

EFFECT OF LUTEIN INTERVENTION IN ALLEVIATING THE POST-RADIATION EFFECTS OF ELECTRON BEAM RADIATION

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ABSTRACT

Ionizing radiations are harmful since they generate free radicals which through a series of reactions may cause damage to genetic material, imbalance in the antioxidant system, or lead to death. Lutein; a carotenoid compound because of its various medicinal properties has been chosen to evaluate its protection against radiation-induced damages. Swiss albino mice were divided into 8 groups of 6 mice each. Irradiation groups received whole-body radiation of 6Gy using an electron beam accelerator. Mice were fed with water/gallic acid/10% dimethyl sulfoxide (DMSO) (w/v)/ lutein respectively. The mice were sacrificed on the 16th day; whole blood was collected in 2% ethylene diamine tetraacetic acid (EDTA) tubes by cardiac puncture method for hematological studies and comet assay. The organs like the brain, lungs, and liver were dissected. Organ homogenates were prepared to perform the antioxidant assay. The femur of the leg was removed and flushed for micronucleus assay. Lutein post-treatment showed significantly increased superoxide dismutase activity in the lung homogenate in comparison to the radiation control. A significant increase in the levels of glutathione was observed in lutein post-treatment when compared to the lutein control in lung homogenate. Also in the lung homogenate of lutein post-treatment, a significant decrease in the levels of malondialdehyde was observed in comparison to the lutein control, and a similar effect was observed in the vehicle control groups. In the comet assay, it was observed that tail moment decreased significantly in lutein post-treatment when compared to its control group whereas no significant changes were observed with %DNA in tail and olive moment. Lutein treatment post-radiation has increased the polychromatic erythrocytes (PCE)/ (PCE+ normochromic erythrocytes (NCE) ratio. Significant changes were observed in the lutein post-treatment group with respect to antioxidant status. Micronucleus assay reveals that lutein treatment post-radiation increases cell multiplication. These results indicate a potent mitigator effect of lutein against radiation-induced antioxidant changes *in vivo*.

Keywords: Antioxidant, Comet Assay, Ionizing Radiation, Lutein, Micronucleus Assay, Mitigation.

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INTRODUCTION

Radiation is a form of energy with a large number of applications. It has wide applications in cancer therapy. Electron beam radiation (EBR) is characterized by high dosage rates and low penetration. It is generated using electricity and magnetism to accelerate electrons to a high energy level. EBR because of its low penetration power is used to treat tumors close to the surface of the body (ex. skin cancer).¹ The radiation-induced damages are irreversible in most cases although reversible effects are seen due to the repair mechanisms in the system. Research is finding its path in identifying novel and ideal radioprotectors that exhibit the least toxic effects at their highest dose when administered orally will be absorbed readily, are available readily in nature, and are cost-effective.² Apart from radioprotectors, mitigators are also found importance to minimize radiation-

induced toxicity. Lutein has proved to exhibit protection against radiation-induced toxicity when pre-administered by protecting the hematological and antioxidant system at a dose of 250mg/kg body weight (b.wt).³ Lutein is a 40-carbon carotenoid that has reported antioxidant property⁴, protects against age-related macular degeneration (AMD)⁵, chemopreventive effects⁶, and antigenotoxic effects.⁷ It also has the potential effect of scavenging the UV radiation-induced free radicals.⁸ Lutein is a dietary component and thus has to be consumed either through diet or as supplements. The bioavailability of lutein is poor and research is being carried out to increase its bioavailability by administering it as nanocapsules.⁹ The present study looks at the potential therapeutic applications of lutein after irradiation.

