Radioprotective Efficacy of Lutein in Ameliorating Electron Beam Radiation-induced Oxidative Injury in Swiss Albino Mice

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Abstract

Background: Lutein, a carotenoid compound, has previously been studied for its antioxidant and medicinal properties as well as the moderate protection it offers against gamma radiation. This study aimed at evaluating the effects of lutein against radiation-induced hematological and biochemical changes in mice.

Methods: The optimized dose of the compound was orally administered for 15 days, and the mice were irradiated (6 Gy) on day 15 after the administration of the compound. The groups were divided (6 mice in each group) into normal control, radiation control, gallic acid control, 10% DMSO control, lutein control, and irradiated groups pretreated with gallic acid, 10% DMSO, and lutein. Gallic acid was used to maintain a standard since it is a proven radioprotector. Within 24 hours post irradiation, the animals were anesthetized and sacrificed. The hematological, biochemical, and antioxidant changes were determined using suitable methods. Data were analyzed by the Kaplan–Meier curve (log-rank test) and ANOVA (the Tukey test). The independent t test was used to compare the independent groups. SPSS (ver. 16) was employed.

Results: Maximum survival was observed with a dose of 250 mg/kg b.wt lutein. The total leukocyte count and the percentage lymphocyte count exhibited a significant decline in the irradiated groups pretreated with gallic acid and lutein in comparison to their controls, whereas the percentage granulocyte count showed a significant rise. Antioxidant activity had markedly declined in the irradiated groups, indicating oxidative stress. Lutein pretreatment reduced the damage and maintained the antioxidant system.

Conclusion: The present study suggests a protective role for lutein in palliating radiation-induced oxidative changes and maintaining the antioxidant system in vivo.

Keywords ● Antioxidants ● Carotenoids ● Lutein ● Oxidative stress

Introduction

Cancer, in today’s world, is alarmingly increasing. This increase in the cancer rate demands a need for radioprotectors which can provide protection at different doses of ionizing radiation (IR), can be orally administered with least toxicity at higher doses, readily...
absorbed, easily available in nature and cost effective.\(^1\)

Radiation has become an important module in the diagnosis and treatment of cancer. It is an important source in the diagnosis and treatment of cancer. There is, however, damage to the genetic material of the cells that are present in the area being exposed to radiation. Thus, there is a limitation in the amount of IR that can be used to treat tumors.\(^2,3\)

Electron beam radiation (EBR) is one such form and has a unique place in the field of radiation oncology, especially in the treatment of skin cancer.

The delivery of agents at the beginning of radiotherapy is expected to target critical biochemical pathways in cells that are yet to be exposed to radiation with a view to either decrease the magnitude of a response or convert the response to an alternate biochemical pathway.\(^4\) Survival after radiation exposure may be dependent on several factors, including the prevention of damage by inhibiting free-radical generation, efficient scavenging of the free radicals, DNA repair, membrane repair, and the replenishment of the severely damaged/dead cells.\(^5\) An effective radioprotector is the one that improves 30-day survival by providing protection against gastrointestinal syndrome, hematopoietic syndrome, or both.\(^6\) Oxidative stress (OS) remains a prominent factor in IR-induced damage; hence, the use of antioxidants both in fundamental research into radiation countermeasures and in clinical radiotherapy needs to be warranted.\(^7\)

OS has been shown to be a chronic component of radiation damage to organs and tissues.\(^7\) Dietary antioxidant supplements are the sources of effective countermeasure to prevent IR-induced OS.\(^8\) Antioxidants function by preventing the accumulation of toxic levels of reactive oxygen species (ROS), which can damage cells by modifying proteins, lipids, and DNA content.\(^9\)

Lutein has previously been studied for its antioxidant and other medicinal properties.\(^10-14\)

Lutein reduces the intestinal damage caused by anticancer drug and, thereby, plays an important role in adjuvant cancer therapy. In a study conducted in 2013 by Chang et al.,\(^15\) pretreatment with lutein inhibited methotrexate-induced ROS generation and apoptosis in intestinal epithelial cells. The bio-accessibility of lutein in the small intestine is greater than that of β-carotene or lycopene. It has been shown that oral supplementation with lutein can accumulate in the skin, thus diminishing ROS generation after ultraviolet (UV) exposure.\(^16\)

Oral supplementation with lutein, lycopene, and β-carotene for 12 weeks reduced lipid peroxidation (LPO) caused by UV-B type of radiation in the human fibroblasts and conferred improvement in the thickness, texture, density, and scaling of skin.\(^17\)

Gallic acid is a potential antioxidant and has previously been shown to confer protective effects against radiation in mice.\(^18\) Thus, gallic acid was used as a standard in the present study. The oral LD\(_{50}\) for dimethyl sulfoxide (DMSO) is 21.4 g/kg body weight (b.wt).\(^19\) It was the solvent chosen for suspending lutein in our study.

