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Research Articles

Role of Rosemary Leaf Extract Against Various Doses of Gamma Radiation

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Abstract

The present investigation reports the radiomodulatory effect of *Rosmarinus officinalis* (rosemary) leaf extract against radiation-induced hematological alterations in Swiss albino mice at various post-autopsy intervals (i.e., between 24 hours to day 30). Treatment of animals with rosemary extract (1000 mg/ kg body wt) prior to irradiation was found to delay the onset of mortality and reduced the symptoms of radiation sickness such as ruffled hairs, lethargy, anorexia and diarrhea in comparison to radiation alone treated animals. Rosemary treated experimental groups exhibited a dose dependent rise (9 < 6 < 3 Gy) in the number of leucocytes (i.e., lymphocytes, monocytes, basophils, eosinophils and neutrophils) by the 30th day post autopsy interval in comparison to the control. Irradiation resulted in a significant increase in lipid peroxidation levels ($p< 0.01$, $p< 0.001$) and a reduction in glutathione levels ($p<0.05$, $p<0.001$) in blood as observed in radiation alone treated animals. Conversely, treatment of mice with rosemary extract exhibited a significant decrease ($p< 0.01$, $p< 0.001$) in lipid peroxidation level and an increase ($p< 0.05$, $p<0.001$) in glutathione content.

Introduction

Radiation protection is at a cross-road after radiation incidents and unacceptable tragedies such as those at Chernobyl and Three Mile Island. Radiation induced damage to the normal tissues can be partially reduced by the use of radioprotectors that reduce the damaging effects of radiation, including radiation-induced lethality (4,22,38). Various workers have investigated the potential application of radioprotective chemicals in the event of planned and unplanned exposure (i.e., clinical oncology, radiation site cleanup, military scenarios, radiological terrorism, radiation accidents, etc.) (18,25,40).

A substantial amount of research has been carried out in the field of chemical radioprotection during the last few decades; however, no safe and ideal synthetic radioprotectors are available to date. Recently, interest has generated in developing the potential drugs of plant origin for the amelioration of radiation effects. Plants and their products are well known to have an advantage over the synthetic compounds in terms of their potential low/no toxicity at the effective dose with minimum or no side effects (3,9,33,34,41). However, the use of medicinal plants suffers from lack of robust scientific evidence to support their use. Therefore, studies supporting or rejecting their role in the treatment of various health disorders is of great need (3).

Rosemary (*Rosmarinus officinalis*), belonging to the family Lamiaceae, is a common medicinal and aromatic plant grown in many parts of the world. It is indigenous to Southern Europe, particularly on the dry rocky hills of the Mediterranean region. Rosemary is used as a culinary herb, a beverage drink, as well as in cosmetics; in folk medicine it is used as a tonic and stimulant, analgesic, anti-rheumatic, carminative, diuretic, expectorant, antiepileptic, antispasmodic in renal colic, dysmenorrhoea, for relief of respiratory disorders, effects on

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human fertility, and the stimulation of hair growth (1). Rosemary has been shown to be safe in toxicity studies in animal models when added as an antioxidant to food (35).

Since time immemorial, the plant has been used traditionally by people for curing various health disorders around the world. Caribs of Guatemala use rosemary to cure various human diseases (12). Rosemary has been described as a wonder-drug in literature and in various medieval drug monographs as well (36,44). Thus, wide acceptability and diverse pharmacological and anti-oxidative properties of the plant stimulated us to evaluate the radio modulatory effect of *Rosmarinus officinalis* in Swiss albino mice exposed to various doses of gamma radiation.

Materials and Methods

Animal care and handling

Male Swiss albino mice (*Mus musculus*), 6-8 weeks old, weighing 20-24 g., from an inbred colony were used for the present study. The animals were provided standard mice feed (procured from Hindustan Lever Ltd., India) and water ad libitum and were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). Four animals were housed in a polypropylene cage with locally procured paddy husk (*Oryza sativa*) as bedding throughout the experiment. Tetracycline-containing water (0.13 mg/ml) was provided once a fortnight and was given as a preventive measure against infections. Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the INSA (Indian National Science Academy), New Delhi, India. The Departmental Animal Ethical Committee approved the present study.