EXPERIMENTAL

Materials and Methods

Lutein (90%) was procured from the China Haihang industry; gallic acid from Sigma and other chemicals were purchased from HiMedia. Inbred female Swiss albino mice weighing about 25±5g were used for the study. They were maintained under standard laboratory conditions and were provided with mice chow and water *ad libitum*. The study was carried out after obtaining ethical clearance from the institute (Ref. KHEMA/IAEC/20/2014). The mice were irradiated with electron beam radiation using the facility available at LeelaNarayana Shetty Memorial Cancer Institute, Mangaluru. The mice were housed in well-ventilated perspex enclosures, and an EBR of 6 greys (Gy) (a sub-lethal dose with 100 cm between the source and the target at 3Gy/min) was administered to them. The compound was administered using oral gavages (0.1mL/10g b.wt) post-irradiation. From the previous studies, 250mg/kg b.wt lutein administered group of mice showed maximum survival and also pretreatment of the compound at this dose has shown radioprotective effects against antioxidant and cytogenetic systems. Thus, 250mg/kg b.wt was chosen to observe the post-treatment/therapeutic effects of lutein. Lutein was dissolved in 10% dimethyl sulfoxide (DMSO). The mice were divided into 8 groups. Group 1 being the standard control-SC (purified water), group 2 radiation control-RC (EBR + distilled water), group 3 gallic acid control- GC [Gallic acid (100mg/kg b.wt)], group 4 gallic acid post-treatment- GR (EBR + Gallic acid), group 5 DMSO control- DC (10%DMSO), group 6 DMSO post-treatment group- DR (EBR + 10%DMSO), group 7 lutein control- LC [Lutein (250mg/kg b.wt)] and group 8 lutein post-treatment- LR [EBR + Lutein (250mg/kg b.wt)]. The animals were dissected on the 16th day. The whole blood was collected in 2% EDTA tubes and used for the study of hematological factors, and comet assay. The mice were dissected for liver, brain, and lungs whose 10% homogenates were used for the antioxidant studies. The serum was divided for the study of liver and kidney function tests. The femur was removed and the bone marrow cells were washed in bovine serum albumin (BSA) and smeared onto slides for May-Grunewald's stain from which the micronuclei were scored.

Hematological Studies

The whole blood collected by cardiac puncture into 2% EDTA tubes was given to the veterinary blood cell counter (Erma Inc.) and the values were generated of different parameters like WBC count, differential cell count, RBC count, hemoglobin, platelet levels, and hematocrit levels.

Total Antioxidant Capacity

The method of Atalani *et al.*¹⁰ was followed. In brief, 500μL of trichloro acetic acid (TCA) was added to 500μL of 10% homogenate. At room temp. the mixture was allowed to stand for 5 min. followed by centrifugation at 3,000rpm for 15mins at 4°C in a cooling centrifuge (Remi C24BL). Phospho-molybdic acid reagent (1mL) was added to 100μL supernatant. For 90mins tubes were kept in the water bath (boiling). The absorbance was measured at 695nm against a reagent blank.¹¹ The results were expressed in terms of gallic acid equivalent.

Enzymatic Antioxidants

Superoxide dismutase (SOD)

The method described by Singh P *et al.*¹² was followed. Homogenates centrifugation was carried out at 10,000 rpm for 20 mins at 4°C and 100 μL supernatant has obtained from a mixture of methionine, riboflavin, and nitro blue tetrazolium chloride was added. Under fluorescence, the mixture was allowed to stand for 10 min. The blue-green colored solution was read at 560nm. The activity of superoxide dismutase was expressed as mU/mL/mg protein.

Catalase

The method of Aebi¹³ was followed. The homogenates were centrifuged at 10,000 rpm for 20 mins at 4°C and 10µL of the supernatant 60mM hydrogen peroxide solution was added and the reaction kinetics was measured at 240nm with 30secs delay for 2 minutes. The activity was expressed as µM/mL/mg protein.

Lipid Peroxidation

Lipid peroxidation (LPO) was measured using the method described by Ohkawa.¹⁴ Homogenate (10%) of 1mL was incubated at 37°C for 10min. One microliter of TCA was added and centrifuged at 2,500 rpm for 15min. To 1mL of the supernatant, 1mL thiobarbituric acid (TBA) was added, and in boiling water bath tubes were kept for 15min. The tubes were allowed to cool and then 1mL of distilled water was added to the tubes. The absorbance was read at 530nm. The results were expressed in terms of microgram equivalents of malondialdehyde (MDA).

Non-enzymatic Antioxidant

Reduced glutathione (GSH)

The method described by Ellman¹⁵ as followed. to 1mL of the homogenate, 5% TCA (w/v) was added. This mixture was allowed to stand for 30 mins and was centrifuged at 2,500 rpm for 15min. 2.5mL of 5,5'-dithionitrobenzoic acid (DTNB) was added to 500µL of the supernatant. The absorbance was measured at 412nm. The results were expressed in terms of microgram equivalents of GSH.

