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Prophylactic and therapeutic potential of tender coconut water intervention on antioxidant status in electron beam irradiated Swiss albino mice

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Ionizing radiation induces oxidative stress due to free radicals production. The *in vitro* study has shown that tender coconut water (TCW) of West Coast tall variety exhibits potent antioxidant property. Here, we attempted to evaluate the potency of TCW in reducing radiation induced oxidative stress in the mice model. The $LD_{50/30}$ dose of electron beam radiation (EBR) for Swiss albino mice was assessed and was found to be 9.33Gy. Therefore, a sublethal dose of 6Gy was selected for further intervention studies to assess the levels of antioxidants. To evaluate the effective dose, the mice were irradiated with a lethal dose of 10Gy with the oral intervention of 50, 100 and 200 µL of TCW/20 g body wt. of mice. Findings of the study suggest that 100 µL/20 g body wt. was found to be effective in decreasing the mortality of irradiated mice. Further, intervention with TCW significantly increased the antioxidant levels compared to that of radiation control group. The results suggest that TCW exhibits radioprotective activity by potentiating the antioxidant levels in mice exposed to a sublethal dose of whole body EBR.

Keywords: *Cocos nucifera*, Ionizing radiation, Nutraceutical intervention, Oxidative stress

Radiotherapy is one of the important modalities used in the treatment of cancer[1-3]. Providing appropriate protection to the surrounding cells from unwanted effects of ionizing radiation therapy is of great concern to prevent the cancer relapse[4,5]. Considerable research efforts have been carried out to obtain an effective, economical and natural radioprotector for protecting the normal cells and selectively sensitize the cancer cells[6]. According to Weiss and Landauer[7] there is an increasing need to search natural products, especially the antioxidant vitamins and phytochemicals, for *in vivo* radiation protection.

The tender coconut water (TCW) from *Cocos nucifera* (L.) Fam. Arecaceae, is a highly nutritious drink composed of unique chemicals, such as sugar, vitamins, minerals, electrolytes, enzymes, amino acids, cytokines and phytohormones[8,9]. Vitamin C in TCW support its antioxidant property; cytokines prevent mutagenesis, and phytohormones promote stem cell proliferation[10]. TCW is useful in conditions like dehydration, digestive disturbances, kidney stones, urinary tract infections, and sterility.

For radioprotection, factors such as stimulation of stem cell proliferation, free radical scavenging, antioxidant, immunostimulation, DNA repair etc., are primarily considered. The tender coconut water (TCW) which has all these properties could be a potential candidate as a radioportector. Hence, in the present study, we investigated weather TCW intervention protects the mice from the oxidative stress induced by the free radicals generated due to the exposure to electron beam radiation.

Materials and Methods

The study was carried out in the Central Research Laboratory, KS Hegde Medical Academy and Radiation studies were carried out in the Department of Oncology, KS Hegde Medical Academy, Mangalore. Fresh tender coconut (West Coast Tall Variety) of 3-4 months was obtained from the local market in Mangalore. Care was taken to avoid coconut with solid endosperm (coconut meat or copra).

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Animal care and handling

The studies performed following CPCSEA guidelines. The study included 6-8 week old inbred, female Swiss albino mice, weighing 25±5 g. Standard conditions of light and dark (10 h light, 14 h dark), temperature, and humidity were maintained. The mice were provided with standard mouse chow and water *ad libitum*. The study was ethically approved by the Institutional Animal Ethics Committee (IAEC), Ref: KSHEMA/IAEC/16/2013.

Radiation specifications

The present study was carried out using the electron beam facility in Department of Oncology, KS Hegde Medical Academy at the distance of 100 cm at the dose rate of 1Gy/min and 15MeV energy by placing in well ventilated Perspex box.

Materials and Methods

Median lethal dose of radiation

Seventy female mice were divided into 7 groups with 10 mice each and a radiation dose of 2, 4, 6, 8, 10, 12 and 14Gy was given, and the animals were observed for 30 days. Mortality in the respective given dose was evaluated and $LD_{50/30}$ value was calculated using Probit analysis[11].

Dose fixation

One tender coconut on an average has 250 mL of water in it. Average human of 60 kg drinks one coconut/day is equivalent to 104 µL of the same to 20 g weighing mice. Accordingly, doses of 50, 100 and 200 µL of TCW were selected for intervention. Thirty female mice were divided into 3 groups with 10 mice each were used for the present study. Group I served as positive control (radiation control), Group II as normal control (control), Groups III-V were orally administered with 50, 100 and 200 µL of TCW for 5 consecutive days. Animals were exposed to lethal dose of 10Gy of electron beam radiation on 6th day and observed for mortality for 30 days. Dose which provided maximum cumulative survival rate according to Kaplan-Meier analysis was selected for further studies.