### Materials and Methods

Lutein 90% was purchased from Haihang Industry, China. All the other chemicals required for antioxidant studies were purchased from HiMedia, Merck, and gallic acid was procured from Sigma.

The present study was ethically approved by the institutional Animal Ethics Committee, K S Hegde Medical Academy (Ref.KSHEMA/IAEC/20/2014). This study is an approach made to evaluate the protective effects of lutein against EBR-induced biochemical changes in Swiss albino mice.

### Irradiation

Radiation facilities at the Department of Oncology, Nitte Leela Narayana Shetty Memorial Institute, Mangalore, were used. EBR was delivered at the dose rate of 3 Gy/min, 15 MeV, with a source-to-target distance of 100 cm. The mice were placed in a well-ventilated Perspex box for irradiation.

### Dose Optimization\(^20\)

Swiss albino mice were divided into 5 groups of 6 mice each. The control group received 10% DMSO (w/v). Lutein was orally administered at doses of 5, 50, 100, and 250 mg/kg b.wt to the respective groups for 15 consecutive days, after which a lethal dose of 10 Gy radiation was given on the 15\(^{th}\) day 1 hour after the administration of the compound. The mice were observed for 30 days post radiation. The group in which maximum animals survived was recorded.

The animals were orally administered with water/gallic acid/10% DMSO/lutein for 15 consecutive days. The groups were normal control (NC, water), radiation control (RC, water+EBR), gallic acid control (GC, 100 mg/kg b.wt of gallic acid), gallic acid pretreatment (GR, 100 mg/kg b.wt of gallic acid+EBR), DMSO control (DC, 10% DMSO), DMSO pretreatment (DR, 10% DMSO+EBR), lutein control (LC, optimized dose-250 mg/kg b.wt), and lutein pretreatment (LR, 250 mg/kg b.wt of lutein+EBR).
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Dissection
On the 15th day, 1 hour after the administration of the compound, the mice were subjected to a sublethal dose of 6 Gy EBR. The mice were anesthetized and dissected within 24 hours of the irradiation. Blood was drawn via the cardiac puncture method into 2% EDTA tubes. The liver, brain, and lungs were dissected out for antioxidant level estimation.

Homogenates (10%) of the tissues were prepared in ice cold PBS, pH 7.4, using a homogenizer. The homogenates were used directly for total antioxidant capacity and LPO assays, and the remaining homogenate was utilized for other antioxidant assays after centrifugation at 10000 rpm for 20 minutes at 4 °C.

Hematological Studies
The hematological parameters were estimated in a blood cell counter. Parameters such as white blood cell count (WBC); red blood cell count (RBC); differential count (percentage granulocyte, monocyte, and lymphocyte counts); and platelet, hemoglobin, and hematocrit values were taken into consideration.

Antioxidant Studies
The method of Prieto et al.21 was followed to measure the total antioxidant capacity. The method described by McCord and Fridovich22 was employed to determine superoxide dismutase (SOD) activity. Catalase activity was determined via the method of Aebi.23 LPO was assessed by the method described by Ohkawa.24 The method of Ellman25 was followed to quantify the glutathione (GSH) levels.

Serum total protein, albumin, urea, creatinine, glutamate oxaloacetate transaminase (SGOT), and glutamate pyruvate transaminase (SGPT) activities were assessed as a part of kidney and liver function tests.

Statistical Analysis
The results for dose optimization were analyzed by plotting the Kaplan–Meier survival curve, followed by the log-rank test, to compare the factors using SPSS software, version 16. The results of the hematological parameters and the antioxidant and biochemical assays were analyzed via the independent \( t \) test. \( P<0.05 \) was considered statistically significant.

Results

Dose Optimization
Lutein, administered to the mice at a dose of 2000 mg/kg b.wt, exhibited no harmful effects with respect to mortality and behavior. Hence mice were fed with 5, 50, 100, and 250 mg/kg b.wt of lutein to optimize the dose for protection against EBR. Based on the Kaplan–Meir plot for survival analysis, the optimum dose was found to be 250 mg/kg b.wt (figure 1). The Mantel–Cox log rank test gave a \( \chi^2 \) value of 32.325, which suggests a highly significant difference in the survival rate between the 6 study groups (\( P=5.1233 \times 10^{-6} \)).

