Palliative effects of lutein intervention in gamma-radiation-induced cellular damages in Swiss albino mice

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Abstract:

Objectives: Radiation-induced hematological, biochemical, and cytogenetic damages to the normal cells are major concerns in the field of radiotherapy. The carotenoids and their derivatives have been the source of antioxidants with wide range of medicinal applications. The objective is to evaluate the protective effects of lutein, a carotenoid, against radiation-induced cellular and tissue damages.

Methods: Swiss albino mice were grouped into 5, 50, 250, and 500 mg/kg b.wt. of lutein treatment groups, a sham and vehicle control group. The groups were irradiated with a lethal dose of 10 Gy γ-radiation. The mortality was recorded for 30 days to optimize the protective dose against radiation. The mice were administered with the compound orally for 15 consecutive days and irradiated with a sublethal dose of 6 Gy. The hematological changes in blood and antioxidant parameters were determined in liver, kidney homogenates, and hemolysate/serum. The hematological parameters were recorded using an automated cell counter. The antioxidants such as malondialdehyde (MDA), glutathione (GSH), superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase were spectrophotometrically determined.

Results: The red blood cell, white blood cell count, lymphocyte count, hemoglobin, platelet levels, and hematocrit value were found to be decreased in the irradiated groups. Lutein pretreatment maintains near-normal levels of these parameters indicating resistance/recovery from the radiation-induced damages. The antioxidant levels were found to be reduced in all the irradiated groups. However, lutein pretreatment (50 mg/kg b.wt.) has increased the catalase activity of hemolysate. Lutein pretreatment has reduced the MDA levels in hemolysate, when administered at doses of 5, 250, and 500 mg/kg b.wt. in comparison to its control.

Conclusion: The study demonstrates the radioprotective potential of lutein by maintaining the hematological and antioxidant homeostasis.

Key words: Antioxidant, carotenoid, hematoprotective, lipid peroxidation, lutein, radioprotection

Radialation is increasingly used for medical and occupational purposes; it is an established weapon in the diagnosis and therapy of cancer. Radiotherapy destroys cells in the area being treated (or “target tissue”) by damaging their genetic material. Radiation also causes formation of free radicals that are unstable molecules which react easily with essential molecules of our body including DNA, fat, and proteins changing their chemical structure. When a free radical attacks a molecule, it will then become a free radical itself, causing a chain reaction which can result in the destruction of a cell. Radiation targets to kill tumor cells, but there is also damage to the normal tissues surrounding the tumor. The biological effectiveness of radiation depends on factors such as linear energy transfer, total dose, fractionation rate, and radiosensitivity of targeted cells or tissues. The goal of the radiation therapy is to minimize the dose delivered to normal cells or tissues thus reducing the damage and maximize the dose effect to tumor cells.

Radioactive elements in their decay process emit a form of nuclear radiation - the gamma radiation. It is a form of ionizing radiation, having high penetration and low ionizing power. Gamma rays have sufficient energy to break the bonds of genetic material, structural components of cells, and other biological molecules. Gamma rays can kill living cells; thus, they are used in external beam radiation therapy to kill tumor...
cells. External beam radiation therapy is given in the form of photon beam. It is the photons from gamma radiation that has the highest energy.

Lutein, a carotenoid that does not exhibit provitamin A activity has in it both β- and ε-ionone rings. It is one of the many carotenoids found naturally occurring in plants such as green leafy vegetables (spinach, kale), corn, egg yolk, and animal fats.[6]

In a study by Craft,[5] it was found that lutein is highly soluble in tetrahydrofuran and least soluble in hexane. The solubility of lutein as reported in dimethyl sulfoxide (DMSO) is 1000 mg/L.

Lutein and zeaxanthin are the only carotenoids found in both macula and lens of the human eye (zeaxanthin predominating in the macula lutea, lutein is present in the retina) having dual function in both tissues to act as powerful antioxidants[5] and to filter high energy blue light[7] protecting the eyes from oxidative stress.

Lutein, in the present study, has been explored for its ameliorative effect against gamma-radiation-induced oxidative damages in Swiss albino mice in comparison to a well-known radioprotector gallic acid.