Biochemical Assay

A serum sample was used to analyze the biochemical parameters. Total protein, albumin, creatinine, and urea levels were estimated using a semi-auto analyzer. The activity of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were measured to determine liver function. The kidney function was evaluated by analyzing the levels of urea and creatinine. Comet assay¹⁶ 1% high melting agarose was layered on slides followed by low melting agarose with the blood sample (20µl). Each layer is left to dry after which the next layer is coated onto the slide. The slide was further coated with a third layer of HMA to avoid the 5 loss or to ensure minimum loss of the sample. The cells were lysed by placing the slides overnight in lysing solution. Alkaline electrophoresis was carried out (20V/400mA, 30min). The slides were treated as neutralizing buffer (Tris buffer, pH-7.5). Ethidium bromide was used to stain the samples and the lysed cells were scored under a fluorescent microscope with the damaged DNA of lysed cells appearing as a comet with tails and the undamaged DNA remaining intact. The degree of damage was assessed using parameters such as olive tail moment, tail moment, and percentage DNA in the tail using the Comet Score software. At least 100 cells were scored per animal in a group. Micronucleus assay¹⁷ PCE- Polychromatic erythrocyte, NCE- Normochromatic erythrocyte, MNPCE- micronucleated polychromatic erythrocyte, MNNCE- micro nucleated normochromatic erythrocyte. The bone marrow cells were washed into freshly prepared 5% BSA. After carefully mixing, the cells were further resuspended in 100 mL BSA after sedimenting at 1,000 rpm for 10 min. This mixture was smeared onto a clean glass slide, subjected to dry and following methanol fixation, staining using May-Grunewald- Giemsa stain followed with every activity for 2-3mins, 4mins and 20mins respectively. The excess stain was removed using purified water and there were slides blinded before scoring for PCE, NCE, MNPCE, and NCEs (MNNCE). Further, [The ratio [PCE/(PCE+NCE)]] was calculated to determine the relative cytotoxicity of EBR and Lutein inhibits the growth of bone marrow.

Statistics

The results of the assays were analyzed by using an independent t-test to test the significant difference between the radiated groups with their respective control group. Also, the radiated groups were compared with the radiation control group to analyze the effectiveness of the compound.

RESULTS AND DISCUSSION

Hematology

The results obtained from the analysis of different hematological parameters are given in Table-1. When compared to normal control white blood cell count has shown a substantial decrease in the RC group. Further, the exposed groups post-treated with gallic acid/ 10% DMSO have shown a substantial reduction (p=0.024,

$p=0.027$ respectively) in WBC levels in contrast to their control groups. The percentage of lymphocytes shows no significant differences between the groups when compared to the respective controls or to the radiation control. Also, there is no significant change in the granulocyte percentage. Whereas, the percentage of monocyte has dramatically raised ($p=0.021$) in the team that received lutein post-irradiation in contrast to the lutein control group. In comparison to the normal control group, the red blood cell count in the radiation control has considerably less ($p=0.013$). In contrast, in the control group, a substantial reduction ($p=0.008$) is also seen in the hemoglobin level of the RC group. In contrast to the RC group, the group that received 10%DMSO post-radiation has shown a substantial increase ($p=0.045$) in the hemoglobin level. In comparison to the control group, the hematocrit percentage has drastically decreased ($p=0.008$) in the group that controls radiation. In the group treated with lutein post-radiation platelet content has significantly reduced ($p=0.006$) in comparison to the lutein function group.

Table-1: Hematological Parameters Evaluated among Various Groups; LY%- lymphocyte; GR%- granulocyte; MO%- monocyte

Groups	WBC($\times 10^3$ cells)	Differential count			RBC ($\times 10^6$ /uL)	Hemoglobin (g/dL)	Hematocrit (%)	Platelet ($\times 10^3$ /uL)
	Mean \pm SD	LY% (Mean)	GR% (Mean)	MO% (Mean)	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
NC	12.17 \pm 2.59	72.97	10.27	16.77	9.29 \pm 0.59	13 \pm 0.44	43.13 \pm 2.25	712.33 \pm 92.2
RC	6.78 \pm 2.78	62.16	12.04	25.8	7.04 \pm 0.1	9.78 \pm 1.11	33.32 \pm 3.86	666.25 \pm 99.22
GC	14.15 \pm 1.2	70.1	9.25	20.65	8.71 \pm 0.057	12.1 \pm 0.14	41.5 \pm 0.28	634.5 \pm 0.707
GR	7.53 \pm 2.91	64.77	10.17	25.07	7.09 \pm 1.3	9.92 \pm 1.94	33.35 \pm 7.1	690 \pm 201.45
DC	14.9 \pm 0.85	62.55	13.9	23.55	8.97 \pm 0.33	11.7 \pm 0.14	39.7 \pm 0.14	646.5 \pm 45.96
DR	6.27 \pm 2.8	50.03	12.75	37.23	8.6 \pm 1.04	11.18 \pm 1.3	40.03 \pm 4.42	692.33 \pm 263.31
LC	9.75 \pm 2.97	68.25	13.33	18.43	8.93 \pm 0.34	12.28 \pm 0.91	41.58 \pm 2.76	878 \pm 122.6
LR	8.45 \pm 0.92	66.76	8.96	24.28	7.94 \pm 2.69	10.46 \pm 3.68	36.34 \pm 12.34	575.75 \pm 218.45