Dose reduction factor

Here, the treatment group received 100 µL of TCW for 5 consecutive days, while the non-treatment group received same amount of distilled water. On 6th day both groups received 7, 8 and 9Gy (since no mortality was observed in 6Gy whole body irradiated mice) of whole body electron beam radiation. The animals were observed for 30 days for mortality.

$$DRF = \frac{\text{LD50/30 with treatment of TCW}}{\text{LD50/30 without treatment of TCW}}$$

Evaluation of antioxidant levels

For evaluation of antioxidants, 30 mice were divided into 5 groups of 6 mice each,and both prophylactic treatment group (PTG) and therapeutic treatment group (TTG) studies were carried out. PTG were orally administered with 100 µL of TCW for 5 days before irradiating with sublethal dose of 6Gy. On 7th day, all animals were sacrificed and blood was collected and used for further studies. TTG were first subjected to 6Gy sublethal dose of radiation and 100 µL of TCW was orally administered for 5 days. On 6th day, the animals were sacrificed.

Determination of total antioxidant concentration

The assay was carried out according to Prieto *et al*.[12], method. In brief, the quantitative assay is based on the conversion of molybdenum(Mo VI) by reducing agents like antioxidants to molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex, the intensity of which can be read spectrophotometrically at 695 nm.

Estimation of reduced glutathione

The assay was carried out using Sharma *et al*.[13], method. This method is based upon the development of a relatively stable yellow colour, when 5,5'-dithiobis (2-nitro benzoic acid) is added to sulphydryl compounds including glutathione.

Estimation of lipid peroxidation

The assay was carried out using Buege *et al*.[14], method. Malondialdehyde (MDA) formed by the breakdown of poly unsaturated fatty acids (PUFA) serves as a convenient index to determine the extent of lipid peroxidation. It reacts with thiobarbutaric (TBA) to give a pink colour which was read at 535 nm.

Estimation of catalase activity

The assay was carried out using the method proposed by Taibur Rahman[15]. The UV absorption of Hydrogen peroxide was measured at 230 nm. The degradation of H_2O_2 by catalase can be measured by decrease in absorbance at 230 nm.

Estimation of superoxide dismutase activity

The estimation of superoxide dismutase enzyme is carried out by Beauchamp and Fridovich method spectrophotometrically[16].

Statistical analysis

All the results are expressed in terms of mean ± standard error mean. The statistical analysis was done

by one-way ANOVA with Dunnett multiple comparison test was performed as a post-hoc test using SPSS version 16.0 and results with *P* value <0.05 was considered as statistically significant.

Results

Calculating Median lethal dose of radiation

A line graph was plotted taking P-value% on Y-axis and $\log_{10}$ value of radiation dose on X-axis. The $LD_{50/30}$ dose was calculated by extrapolating a line from the % Probit value 5 from Y-axis to $\log_{10}$ radiation dose on X-axis as depicted in Fig. 1. The $LD_{50/30}$ was found to be $\log_{10}$ value of 0.95, Antilog of 0.97 is 9.33. Hence, according to the graph, the $LD_{50/30}$ value of radiation was found to be 9.33Gy.

Dose fixation

According to the Fig. 2, 100 µL/20 g body wt. of TCW intervention showed highest cumulative survival rate. Accordinly, the dose was selected for further studies.

Calculating dose reduction factor

Fig. 3 shows that the $LD_{50/30}$ dose for radiation with administration of distilled water was 8.13Gy (log10 value of the dose is 0.91). Whereas in the treatment groups, it was 8.75 (log10 value of the dose is 0.94). The DRF value was 1.08.

