (SOD) in mice liver tissue. Bars represent means ± SEM (n=6) of three separate experiments. Radiation dose used was 100 µGy. [a]indicated significant differences when compared to radiation group (p=0.001).

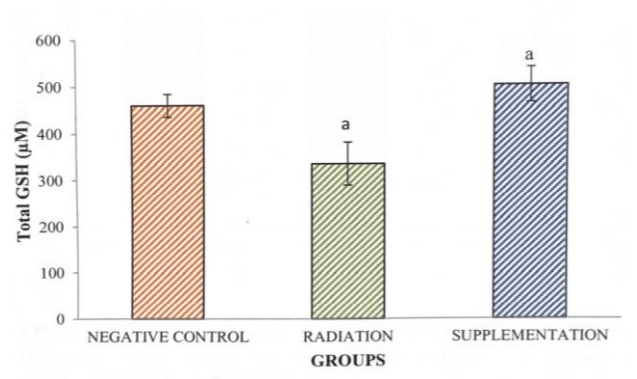

Figure 3: Effect of 50% Carica papaya L. juice on radiation-induced total GSH level mice liver tissue**.** Bars represent means ± SEM (n=6) of three separate experiments. Radiation dose used was 100 µGy. [a]indicated significant differences when compared to radiation group (p=0.015).

4.0 DISCUSSION

Exposure to ionizing radiation (IR) is found to be harmful to any living organism and even low-dose ionizing radiation (LDIR) may cause deterioration and trigger primary radio-adaptive responses. It was documented that exposure of ionizing radiation may stunt the activities of endogenous antioxidant enzymes. Antioxidant enzymes considered as first line defense system and also functions in balancing redox reaction, as well as preventing interference in normal biochemical process [9, 10] showed that diet contents may assist endogenous antioxidants activity to

improve antioxidative defense system and hence prevents oxidative stress.

Mehdipour et al. [11] stated that oxidative stress formed in the body is due to either excessive proliferation of ROS or the body itself has lost antioxidant potential. ROS are capable in altering many biomolecules including lipids, proteins and nucleic acid in means of changing their structure and action. Primary defense systems such as superoxide dismutase, glutathione and catalase are mainly act in scavenging of reactive oxygen species (ROS) to avoid lipid peroxidation [12]. SOD act against oxidative damage by catalyzing the dismutation of superoxide anion into H_2O_2 and clears away ROS, the toxic material of H_2O_2 is then transformed into H_2O and O_2 by catalase [13]. Besides that H_2O_2 also could be neutralized by the action of glutathione peroxide by donating hydrogen atom to H_2O_2 to form water.

SODs are enzymes encoded on chromosome 21 and the only enzymatic system that is responsible in quenching O^-_2 to oxygen and H_2O_2 making the enzyme significant against oxidant stress [14-16]. This study showed increment of SOD inhibition activity in supplementation group compared to radiation and negative control groups. Increment is in agreement with Zhao et al. [17], in which administration of anthocyanin extracted from *Lonicera caerulea var. edulis* (ALC) significantly increased SOD and glutathione peroxidase (GPx) activities in ICR mice's liver. It seems that treatment with papaya before irradiation protects liver from damage and hence offers radioprotection at biochemical level, thus maintaining cellular functions.

Apart from that, papaya also may have adequate phytochemicals including flavonoids which able to help increase SOD inhibition activity in treatment group. Study by Gayosso-Garcia Sancho et al. [18] and Maisarah et al. [19] showed that during papaya ripening process, the chlorophyll started to diminish, in conjunction with carotenoid synthesis which is responsible for increment of yellow-orange colour of papaya. Lycopene, an important key intermediate in biosynthesis of many carotenoid components such as beta carotene and xantophylls [20] and has a strong ability to scavenge free radicals and is responsible in quenching singlet molecular oxygen [21]. This result supports the increment of SOD inhibition activity in liver due to the presence of antioxidant component in papaya (*Carica papaya* L.,) which can increase SOD inhibition in supplementation group.

GSH was found abundantly in liver compared to other tissues because hepatocytes are the main cells producing GSH. In the present study, 50% papaya (*Carica papaya* L.,) supplemented mice resulted in increase of GSH level in supplementation mice resulted in increase of GSH level in supplementation group compared to both the radiation and negative control groups. This result is supported by [22] which stated that supplementation with 250 mg/kg papaya (*Carica papaya* L.,) juice for 20 consecutive days has significantly increase GSH level up to 8Gy irradiated male albino rats. This condition may be due to papaya (*Carica papaya* L.,) contains high concentration of flavonoids and alkaloids. Flavonoids are potent free radical scavengers because of the phenolic hydroxyl groups [23]. The present result also supported by Azarkan et al. [24] which has stated that papaya (*Carica papaya* L.,) contains abundant antioxidant phytochemicals including lycopene and β-carotene which able to reduce the usage of antioxidant enzymes in the process of fighting oxidative stress, hence capable in increasing total GSH level in treatment group.

5.0 CONCLUSION

The results from the present study show the protective effect of 50% papaya (*Carica papaya*) juice in alleviating LDIR-induced oxidative stress in male Balb/c mice's liver tissue. Oxidative stress is regulated by enzymatic and non-enzymatic antioxidant system in body. The increase trend SOD inhibition activity and total GSH which was observed in supplementation group compared to radiation and negative control group showed marked radioprotective effect of 50% papaya (*Carica papaya L.*) juice against ROS produced prior to LDIR exposure.

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