Hematological Parameter Analysis
The hematological parameters, analyzed for their mean, standard deviation, and differential count in percentage, are presented in table 1. The WBC was reduced in the irradiated groups in comparison to their respective controls. A significant reduction in the WBC levels was observed in the LR and DR groups when compared to their control groups (\( P=0.001 \) and \( P=0.047 \), respectively).

Among the differential cell count parameters, the percentage granulocyte count rose significantly in the RC group (\( P=0.001 \)). Similarly, the percentage granulocyte count in the DR and LR groups also exhibited a significant increase (\( P=0.005 \) and \( P<0.001 \)). In the GR group, the increase was significant as compared to its control (\( P<0.001 \)). A significant increase in the percentage monocyte count was observed only in the LR group compared to its control group (\( P=0.011 \)). However, the percentage lymphocyte count in the irradiated groups decreased significantly in comparison to the RC group (\( P=0.001 \)). A significant reduction in the percentage lymphocyte was found in the LR and DR groups (\( P<0.001 \) and \( P=0.005 \), respectively). In the GR group, the percentage lymphocyte had a significant drop in comparison with its control group (\( P<0.001 \)).

However, no statistically significant changes were observed in the RBC, hemoglobin,
and hematocrit levels. The platelet count demonstrated a significant increase in the GR group when compared to its control (P=0.03). There was only a very small difference in the mean values of the different hematological parameters between the LR group and its control group. Additionally, a slight increase in the RBC level was observed in the DR group, which was significantly higher than that in its respective control group.

Antioxidant System Analyses

The analyses carried out indicated that there was a decrease in the total antioxidant capacity (TAC) of all the 4 irradiated groups in comparison to their respective control groups (figure 2). The independent t test performed over the results obtained for the liver homogenate indicated a significant reduction in the TAC of the irradiated groups when compared to their respective control groups with P values of <0.001, 0.017, <0.001, and 0.002 for the NC-RC, GC-GR, DC-DR, and LC-LR groups, respectively. The results of the irradiated groups, when compared to the RC group, showed a significant increase (P=0.017, P=0.004, and P=0.038 for the GR, DR, and LR groups, respectively). The brain homogenates also showed a decreased TAC when compared to the controls. The P values obtained for comparison between the respective control and irradiation groups only.

### Table 1: Results of important hematological parameters evaluated among the different study groups

| Groups | WBC mean±SD | P value | Differential count | P value | Meant±SD | P value |
|--------|-------------|---------|---------------------|---------|----------|---------|
|        |             |         | LY%                 |         | RBC      |         |
|        |             |         | P value              |         | Hgb      |         |
|        |             |         | GR%                 |         | HCT      |         |
|        |             |         | MO%                 |         | PLT      |         |
| NC     | 16.33±5.68  | 0.089   | 69.53               | 0.001   | 10.63    | 0.193   |
| RC     | 10.65±1.27  | 56.22   | 71.22 <0.001        | 17.3    | 11.48    | 0.672   |
| GC     | 14.32±3.51  | 0.17    | 14.42±3.51          | <0.001  | 9.53±0.8 | 0.03    |
| GR     | 10.42±5.14  | 0.195   | 9.12±1.44           | 0.001   | 11.77±1.99| 0.001   |
| DC     | 12.42±3.78  | 0.047   | 12.42±3.78          | 0.005   | 10.68    | 0.827   |
| DR     | 7.76±2.69   | 0.001   | 65.98 ±5.28         | 23.33   | 43.2±2.15| 0.001   |
| LC     | 18.86±5.16  | 0.001   | 18.86±5.16          | <0.001  | 9.2      | 0.111   |
| LR     | 7.83±2.04   | 0.001   | 53.8                | 33.48   | 12.8     | 0.919   |

*P<0.05 is considered statistically significant. NC: Normal control; RC: Radiation control; GC: Gallic acid control; GR: Irradiated group pretreated with gallic acid; DC: DMSO control; DR: Radiated group pretreated with DMSO; LC: Lutein control; LR: Lutein pretreated group; WBC: White blood cell; LY: Lymphocyte; GR: Granulocyte; MO: Monocyte; RBC: Red blood cell; Hgb: Hemoglobin; HCT: Hematocrit; PLT: Platelet; *P values given in the table were obtained for comparison between the respective control and irradiation groups only.
significant drop in the irradiated groups with P values of 0.002, 0.009, 0.003, and 0.001 for the NC-RC, GC-GR, DC-DR, and LC-LR groups, correspondingly.

Catalase activity (figure 4) did not exhibit any statistically significant difference between the liver and lung homogenates. In the brain homogenates, the activity significantly decreased...
in the GR (P<0.001) and DR (P=0.006) groups. There was a significant reduction in the activity in the RC group in comparison to the NC group (P=0.036). The LR group revealed a significant rise in the activity when compared to the RC group (P=0.004).