Total Antioxidant Capacity

The total antioxidant level in the homogenates is depicted in Fig.-1. In the liver homogenate, there is a substantial increase ($p=0.029$) in the total antioxidant levels in the gallic acid post-treatment group in comparison to the radiation control group. The brain homogenate of the DMSO post-treatment group (radiation+10%DMSO) displayed a substantial increase ($p=0.004$) in the total antioxidant capacity in comparison to its control group. Compared to the control group with $p=0.000$ similar effect was observed in the lutein post-treatment group. Also, the DMSO Post-treatment group has demonstrated a notable improvement ($p=0.042$) in antioxidant levels when to the radiation control group. The lung homogenate of the gallic acid, DMSO, and lutein post-treatment groups have all shown significantly reduced ($p=0.023$, 0.002, 0.028 respectively) antioxidant levels in contrast to its several control groups.

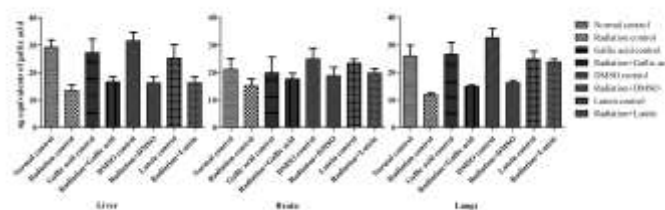


Fig.-1: In Liver, Brain, and Lung Homogenates, Mean Total Antioxidant Capacity with SD was Determined

Superoxide Dismutase

The activity of enzyme superoxide dismutase in SOD units is depicted in Fig.-2. In the liver homogenate, compared to the appropriate control groups with $p=0.024$, 0.000, 0.002, and 0.008 the activity was found to be significantly reduced in the radiation control group, gallic acid, DMSO, and lutein post-treatment groups. Also, the activity in the gallic acid post-treatment group was significantly increased ($p=0.032$) contrasted with the RC group. The activity of the enzyme in the brain homogenate was found to be considerably decreased ($p=0.021$) in the RC group in comparison to that of the normal control group. Also, a substantial reduction

($p=0.005$) in the activity was found in the DMSO comparison of the post-treatment group with its control group. The activity was found to be significantly reduced in the gallic acid and DMSO post-treatment groups compared to the RC groups with respective p -values of 0.014 and 0.022. In the lung homogenate, it was observed that the gallic acid and lutein post-treatment groups showed a significant increase in activity in comparison to the RC group with $p=0.03$ and $p=0.045$ respectively.

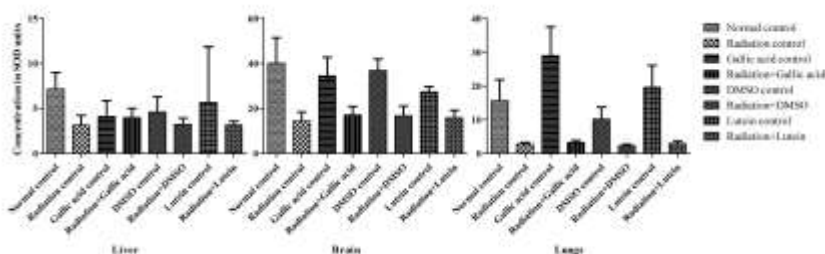


Fig-2: Mean SOD Activity with SD in Liver, Brain, and Lung Homogenates among the Various Groups

Catalase

The results for the catalase activity are depicted in Fig.-3. In the liver homogenate, it was found that the activity notably reduced ($p=0.022$) in the lutein post-treatment group in comparison to the lutein control group. The brain homogenates displayed a notably increased ($p=0.000$) activity in the RC group in contrast to the normal control group. In the gallic acid and DMSO post-treatment groups, the activity was found to be notably reduced ($p=0.000$) in comparison to the RC groups. Whereas, lutein post-treatment has shown a notable increase ($p=0.011$) in the activity in contrast to the RC group. In the lung homogenate, the activity was found to be notably reduced ($p=0.039$) in the RC group in comparison to the normal control group. The DMSO and lutein post-treatment groups have shown a notable reduction ($p=0.000$) in the activity in contrast to their respective control groups.