**Methods**

Lutein (90% pure) was purchased from Haihang Industry, Jinan city, China; gallic acid from Sigma Co., and all the other chemicals required were purchased from Merck and Himedia.

**In vivo Studies: Swiss Albino Mice**

Inbred Swiss albino mice, 6–8-week-old, weighing 25 ± 5 g was used in the study. The animals were maintained under standard conditions of light and dark (10 h light, 14 h dark), temperature, and humidity. The mice were given standard mouse chow and water ad libitum. The study was ethically approved by the Institutional Animal Ethics Committee (IAEC), Ref: KSHEMA/IAEC/02/2014.

**Irradiation of Mice**

The mice were irradiated using Gamma chamber 5000, which is a compact self-shielded Co-60 research irradiator facility available in the Centre for Application of Radioisotopes and Radiation Technology (CARRT), Mangalore University. It has a dose rate maximum of 9 kGy/h approximately at the center of sample chamber. The mice were placed in a well-ventilated 3 cm × 6 cm perspex box for irradiation.

**Acute Toxicity Determination**

A single high dose of lutein suspended in 10% DMSO at the limit test dose of 2000 mg/kg b.wt. according to the Organisation for Economic Co-operation and Development guidelines 423 (OECD 2001) was administered orally at 1 mL/100 g b.wt. to 6 Swiss albino mice weighing 25 ± 5 g. It was observed for mortality and general behavior for about 3–4 days. Animals were checked for mortality; further dosing at the next higher or next lower level is carried out according to the OECD guidelines until the study objective is achieved. Ten percent DMSO was chosen as a solvent since lutein formed a clear suspension in it and it is also proven to be nontoxic to mice.

**Dose Optimization for Radioprotection**

Mice were divided into five groups of 6 mice each. Ten percent DMSO was administered to the control group and lutein at different doses of 5, 50, 250, and 500 mg/kg b.wt. was administered for 15 consecutive days to the respective groups and was irradiated 1 h after administration of 10% DMSO/lutein (5, 50, 250, and 500 mg/kg b.wt.). 10 Gy was selected for survival studies to calculate LD₅₀ as it kills 50% of the animals within 30 days due to gastrointestinal syndrome and bone marrow syndrome. The mice were monitored for 30 days and any sign of radiation sickness and mortality was recorded. The survival function thus obtained determines the survival of the whole organism. The results were plotted in Kaplan–Meier curve using SPSS software version 16 (SPSS Inc, Chicago, IL, USA). Based on these results, the optimum dose of the compound for radioprotection was obtained. The mice were further divided into ten groups of six mice each.

- **Group I:** Normal control (NC) (received standard mice feed and water ad libitum daily)
- **Group II:** DMSO control (10% DMSO for 15 consecutive days)
- **Group III:** Radiation control (RC) (10% DMSO for 15 consecutive days + radiation)
- **Group IV:** Lutein control (LC) (received highest dose, i.e., 500 mg/kg b.wt. for 15 consecutive days)
- **Group V, VI, VII, and VIII:** Lutein (5, 50, 250, and 500 mg/kg b.wt., respectively) for 15 consecutive days + radiation
- **Group IX:** Gallic acid control (GC) (100 mg/kg b.wt. gallic acid for 15 consecutive days)
- **Group X:** 100 mg/kg b.wt. gallic acid for 15 consecutive days + radiation

A sub-lethal dose of radiation (6Gy gamma radiation) was given to the groups for hematological and antioxidant studies.

**Dissection of Mice**

The mice were dissected within 24 h of irradiation. They were anesthetized and the whole blood was collected by cardiac puncture for hematological estimations. The organs – liver and kidney were dissected and homogenized for antioxidant estimations.

**Tissue Homogenation**

The tissues of liver and kidney were washed with ice-cold phosphate-buffered saline (PBS) and a 10% homogenate was prepared in ice-cold PBS, pH 7.4 with Remi (RQ-127A) homogenizer. The homogenized samples were then centrifuged for 20 min at 10,000 rpm at 4°C in Remi cooling centrifuge (C24BL). The supernatant was separated and used for all the estimations.

**Hematological Studies**

The hematological studies were done using Erma veterinary blood cell counter (PCE-210VET) using the whole blood collected in 2% ethylenediaminetetraacetic acid tubes.