The GSH levels had a rise in the irradiated groups (figure 5). In the brain homogenates, the levels showed an increase in the RC (P<0.001) and DR (P<0.001) groups. In comparison to the RC group, GSH significantly increased in the GR (P=0.031), DR (P<0.001), and LR (P<0.001) groups. The GSH levels were highly raised in the GR group (P<0.001). In the liver homogenate, the levels were raised with a high statistical significance (P<0.001) in the RC and GR groups by comparison to their control groups. In the LR group, in comparison to the RC group, the levels were reduced (P<0.001). In the lung homogenates, the GSH levels were elevated significantly in the GR, DR, and LR groups (P=0.049, P<0.001, and P<0.001, correspondingly).

The malondialdehyde (MDA) levels were estimated as an indicator of LPO (figure 6). Increased MDA levels indicate higher rates of LPO. In the brain homogenates, the GR group showed significantly higher levels of LPO (P=0.007). The formation of MDA was significantly decreased in the LR group in comparison to the RC group (P=0.015). The lung homogenates in the RC and DR groups revealed a significant decrease in LPO when compared to their controls (P=0.007 and P=0.002, respectively).

Liver and Kidney Function Test

Table 2 depicts the summarized results of the liver and kidney function tests performed for the LR, GR, and DR groups and their corresponding controls. The findings revealed no statistically significant results for the serum total protein, albumin, SGPT, and creatinine levels. The SGOT levels were elevated in the RC group when compared to the NC group (P=0.007). Also, a significant increase in the SGOT levels was observed in the LR group when compared to its control group (P=0.049). The concentration of urea was reduced in the RC (P=0.019), GR (P=0.001), DR (P=0.022), and LR (P<0.001) groups in comparison to their respective controls.

Discussion

Total-body irradiation of animals leads to multiple organ dysfunctions due to gastrointestinal, cerebrovascular, or hematopoietic system toxicity. Carotenoids have been studied extensively for their major role in offering radiation protection by directly scavenging the free radicals or by delaying the propagation phase of oxidation. The role of lutein has been studied comprehensively...
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In the present study, lutein showed maximum survival against a lethal dose of radiation in the group treated with 250 mg/kg b.wt. A study in 2010 by Sindhu ER et al. reported a significant increase in chloramphenicol acetyltransferase, in relation to antioxidant and anti-apoptotic pathways. The study of lutein in countering the radiation-induced OS in in vivo models can provide direct information about the nature and extent of the radiation protection that it offers.
SOD, GSH, and GSH reductase levels in mice treated with 250 mg/kg b.wt of lutein. The antioxidant supplementation by lutein might be the major attribute in the survival against a lethal dose of radiation.

Previous studies have reported that pretreatment with lycopene to gamma-irradiated lymphocytes prevented LPO, improved antioxidant status and, thus, prevented damage to lymphocytes. Thiobarbituric acid reactive substances were reduced and GSH, SOD, chloramphenicol acetyltransferase, and glutathione peroxidase were significantly increased in lycopene treatment. Whole-body EBR in mice induced a marked reduction in WBC and a marked elevation in micronucleus formation (polychromatic erythrocytes) of bone marrow. Likewise in the present study also, the lung was found to be radiosensitive when compared to the liver, kidney, and brain. The kidney and lung appeared small in size in the irradiated groups in comparison to their controls. Hepatomegaly was seen in the irradiated groups. The brain of the irradiated groups was tender. The lungs of the irradiated groups turned to a bluish black color, indicating apparent cyanosis.

IR can indirectly induce DNA damage by producing ROS such as superoxide anions and hydrogen peroxide. SOD is the key antioxidant enzyme in the metabolism of oxygen-free radicals in that it catalyzes the dismutation of superoxide anion radicals to oxygen and hydrogen peroxide. SOD breaks down the radicals of superoxide anions into $\text{H}_2\text{O}_2$ and $\text{H}_2\text{O}$. It provides the 1st line of defense against oxygen-derived free radicals, whereas chloramphenicol acetyltransferase and glutathione peroxidase are part of the next step of the antioxidant defense mechanism, converting $\text{H}_2\text{O}_2$ to water. A study showed that mice exposed to whole-body EBR exhibited significant reductions in the glutathione S-transferase, catalase, SOD activity, GSH, total thiols, and TAC as well as increased LPO in their liver. The ability of lycopene to reduce gamma radiation-induced DNA damage has been attributed to its antioxidant-sparing action. Beta-carotene pretreatment in rats exposed to gamma rays protected the liver and blood cells of the animals against free-radical damage induced by radiation. In the present study, the SOD activity was found to be high in the brain tissues when compared to the lung and liver tissues. In the RC group, the activity showed a decline when compared to the NC group. Lutein pretreatment maintained near-normal levels of SOD activity in comparison with the control groups in the liver and lung tissues, whereas the activity was significantly reduced in the brain homogenates. Lutein treatment, prior to the exposure of the mice to EBR, maintained the catalase activity to near-normal levels in the brain tissues. The catalase activity was found to be highest in the liver when compared to that of the brain and lungs. The mild response of lutein toward the antioxidant enzyme might be