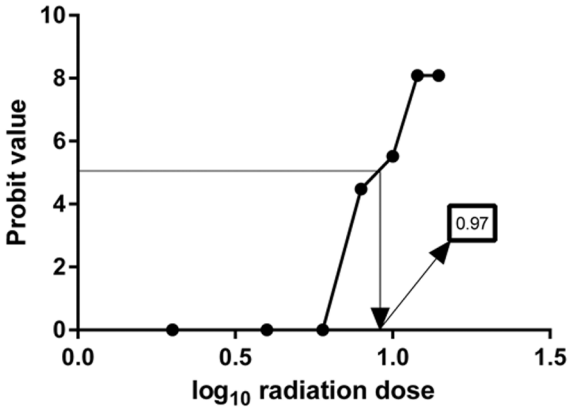


Fig. 1—Probit Graph depicting the LD50 value

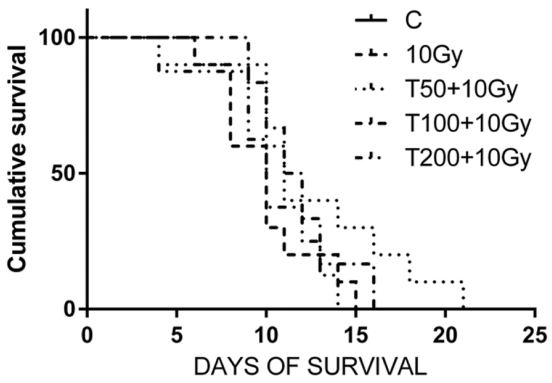


Fig. 2—Kaplan- Meier graph for various doses of TCW

Evaluation of antioxidant levels in mice

Determination of total antioxidant concentration

In prophylactic study groups, no significant difference in the total antioxidant levels was observed. Therapeutic group showed a significant decrease in the same in radiation control group when compared with control group. Treatment with TCW significantly increased the total antioxidant concentration as shown in Fig. 4.

Estimation of reduced glutathione

As shown in Fig. 5A, no significant difference in the level of reduced glutathione was observed between radiation control group and treatment groups.

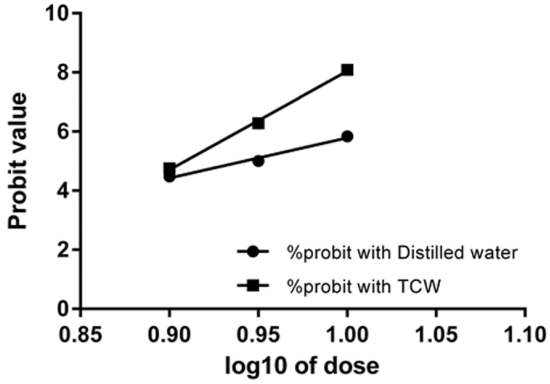


Fig. 3—Probit analysis graph for both distilled water and TCW intervention

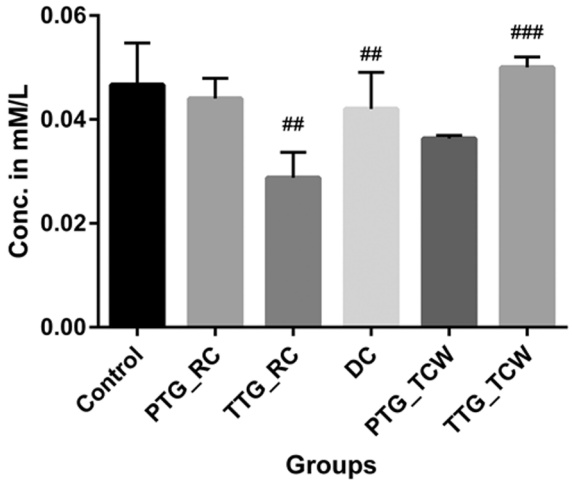


Fig. 4—Total antioxidant concentration in mM/L. [RC, DC, TCW, PTG, TTG refer to Radiation control, Drug control, Tender coconut water administered, prophylactic and therapeutic groups, respectively. *,**, ***, **** and $^{\#}$, $^{\#\#}$, $^{\#\#\#}$, $^{\#\#\#\#}$ represents *P* value <0.05, 0.01, 0.001 and 0.0001 of PTG and TTG, respectively]

Estimation of lipid peroxidation

Fig. 5B reveals a significant increase in the lipid peroxidation was observed in radiation control groups of both prophylactic and therapeutic study groups. A significant reduction in the Lipid peroxidation was observed in treatment groups.

Estimation of catalase activity

As indicated in Fig. 5C, a significant decrease in the catalase activity was observed in radiation control groups of both prophylactic and therapeutic study groups. A significant improvement in the level of catalase was observed in the treatment groups.

Estimation of superoxide dismutase activity

As shown in Fig. 5D in prophylactic studies, significant decrease in SOD activity was observed in the radiation control group when compared to the control. Significant improvement in the enzyme activity was observed in the TCW intervention group. In the therapeutic study groups too, a significant decrease in the SOD activity was observed in the radiation control group. Upon TCW intervention, though the improvement in the enzyme activity was observed, the results were not statistically significant.

Discussion

Ionizing radiation induces damage to the living cells directly by inducing double strand and single strand DNA breaks and indirectly by the process of radiolysis of the water[16]. Radiolysis of water leads to a cluster of reactions producing free radicals which is responsible for imbalance in the oxidation-reduction potential leading to the oxidative stress. Thus, from past few years, researchers are evaluating various herbs and herbal formulations which possess rich antioxidant properties for their radioprotective potential[17]. In the present study, we attempted to evaluate the radioprotective efficacy of TCW.