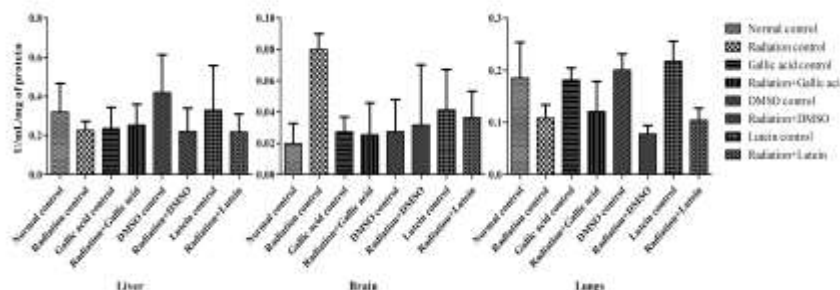


Fig-3: Mean Catalase Activity with SD in the Homogenates of Liver, Brain, and Lungs among the Various Groups

Lipid Peroxidation

The level of malondialdehyde which indicates the level of lipid peroxidation is given in Fig.-4. The gallic acid and DMSO post-treatment groups have shown significantly reduced ($p=0.002$ and $p=0.031$ respectively) levels of lipid peroxidation in comparison to the same control groups in the liver homogenates. The gallic acid post-treatment has significantly reduced ($p=0.023$) the level of lipid peroxidation whereas, lutein post-treatment has significantly increased ($p=0.000$) the malondialdehyde levels in comparison to that of the radiation control group. In the brain homogenates, the level of formation of lipid peroxidation product is significantly increased ($p=0.000$) in comparison to the RC group, the normal control group. When compared to the control groups lipid peroxidation is also significantly increased ($p=0.01$ and 0.004 respectively) in the gallic acid and DMSO post-treatment group. In the DMSO and lutein post-treatment groups the peroxidation levels are increased in comparison to the radiation control groups with $p=0.012$ and 0.000 respectively. Lung homogenate of DMSO and lutein post-treatment groups have shown significantly reduced ($p=0.000$) levels of lipid peroxidation in contrast to the corresponding control groups. Also significantly reduced ($p=0.000$) levels of lipid peroxidation were observed in the DMSO and lutein post-treatment groups in comparison to the radiation control group.

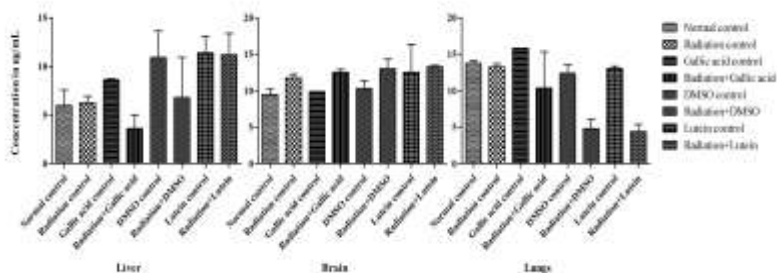


Fig-4: Mean MDA Levels with SD in the Homogenates of Liver, Brain, and Lungs among Various Groups

Glutathione

The glutathione levels are indicated in Fig.-5. The liver homogenate showed significantly reduced ($p=0.027$) levels in the RC group in comparison with the normal control group. Significantly reduced ($p=0.000$) levels of GSH were also observed in the lutein post-treatment group when compared to the radiation control group. The brain homogenate of the radiation control group has shown considerably less ($p=0.012$) levels of GSH in comparison to the normal control group. The gallic acid post-treatment has been shown to significantly increase ($p=0.015$) the GSH levels when compared to the radiation control group. Lung homogenate of gallic acid post-treatment group has shown significantly reduced ($p=0.049$) levels of glutathione in contrast to its control group. Whereas significant increased ($p=0.003$) levels of GSH have been observed as compared to the lutein post-treatment group and the lutein control group. DMSO and lutein post-treatment has increased the levels of glutathione significantly ($p=0.02$ and $p=0.001$ respectively) in contrast to the RC group.

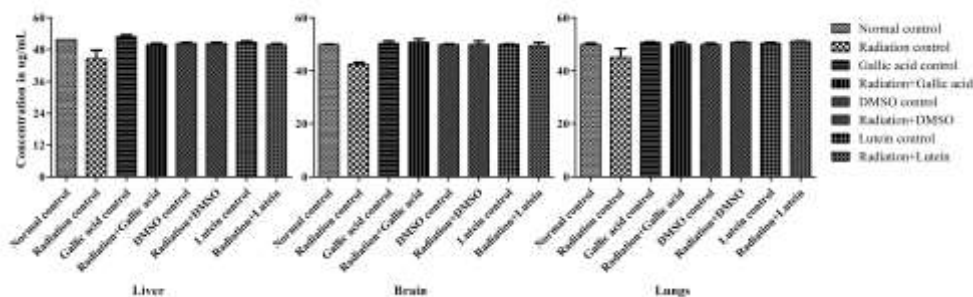


Fig-5: Mean Reduced Glutathione (GSH) Levels with SD in the Homogenates of Liver, Brain, and Lungs among Various Groups

Biochemical Assays

The results of biochemical parameters are summarized in Table-2.