**Antioxidant Studies**

Spectrophotometric methods were used for the measurements which were recorded in Systromics PC-based double-beam ultraviolet spectrophotometer 2202.

**Total Antioxidant Capacity**

The total antioxidant capacity assay is a nonspecific assay that measures the level of total antioxidants present in the system
that scavenge the free radicals. One hundred microliter of sample was treated with 100 μL of trichloroacetic acid (TCA). The mixture was allowed to stand for 5 min and centrifuged at 3000 rpm and the supernatant was separated. One hundred microliter of supernatant was taken and 1 mL of molybdic acid reagent was added. The mixture was kept in a boiling water bath for 90 min. The absorbance was read at 695 nm. The molybdic acid reagent contained 0.6 M sulfuric acid, 28 mM sodium dihydrogen orthophosphate, and 4 mM ammonium heptamolybdate.[8]

Superoxide Dismutase
The superoxide dismutase (SOD) activity was determined by treating 0.1 mL of sample with a mixture of methionine, riboflavin, and nitro blue tetrazolium chloride. This mixture was allowed to stand 10 min under bright light. The blue-green-colored solution was read at 560 nm.[8] The activity of SOD was expressed in units/mg of protein for homogenates.

Glutathione Peroxidase
The glutathione peroxidase activity was measured by treating 0.25 mL of sample with 0.2 mL of 400 mM phosphate buffer, 0.05 mL of 10 mM sodium azide, 0.1 mL of 4 mM reduced GSH, and 2.5 mM of hydrogen peroxide. The reaction mixture was incubated at 37°C for 30 min and centrifuged at 3000 rpm for 10 min. One hundred microliter of the supernatant was treated with 0.25 mL of 10% TCA, 1.5 mL of 0.3 M phosphate buffer, 1 mL distilled water, and 0.25 mL of 5,5'-dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent). The absorbance was measured at 412 nm and the activity expressed in units/mg of protein for homogenates.[8]

Catalase
The catalase activity was determined by treating 10 μL of sample with 3 mL of 60 mM hydrogen peroxide. The kinetic measurement was taken at 240 nm with 30 s delay for 2 min.[8] The activity was expressed in units/mg of protein for homogenates and units/mg of hemoglobin for hemolysates.

Glutathione-S-transferase
Twenty microliters of sample was treated with the buffer mixture containing 0.1 M sodium phosphate buffer (pH 6.5), 20 mM GSH (reduced), and 20 mM 2,4-dinitrochlorobenzene. The kinetic measurement was recorded at 340 nm with 30 s delay for 5 min.[8]

Lipid Peroxidation and Membrane Stabilization
Formation of malondialdehyde
The formation of malondialdehyde (MDA) was estimated by diluting 0.1 mL sample with 0.4 mL of distilled water and 1 mL trichloroacetic acid–thiobarbituric acid reagent. The reaction mixture was kept in a boiling water bath for 15 min. The endpoint was measured at 535 nm and calculated using MDA standard curve.[8]

Reduced glutathione
The reduced GSH was estimated by treating 0.1 mL of sample with 1.5 mL of precipitating solution containing metaphosphoric acid and sodium chloride. The mixture was allowed to stand for 10 min and centrifuged. 0.5 mL of this supernatant was treated with 2 mL of 0.3M phosphate solution and 0.25 mL of 5,5'-dithio-bis-(2-nitrobenzoic acid). The absorbance was read at 412 nm within 10 mins and calculated using a GSH standard curve.[8]

The results were analyzed by one-way ANOVA, post hoc Tukeys test, using SPSS software version 16.

Results

Acute Toxicity Test
In the acute toxicity study, lutein up to 2000 mg/kg b.wt. orally exhibited no toxic effect or death in mice.

Optimization of Lutein Dose for Radioprotection
Survival assay revealed that lutein when administered orally at concentrations of 5, 50, 250, and 500 mg/kg b.wt. for 15 consecutive days and then irradiated.

Kaplan–Meier plot is depicted in Figure 1. Maximum and prolonged survival was observed in the group treated with 50 mg/kg b.wt. lutein after irradiation with a lethal dose of 10 Gy gamma radiation.