Present study result suggests that 9.33Gy of whole body electron beam radiation as the median lethal dose for Swiss albino mice. Sato T *et al*.[18] subjected mice to 7, 7.5 or 8Gy whole body radiation, and observed survival rates of 67, 47, and 0%, respectively in untreated animals. Whereas Madhu *et al*.[19], showed $LD_{50/30}$ of electron beam radiation to be 10 Gy. The difference may be because of different instrument and difference in dose rate.

To start an intervention study, it is necessary to determine an effective drug dose. The effective dose for radiation was calculated using Kaplan–Meier analysis. Three different doses were selected.

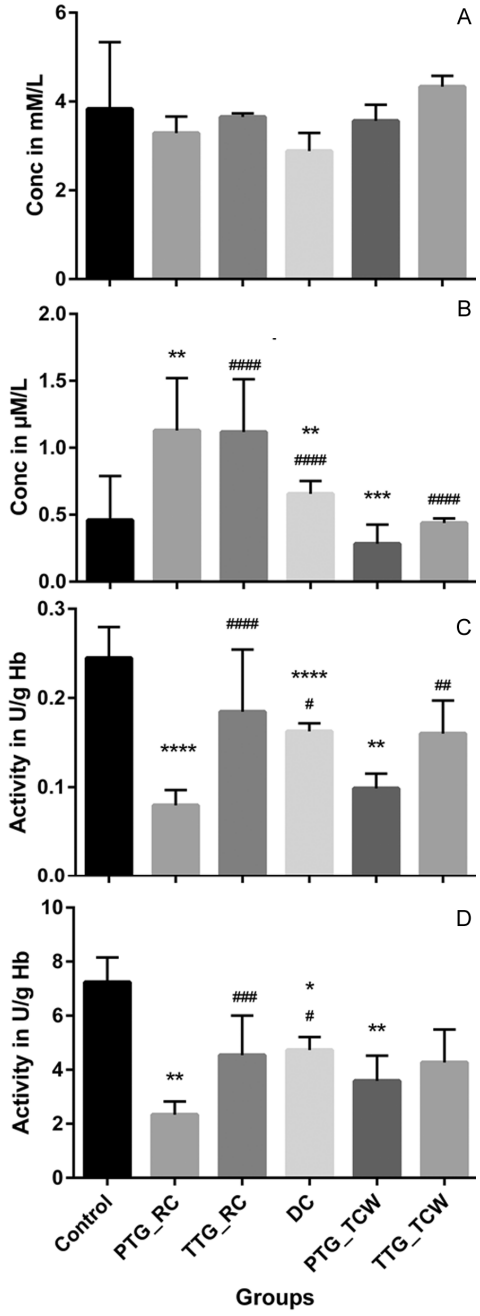


Fig. 5—Antioxidant level in PTG and TTG. Levels of (A) Reduced glutathione; (B) Melondialdehyde; and activity levels of (C) catalase; and (D) superoxide dismutase activity. [Here RC, DC, TCW, PTG, TTG refers to Radiation control, Drug control, Tender coconut water administered, prophylactic and therapeutic groups respectively. ***, ***, **** and #, ##, ###, #### represents *P* value <0.05, 0.01, 0.001 and 0.0001 of PTG and TTG, respectively]

The results of the present study showed the highest cumulative survival rate was observed in 100 µL/20 g body weight of TCW. Thus it was selected for further intervention studies.

Blood antioxidant level assessment is the important parameter to check radioprotective property of the herbal formulation1[20-22]. In the prophylactic study, radiation control group showed a significant decrease in the SOD and catalase level, increase in the MDA level when compared to the control group. Radiation control group of therapeutic study showed a significant decrease in the SOD and total antioxidant concentration when compared with the control group. From the above results we can interpret that selective antioxidants get activated based on the free radical generated and duration from the time of exposure to electron beam radiation.

Prophylactic study results showed significant decrease in the lipid peroxidation in the TCW intervention group. In therapeutic study, significant improvement in SOD and total antioxidant concentration was observed in TCW treatment groups. Another study showed significant decrease in the levels of SOD and catalase, but moderate decrease of GSH levels were found in the serum of irradiated animals[23] which is in alignment with the results of our present study.

Dose reduction factor (DRF) is one of the important factors to determine the radioprotective efficacy of a radioprotector. According to the present study, DRF value of TCW was found to be 1.08Gy, which can be recommended for intervention as adjuvant therapy.

Conclusion

Findings of the above study suggest that tender coconut water (TCW) intervention significantly ameliorated the reduction of antioxidant status in irradiated mice model. The study also reccommends that the dosage of intervention, 100 µL TCW/20 g of body wt. of mice, to be optimum concentration for effective radioprotective effect. Further, finding of the survival study shows that the DRF value for TCW intervention was 1.08Gy indicating that TCW could be one of the potential radioprotective nutracuetical agents.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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