Table-2: Biochemical Parameters Measured Among Various Groups

Groups	Total Protein (g/dl)	Albumin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	SGOT (U/L)	SGPT (U/L)
NC	5.23±0.39	3.29±0.21	37.03±1.23	3.3±0.32	60.8±0.57	29.97±3.72
RC	5.23±0.29	2.96±0.23	35.08±11.09	3.02±0.39	68.85±16.05	28.64±2.76
GC	5.09±0.45	3.04±0.01	34.2±0.9	2.03±0.3	71.0±8.3	24.3±2.3
GR	5.42±0.37	2.98±0.37	37.52±8.25	2.7±0.2	153.65±34.38	32.95±7.11
DC	5.35±0.48	2.5±0.3	35.8±2.12	2.64±0.28	95.05±7.42	32.55±13.51
DR	5.91±0.42	2.41±0.39	20.5±2.2	2.93±0.29	81.5±11.25	24.7±3.1
LC	5.84±0.34	2.59±0.4	24.47±6.71	2.63±0.2	100.07±34.37	28.2±14.99
LR	5.91±0.66	2.65±0.52	25.18±5.1	2.55±0.32	95.3±13.7	40.2±20.9

Comet Assay

The results (Fig.-6-8) show that the RC groups have a noticeably higher percentage of DNA in the tail, tail moment, and olive tail moment, with p values of 0.013, 0.034, and 0.008 accordingly. The gallic acid post-

treatment group has also shown a considerable increase in the above-mentioned parameters ($p=0.006$, 0.026 , and 0.003 respectively) in comparison to its control group. The DMSO post-treatment group has shown a decrease in the olive tail moment in contrast to its control group with a p -value of 0.032 . The increase in the olive tail moment in the gallic acid post-treatment group is significantly higher ($p=0.04$) than that of the RC group. There is a decrease in the % Olive tail moment, DNA in the tail, and tail moment lutein post-treatment group in comparison to its control group; but the changes are not significant.

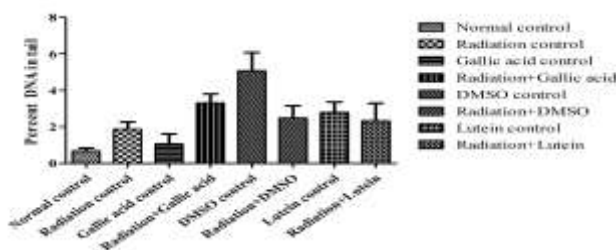


Fig-6: Showing the % DNA in Tail

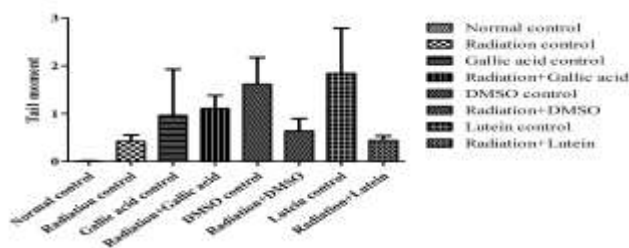


Fig-7: Showing the Tail Moment

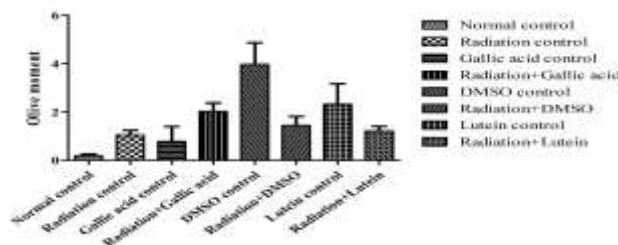


Fig-8: Showing the Olive Moment

Micronucleus Assay

The results (Table-3) obtained for the micronucleus assay shows that the percent formation of the micronucleated polychromatic erythrocyte is significantly higher ($p=0.05$) in the RC group in contrast to the normal control that received water. MNPCE% has significantly increased ($p=0.003$) in the group that received gallic acid post-radiation in comparison to its control group. Micro nucleated normochromatic erythrocyte percent formation is significantly increased ($p=0.02$) in the RC group in contrast to the normal control group. In the groups treated with gallic acid and lutein, post-radiation the percent formation of MNNCE decreased significantly ($p=0.02$ for each).