### Table 2: Results for the biochemical parameters measured among the different study groups

| Groups   | Serum protein | Albumin | SGOT | SGPT | Urea | Creatinine |
|----------|---------------|---------|------|------|------|------------|
| NC       | 5.72±0.69     | 3.22±0.48| 127.3±25.82 | 49.0±10.04 | 47.4±7.3 | 0.58±0.08  |
| RC       | 5.59±0.63     | 3.2±0.2  | 257.5±88.18** | 40.1±6.21 | 36.2±6.66* | 0.61±0.07  |
| GC       | 5.3±0.53      | 3.09±0.32| 159.6±43.27 | 52.5±9.43 | 40.4±6.93  | 0.53±0.03  |
| GR       | 5.41±0.78     | 3.28±0.23| 142.17±74.44| 37.6±12.36 | 26.2±7.11**| 0.54±0.06  |
| DC       | 5.27±0.43     | 3.34±0.24| 145.33±33.2 | 43.65±7.8  | 40.97±9.44 | 0.57±0.03  |
| DR       | 5.24±0.58     | 3.09±0.18| 216.88±85.52| 36.28±8.93 | 29.13±5.05*| 0.56±0.04  |
| LC       | 5.57±0.61     | 3.10±0.3 | 139.83±50.44| 39.37±5.55 | 34.43±3.91 | 0.57±0.04  |
| LR       | 5.62±1.08     | 2.95±0.19| 229.53±84.16*| 44.27±15.32| 22.6±6.88***| 0.5±0.03   |

***P<0.001, **P<0.01, and *P<0.05 are considered significant with respect to their control groups. NC: Normal control; RC: Radiation control; GC: Gallic acid control; GR: Irradiated group pretreated with gallic acid; DC: DMSO control; DR: Radiated group pretreated with DMSO; LC: Lutein control; LR: Lutein pretreated group.

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attributed to its cytoprotective potential, which resulted in normal enzymatic activities.

The GSH level was the highest in lungs. The levels of GSH were raised in the irradiated groups, indicating the pro-oxidative nature of action. The administration of lutein prior to irradiation increased the GSH levels significantly in the lungs. In the liver and brain, the increase was not statistically significant. GSH plays a key role in maintaining the integrity and function of the membrane. The enhancement in GSH levels indicates a potent response in activating the endogenous GSH levels by lutein.

MDA is one of the major biomarkers known in the pathophysiological mechanism of whole-brain radiation-induced impairment of cognitive function due to increased OS. In the present study, there was an increase in the brain MDA levels in the irradiated groups and a near-normal range was maintained in lutein treatment and gallic acid treatment compared to the irradiation groups. The oxidized lipid membrane breaks up into small molecules of MDA, which is a biomarker of LPO. The enhancement in GSH levels can stabilize the membrane and hence reduce the percentage of the formation of MDA.

The changes in the biochemical parameters of the liver and kidney function in response to radiation are dependent on the dose and the duration post irradiation. A significant increase was seen in the SGOT level, which is a generalized marker of tissue damage. These changes were not observed in the LR and GR groups, indicating a lesser extent of tissue damage.

Lutein was found to scavenge superoxide and hydroxyl radicals effectively and also was shown to stabilize the membrane of erythrocytes against heat-induced lysis in our previous study. The present study supports the antioxidant potential of lutein in vivo. As IR produces singlet oxygen species, which might have escaped the scavenging action of lutein, it could be the reason for the delay in producing certain effects which were expected from lutein. Specific studies on lutein may help us further clarify its role as a radioprotector and studies are required to understand the dose-dependent response of lutein to IR and its subsequent biochemical effects.

**Conclusion**

The present study was carried out to provide an insight into the use of lutein as a radioprotector. Lutein maintained the hematological system and also the antioxidant system in vivo. There is further scope for gene-level studies. Lutein, if proven capable of enhancing or maintaining the antioxidant and hematological system, can be a boon in the field of radiotherapy on the strength of its use as a cost-effective radioprotector.

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**Conflict of Interest:** None declared.

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