Irradiation

The cobalt teletherapy unit (Co-60) at the Cancer Treatment Centre, Radiotherapy Department, SMS Medical College & Hospital, Jaipur, India, was used for irradiation. Unanaesthetized animals were restrained in well-ventilated Perspex boxes and exposed to various doses of gamma radiation (i.e., 3, 6 and 9 Gy) at a distance (SSD) of 80 cm from the source at a dose rate of 0.85 Gy/min.

Taxonomic description of the plant

Rosemary is an evergreen shrub growing to 1.5 m by 1.5 m at a medium rate. The leaves of rosemary are about 1 inch long, linear, revolute, dark green above and paler and glandular beneath, with camphoraceous aromatic odour. The scented hermaphrodite flowers are small and pale blue. Much of the active volatile principle resides in their calyces. There are various other varieties of the plant, but the green-leaved variety is the kind used medicinally (13).

Preparation of plant extract

The identification of the plant *Rosmarinus officinalis* (family: Lamiaceae) was done by a botanist, Dr. Deepak Acharya, (Voucher Specimen No: DDC/2001/DEPTBT/ ACHARYA2430) of the Department of Botany, Danielson College, Chhindwara, Madhya Pradesh (India). The non-infected leaves of the plant were collected, carefully cleaned, shade dried and powdered in a grinder. The plant material was prepared by extracting 200 gm of leaf powder with double distilled water by refluxing for 36 hrs (12 hrs. x 3) at 55 ± 5°C. Pellets of the extract were obtained by evaporation of its liquid contents in the incubator. An approximate yield of 22 % extract (w/w) was obtained.

The required dose for treatment was prepared by dissolving the drug pellets in double distilled water and administered by oral gavage with a micropipette (100 µl/ animal) at a dose of 1000 mg/ kg body wt./animal (1000 mg of 22% of original plant weight). Henceforth, rosemary leaf extract will be called RE.

Experimental Design

Optimum dose determination

A dose selection of *Rosmarinus officinalis* (RE) was done on the basis of a drug tolerance study. For this purpose, various doses of RE extract (100, 200, 400, 800, 1000, 1500 and 2000 mg/kg body wt.) were tested for their tolerance (once in a day for 5 consecutive days) in Swiss albino mice. One hour after the last administration of RE, mice were exposed to 8 Gy gamma irradiation. All these animals were then observed for 30 days for scoring signs of radiation sickness or mortality. Thus, the optimum tolerated dose of RE (1000 mg/ kg b. wt.) was determined and used for further detailed experimentation (Fig 1).

The $LD_{50/30}$ and Dose Reduction Factor

The efficacy of any protective agent is evaluated by the determination of its dose reduction factor (DRF). The DRF of *R. officinalis* extract (RE) based on $LD_{50/30}$ survival experiment was calculated after irradiating a large number of Swiss albino mice to different doses of gamma rays in the presence (experimental) or absence (control) of RE. The percentage of mice surviving at each radiation dose till 30 days following such exposures was used to construct survival dose response curves. Regression analysis was done to obtain $LD_{50/30}$, and dose reduction factor was computed as:

$$DRF = \frac{LD_{50/30} \text{ of Experimental Animals}}{LD_{50/30} \text{ of Control Animals}}$$

The $LD_{50/30}$ values for control and experimental animals obtained from the survival data are 6.85 and 10.47 respectively. The dose reduction factor of *R. officinalis* against radiation treatment was calculated on the basis of the survival experiment and was measured as 1.53.