Table-3: Micronucleus Assay

Groups	MNPCE%	MNNCE%	PCE/(PCE+NCE)
NC	0.98	0	0.96±0.01
RC	3.88	49	0.97±0.01
DC	2.92	8.33	0.97±0.02
DR	3.6	31.25	0.97±0.02
GC	0.74	0	0.98±0.01
GR	4.47	40	0.99±0.01
LC	3.66	46.98	0.95±0.02
LR	3.97	0	0.99±0.01

The PCE/(PCE+NCE) ratio is significantly reduced ($p=0.002$) in the RC group in contrast to the normal control group. This is an indication of increased cytotoxicity in the radiation control group. Whereas, this ratio has significantly increased ($p=0.001$) in the group which was treated with lutein, post-irradiation. Also in comparison to the radiation control, the ratio is found to be significantly increased ($p=0.018$, 0.001 respectively) in the groups post-treated with gallic acid and lutein. The ratio in the lutein post-treated group is also found to be significantly increased ($p=0.022$) in comparison to the gallic acid post-treated group. The increase in the ratio is an indicator of increased cell multiplication in the group due to the administration of gallic acid/ lutein. Radio protectors are those compounds that are given before or during radiation treatment/ radiotherapy; whereas, mitigators are compounds that are given to minimize the toxicity of cells after radiation treatment. Rather than providing a prophylactic action, a compound proves its potential for radiotherapy, if it is of therapeutic use. From our previous studies, lutein has been shown to enhance the hematological status in the irradiated group pre-treated with lutein at a dose of 250mg/kg b.wt when compared to the radiation control group. At a dose of 250mg/kg b.wt lutein, mice have shown maximum survival and thus have been chosen as the optimized radioprotective dose. Radio protectors and radiation mitigators play a key role in radiotherapy; in that, it determines the patient's quality of life.¹⁷

Radiation mitigators play a key role in reducing the toxicity induced by radiation by interrupting the cytokine cascade which may lead to vascular death, ongoing mitotic cell death, etc., or brings about hindrance to the continuation of radiation-induced damages.^{18,19} There are no mitigators of radiation that are approved by the Food and Drug Administration (USFDA) to treat acute radiation syndrome in humans. However, amifostine with LD₅₀ of 2.1 is a radioprotector approved by FDA and is in use to reduce radiation-induced toxicity in clinical practice. It, therefore, serves as a gold standard for the compounds that are being tested for their efficacy to be used as radioprotectors.²⁰ There is thus a thirst in the field of research for the finding of a compound which could be of therapeutic use and serve as a mitigator to reduce radiation-induced damages. The WBCs are the major group of cells that are affected by radiation. A dose-dependent decrease in the WBC population was also observed.²¹ The decrease in the percent distribution of a particular cell population depends on the time and duration after exposure to radiation. It is relative to the change in other cell population levels. Proliferating lymphocytes are more sensitive to radiation than other cell types and return to their normal levels within a few days post-radiation at sub-lethal doses.²² The increase might be apparent in the monocyte population which may be due to a relative decrease in lymphocyte and neutrophil populations. The alteration in the differential cell population may also have an impact on the immune response.²³ Decrease in the RBC parameters and hemoglobin levels indicate the fragility of RBC which might be due to decreased membrane stability. The decrease in the cellular volume in blood might cause alterations in the viscosity and this might lead to platelet aggregation hence a reduced platelet count is observed. If the cellular integrity is intact and the blood cell population remains unaltered, the platelet count remains within normal levels. Gallic acid and beta carotene supplementation were found to enhance the antioxidant capacity in paracetamol-induced liver toxicity in mice and D-galactosamine-induced liver toxicity in rats respectively.²⁴ The total antioxidant capacity provides substantial evidence about oxidant-reductant homeostasis. An increase in the free radicals and the corresponding antioxidant response may reduce the total antioxidant levels. This effect is seen in the liver, brain, and lung homogenates of a radiation control group which seems to be normalized in the gallic acid, DMSO, and lutein treatment groups. After responding to free radicals, the antioxidant may not be able to regenerate the active functional group and thus the overall capacity could have remained in the lower levels. To clarify this further, the enzymatic and non-enzymatic antioxidants were estimated. The SOD forms the first line of antioxidant defense in scavenging free radicals.²⁵