Analyses of the hematological parameters
A significant decrease in the RBC count was observed in RC when compared to the NC group (P < 0.001). The irradiated groups pretreated with gallic acid and different doses of lutein have significantly maintained the RBC levels similar to their control when compared to the RC group (P < 0.001) [Figure 2].

Mean hemoglobin level in different study groups is depicted in Figure 3 which was not found statistically significant. However, lutein treatment has maintained the hemoglobin levels to normal levels in comparison to its control group. Pretreatment of animals with gallic acid has increased the hemoglobin level in comparison to its control.

There is a significant reduction in the white blood cell (WBC) count of RC group when compared to the normal (P < 0.01), whereas near-normal levels were seen in the group pretreated with 500 mg/kg b.wt. lutein (P < 0.05) [Figure 4].
Differential cell count indicates that percentage of lymphocyte has decreased whereas the monocyte and granulocyte percentage has increased in the irradiated groups, but the results are not found statistically significant [Figure 5]. However, this variation is not seen in the gallic acid-treated group.

A significant decrease \( (P < 0.001) \) in the hematocrit values in the RC when compared to untreated normal group was observed. The irradiated groups pretreated with gallic acid and 500 mg/kg b.wt. lutein have maintained the values to near normal when compared to the RC \( (P < 0.01, 0.05, \) respectively) [Figure 6].

There is a significant decrease in platelet levels in the RC group when compared to NC \( (P < 0.05) \); however, platelet levels closer to normal were observed in the group pretreated with gallic acid and lutein 5 mg/kg b.wt. \( (P < 0.05) \) [Figure 7].

**Results of enzymatic and nonenzymatic antioxidants**

Antioxidant studies revealed statistically no much significant difference between the groups.

**Total antioxidant capacity**

The results of mean total antioxidant capacity with standard deviation under different study groups are depicted in Figure 8. Statistical analyses carried out showed no significant difference in total antioxidant capacity among the different study groups in comparison to their respective control groups. The total antioxidant capacity of the liver and kidney homogenates has reduced in all the irradiated groups. However, 50 mg/kg b.wt. lutein pretreatment group has shown an increase in total antioxidant capacity in the hemolysate when compared to the LC group.

**Superoxide dismutase**

The results of SOD activity are depicted in Figure 9. The results reveal that the mean activity among different study groups was not found statistically significant from their respective controls. A decreased activity in the hemolysate, liver and kidney homogenates of all the irradiated groups were observed. However, gallic acid pretreatment resulted in increase of SOD activity in hemolysate and the organ homogenates. Lutein pretreatment both at the dose of 50 and 250 mg/kg b.wt. have found to increase SOD activity in comparison to its control group.

**Glutathione peroxidase**

The mean along with the standard deviation of glutathione peroxidase activity is depicted in Figure 10. GPx activity was found to be increased in the hemolysate of lutein pretreated groups at different doses of 5, 50, 250, and 500 mg/kg b.wt. Similarly, gallic acid pretreatment increased the activity in hemolysate and liver homogenate. However, mean activities were not found to be statistically significant.
Catalase activity was higher in the group treated with 50 mg/kg b.wt. lutein, when compared to that of RC in hemolysate ($P < 0.05$) [Figure 11].

Glutathione-S-transferase
The activity of GST among the different study groups is given in Figure 12. A reduced activity was observed in all the irradiated groups in comparison to their respective control groups. The gallic acid pretreatment has shown an increase in GST activity of kidney homogenate in comparison to GC group. However, the statistical analyses carried out gave no statistical significant change.

Lipid peroxidation and membrane stabilization
Formation of malondialdehyde
There is a significant increase ($P < 0.01$) in the serum MDA levels in RC group when compared to that of normal indicating increased lipid peroxidation, whereas it was reduced in the groups treated with 5, 250, and 500 mg/kg b.wt. lutein ($P < 0.05$) and gallic acid ($P < 0.05$) [Figure 13].

Glutathione
Reduced GSH levels remained in the normal range in the group treated with gallic acid prior irradiation when compared to that of RC in serum ($P < 0.01$) [Figure 14].