Modification of radiation response

A total of 48 animals used for the experiment were assorted into 4 groups. Mice of group 1 (sham irradiated) were orally administered double distilled water (DDW) at a dose of 1000 mg/ kg body weight, volume equal to RE. Animals belonging to group 2 (RE alone) were given daily rosemary extract at a dose of 1000 mg/ kg/ animal for 5 consecutive days, one hour before irradiation. Animals of group 3 (radiation control) were exposed to various doses of gamma rays alone (i.e., 3, 6 and 9 Gy) one hour after DDW treatment on day 5. Group 4 (RE experimental) received RE (1000 mg/ kg body wt./ animal) as in group 2. One hour after last administration of RE, mice were exposed to various doses of gamma rays, (i.e., 3, 6 and 9 Gy), respectively. These animals were observed daily for any sign of sickness, morbidity, behavioral toxicity and mortality. A minimum of 6 animals from each group were necropsied on days 1, 3, 5, 10, 20 and 30 post-treatment intervals to study hematological and biochemical parameters.

Hematological study

For the study, blood was collected from the orbital sinus of animals from each group in a vial containing 0.5 M EDTA. Differential leucocyte counts (lymphocytes, monocytes, basophils, eosinophils and neutrophils) were determined by adopting standard procedures. The number of each type of leucocytes (lymphocytes, monocytes, basophils, eosinophils and neutrophils) and the total number of leucocytes counted were recorded. The percentage of each type of leucocyte was calculated by the formula:

$$\frac{\text{Number of type of leucocyte}}{\text{Total number of leucocytes counted}} \times 100$$

Biochemical determinants

Biochemical alterations were studied in animals of all the groups at one hour post- exposure to gamma radiation. The level of glutathione (GSH) was determined in blood by the method of Beutler *et al*. (5). The lipid peroxidation (LPx) level in the serum was measured by the assay of thiobarbituric acid reactive substances (TBARS) according to the method of Okhawa *et al*. (30).

Statistical analysis

The result for all the groups at various necropsy intervals were expressed as mean ± standard error of the mean (S. E. M.) to evaluate whether the mean of the sample drawn from experimental (RE experimental) deviated significantly from respective control (Irradiation control). Student's 't' test was used by the method of Bourke *et al*. (7). The significance level was set at different levels as $p<0.05$, $p<0.01$ and $p<0.001$.

Results

Data are presented in Tables 1-3 and Figures 1-4. The radioprotective effect of rosemary leaf extract (RE) was studied in mice treated with 1000 mg/ kg body wt. RE before exposure to 3, 6 and 9 Gy of gamma radiation. No noticeable signs of behavioral change, sickness or mortality were observed in Sham irradiated/ RE-treated group. Animals exposed to 3 and 6 Gy gamma rays alone did not show mortality throughout the experimental period, but slight laziness was observed in some animals. Animals exposed to 9 Gy gamma rays exhibited epilation, ruffled hair, watering of eyes, weight loss and became lethargic. No animal could survive in the 9 Gy irradiated alone group beyond day 10. Animals pretreated with RE did not exhibit mortality or any symptoms of radiation sickness. General health, activeness, food and water intake were found to be normal in RE pretreated irradiated animals.

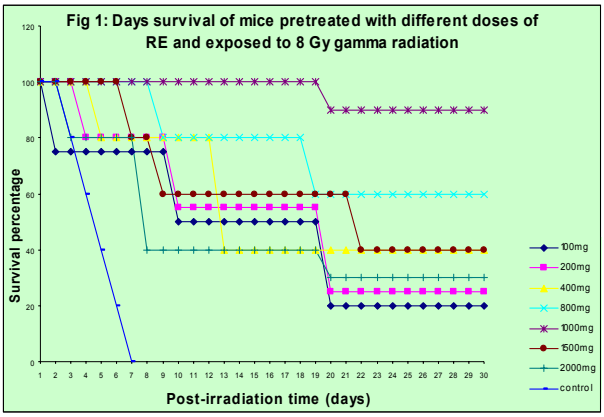


Fig 1: Days survival of mice pretreated with different doses of RE and exposed to 8 Gy gamma radiation