The decrease in SOD levels was found in liver, lung, and brain homogenates of the radiation control. The intervention of gallic acid and lutein was found to normalize the SOD levels in the liver, lung, and brain tissue. SOD acts by catalyzing the conversion of superoxide radicals to hydrogen peroxide radicals which later stimulates the activity of catalase and GPx which convert the hydrogen peroxide radicals into water.²⁶ There was a reduction in catalase activity in the lung, liver, and brain homogenates of radiation control. The lutein and gallic acid intervention has shown a near-normal catalase level. Thus decrease in enzymatic antioxidants of the liver, lung, and brain tissue in the radiation control indicates radiation-induced oxidative stress. Further, the results of GSH indicate increased levels in radiation control which remained decreased in the lutein treatment groups. An increase or decrease in the GSH levels may have a dual role in interpreting the

antioxidative response against radiation. A decrease in GSH indicates a pro-oxidative role of GSH and an increase indicates an antioxidative role that is dependent on numerous environmental factors.²⁸ GSH maintains the integrity of cell membranes by preventing oxidation of membranes.²⁷ Ionizing radiation has been shown to cause membrane oxidation by the formation of short-chain peroxides of the lipid bilayer. This is indicated by the increased formation of malondialdehyde (MDA) which are thiobarbituric acid reactive substances (TBARS). In our study, there is a rise in the MDA formation in the liver, lung, and brain homogenates of the radiation control group indicating radiation-induced lipid peroxidation. The corresponding decrease observed in lutein and gallic acid treatment indicates membrane stabilization. The non-availability of GSH could be the reason for the groups which did not show any changes or which led to a further increase in MDA formation. Thus, it can be understood from the present study that radiation-induced oxidative stress and the effect of a mitigator depend on the antioxidative response of the non-enzymatic and enzymatic antioxidants *in vivo*. The effect of ionizing radiation on the DNA is more severe as it may have teratogenic effects.²⁹ The risk of mutation and consequent development of cancer is more often seen after exposure to radiation.³⁰ Thus any mitigator/protector are more often expected to protect the DNA either directly or indirectly. The radiation-induced DNA damage is observed in the radiation control indicated by the elevated single strand breaks (SSB) as determined by comet assay. The subsequent treatment with lutein has reduced the formation of SSB. The changes in the nuclear content are determined by bone marrow micronucleus formation (MN). During the hematopoiesis, when the immature RBCs undergo cell division, the centrosome fails to function and the MN does not get discharged from the protoplasm and remains within the cell.³¹ Thus MN formation is a marker of genomic instability and in the present study; it is reflected by the increased MN formation in the radiation control group. A smaller ratio of PCE/(PCE+NCE), indicates greater cytotoxicity due to radiation or chemicals with which mice are treated whereas, a higher ratio is an indication of increased cell multiplication in mice due to treatment with a compound/chemical.³² Lutein intervention has reduced the radiation-induced MN formation. Thus, the mitigator role of lutein is mediated by the *in vivo* enzymatic and non-enzymatic antioxidants which scavenge the free radicals and prevent DNA damage.

Though few results indicate an increase in the DNA damage parameters of the intervention groups, it might be attributed to the non-availability of GSH which offers direct protection to the DNA. Post-irradiation treatment with lutein in the present study has been shown to increase the SOD activity of the brain homogenate in comparison to groups that received gallic acid and DMSO post-treatment. Also, an increased level of GSH was found in the lung homogenate of the lutein post-treated group when compared to the radiation control group. There was not much variation found in the overall amount of antioxidant power of the lutein post-treatment group with respect to its control group.

However, reduced catalase activity, unaltered total antioxidant capacity, and increased levels of lipid peroxidation observed in the lutein post-irradiation treatment group may lead to continued intracellular damage and apoptosis. The results indicate the overall radio mitigative potential of lutein when orally administered at 250mg/kg b.wt post-irradiation by protecting the antioxidant system rather than providing protection to the hematological system which is observed in the case of lutein pre-treatment at this dose.

CONCLUSION

Post-irradiation treatment with lutein in the present study has been shown to increase the SOD activity of the brain homogenate in comparison to groups that received gallic acid and DMSO post-treatment. Also, an increased level of GSH was found in the lung homogenate of the lutein post-treated group when compared to the radiation control group. There was not much variation found in the total antioxidant capacity of the lutein post-treatment group with respect to its control group. However, reduced catalase activity, unaltered total antioxidant capacity, and increased levels of lipid peroxidation observed in the lutein post-irradiation treatment group may lead to continued intracellular damage and apoptosis. The results indicate the overall radiomitigative potential of lutein when orally administered at 250mg/kg b.wt post-irradiation by protecting the antioxidant system rather than providing protection to the hematological system which is observed in the case of lutein pre-treatment at this dose.

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