Discussion
Gamma radiation has been widely used as a therapeutic source in the treatment of different types of cancer.\[9\] It has a broad range of applications and mediates various forms of cancer cell death such as apoptosis, necrosis, autophagy, mitotic catastrophe, and senescence.\[10\] Apart from its utilities, gamma radiation has its shortcomings as it affects not only the tumor cells but also the normal cells. The unexposed cells have also been shown to display the radiological changes when placed with the exposed cell media by the intercellular communication and also through the factors released by the exposed cells into the media.\[11\] The major studies have revealed that gamma radiation at sublethal doses, inflicted damages to the cell membrane,\[12\] increases the lipid peroxidation and reduces the total antioxidant capacity.\[13\] These effects are caused by the free radicals generated by radiolysis of water within the cell. The antioxidants which have been known to scavenge these free radicals are currently the subject of our interest as they have proven to be effective radioprotectors.\[14\] The carotenoids such as β-carotene and retinol have been previously studied for their protective effects against radiation-induced biochemical damages.\[15\] In the present study, lutein has been evaluated as a radioprotector against gamma radiation-induced...
biochemical changes although lutein has been shown to be a moderate antioxidant in vitro in the previous studies.\(^{[16]}\)

The direct effect of any radioprotector is to enhance the survival. Hence, in the present study, lutein was optimized for its optimum radioprotective dose which was found to be 50 mg/kg b.wt. of mice. Further, the study was extended to sublethal radiation dose effects. At sublethal dose of radiation, the survival is not affected, but there are dose-dependent damages seen in many organs and tissues in the body. The hematological parameters which are normally affected by radiation are hemoglobin, red blood cell count, WBC count, and other red cell indices which are reduced in irradiated conditions.\(^{[17]}\) The major biochemical changes seen are decrease in GSH, the antioxidant enzymes catalase, SOD,\(^{[18]}\) and increased lipid peroxidation indicated by the MDA formation.\(^{[19]}\)

In the present study, there is a decrease in the hematological parameters like hemoglobin, red blood cells and WBC counts in the irradiated groups compared to the nonirradiated groups. There is a decrease in the total antioxidant capacity, GSH levels in the irradiated groups. The antioxidant enzymes have not shown any significant decrease but are present in the lower range which indicates an altered function in the irradiated groups. Further, there is increase in the MDA levels in the irradiated groups which indicate membrane lipid peroxidation. Lutein treatment prior irradiation has reduced the radiation-induced effects by maintaining the hematological parameters, total antioxidant capacity, GSH, antioxidant enzyme levels, and reduced the MDA level indicating decrease in lipid peroxidation.

The major drawback with the carotenoids is their poor bioavailability\(^{[20]}\) as they need specific transport processes for their absorption and the desired effect.\(^{[21]}\) Lutein’s bioavailability and hydrophilicity may be increased by administering it as poly (lactic-co-glycolic acid)-polyethylene glycol nanocapsules\(^{[22]}\) or by combining lutein with polyvinylpyrrolidone (lutein-loaded particles) which increases the stability when compared to that of free lutein against light by 3.3 times, heat by 1.7 times, and oxygen by 4 times.\(^{[23]}\)
Conclusions

Lutein, a xanthophyll pigment which is a dietary compound, in the present study has revealed protective property against radiation-induced hematological parameters and enzymatic, nonenzymatic antioxidants. Lutein has tried to maintain near-normal level of red blood cell count and at 500 mg/kg b.wt., it has also maintained the WBC and the hematocrit levels. There was also a significant difference in the platelet levels of mice treated with 5 mg/kg b.wt. lutein in comparison to the RC group.

Oral administration of lutein at a dose of 50 mg/kg b.wt. has increased the catalase activity of hemolysate significantly in comparison to the RC group. At doses of 5, 250, and 500 mg/kg b.wt. lutein, the MDA levels were found to be decreased significantly in comparison to the RC group which indicates a reduced risk for membrane lipid peroxidation. Oral administration of lutein for 15 days shows protective effects in mice against the radiation-induced hematological damages and alteration of antioxidant system. This may suggest the use of lutein as a radioprotector in the near future, either alone or as a formulation.

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Conflicts of Interest

There are no conflicts of interest.

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