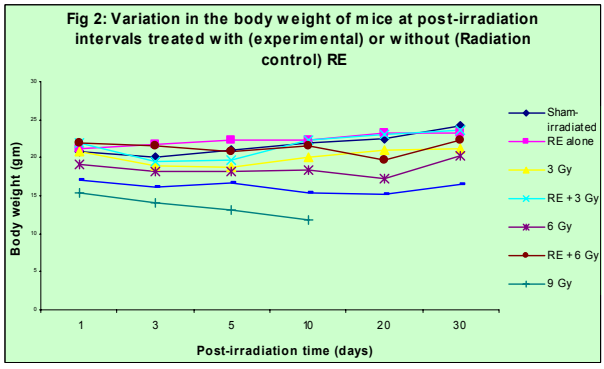
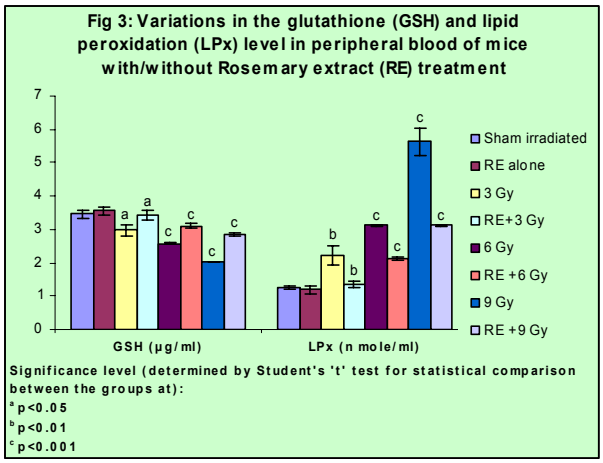
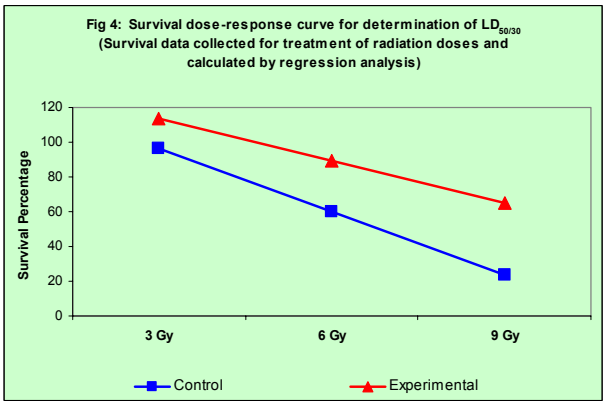


Fig 2: Variation in the body weight of mice at post-irradiation intervals treated with (experimental) or without (Radiation control) RE





After whole body exposure to different doses of gamma radiation (i.e., 3, 6 and 9 Gy), lymphocyte percentages remained significantly lower than normal, and could not regain a normal value even by the last day of autopsy interval (day 30). No significant changes in monocytes, eosinophils and basophil counts were registered in any of the groups. However, monocytes followed a pattern similar to lymphocytes. Following irradiation, a significant increase above normal was observed in neutrophil counts. A normal value could not be restored in any of the irradiated groups till day 30 post-exposure.

Daily administration of 1000 mg/ kg of RE for 5 consecutive days rendered recovery in the different types of leucocytes (i.e., lymphocytes, monocytes, eosinophils, basophils and neutrophils) in comparison to irradiated alone groups, and values close to normal were registered in a dose dependent manner (3> 6> 9 Gy) by post-treatment day 30.

Biochemical determinants

There was no significant difference observed in the levels of glutathione (GSH) and lipid peroxidation (LPx) in the blood content of sham irradiated (group 1) or RE alone treated animals (group 2). In concomitant treatment of RE and radiation (group 4), GSH was found to be further lowered than the radiation treated group. A significant elevation in the values of blood GSH as compared to group 3 was estimated in RE experimental animals. An increase in LPx levels above normal was evident in serum of irradiated mice, while a significant decrease in such values was evident in the RE pretreated irradiated group.

Discussion

Radiation injuries are manifested as a result of enhanced production of free radicals due to oxidative stress. Exposure to radiation causes ionization of molecules in the cells, which sets off potentially damaging reactions *via* free radical production (23). Free radical mediated processes and oxidative stress have been implicated in the pathogenesis of the aging process and various diseases such as atherosclerosis, liver damage, arthritis, cancer, and neurodegenerative disorders (2). The prevailing view is that intake of antioxidant nutrients can reduce the risk of free radical-related health problems and may prove to be protective against ionizing irradiation.

The present study revealed that the number of lymphocytes declined in a dose dependent manner after exposure to 3, 6 and 9 Gy gamma irradiation. A rapid depression was observed at early intervals which may be attributed to direct destruction of such cells in peripheral blood of mice (31). No significant changes in monocytes, eosinophils and basophils were observed after whole body exposure to different doses of gamma radiation. It may be attributed to the fact that mature granulocytes are radioresistant whereas lymphocytes are extremely sensitive to radiation (8). Furthermore, neutrophil granules altered inversely as compared to lymphocytes. These cells exhibited an early rise while lymphocytes and monocytes declined soon after exposure thus showing an opposite behavior. This can be explained by an abortive rise phenomenon as described earlier by workers (26,39).

According to Hall (14), by the time the number of circulating cells in the blood reaches minimum value as the mature circulating cells begin to die off, the supply of new cells from the depleted precursor population becomes inadequate to replace these, thereby making radiation effects become apparent. Also, this abrupt increase may have appeared due to an abortive rise phenomenon (6,26) or can be interpreted as stimulation effect (11). Hastening the maturation of granulocyte precursors in bone marrow and their release into general circulation can be attributed to a rise in neutrophil counts (29,42).

It is evident from the present study that administration of RE reduced radiation sickness and

mortality, and provided protection to differential leucocytes counts (i.e., lymphocytes, monocytes, basophils, eosinophils and neutrophils) in the peripheral blood of mice from the damaging gamma radiation. It has been observed that rosmarinic acid (found in rosemary) is effective in relation to blood circulation and to improve hemodynamics in occlusive arterial diseases (1). Rosemary has been found to contain certain antioxidative (32) and free radical scavenging activity (45) in its active compounds like caffeic acid, carnosolic acid, chlorogenic acid, rosmanol, rosmarinic acid, carnosol, different diterpenes (16,43), rosmari- diphenol, rosmariquinone (17) and other natural antioxidants such as ursolic acid, alkaloid rosmaricine and glucocolic acid (21). In a recent study, carnosic acid was found to render protection to UVA irradiated human skin fibroblasts (29).

The basic effect of radiation on cellular membranes is believed to induce lipid peroxidation (LPx) by the production of free radicals that have the potential to damage DNA and cause cell death (24,27). The level of radiation-induced LPx increased considerably in a dose dependent manner in the entire group 3 irradiated animals, whereas a decrease in the values was observed in RE-treated group 4. The inhibition observed in the LPx level in blood of RE administered animals may have been responsible for the observed radioprotection by plant extract. This view is supported by the investigation of an anti-lipoperoxidant activity of young sprouts of *Rosmarinus officinalis* that significantly reduced the formation of malondialdehyde in rat hepatocytes1 (9). Sotelo-Felix *et al.* proposed that carnosol could scavenge free radicals induced by carbon tetrachloride, consequently avoiding the propagation of lipid peroxides in the liver of mice (37).

Studies conducted by Haraguchi *et al.* report an inhibition of superoxide and lipid peroxidation by 4 diterpenoids from rosemary, i.e. carnosic acid, carnosol, rosmanol and epirosmanol (15). Del Bano *et al.* investigated the efficacy of carnosic acid, carnosol and rosmarinic acid (active constituents of rosemary) and found these to be radioprotective against chromosomal damage induced by γ-rays (10). The exact mechanism of action of rosemary is yet to be elucidated; however, it may act as a protective by scavenging free radicals triggered by radiation.

Glutathione (GSH) is one of the antioxidant enzymes that act as the first line of defense against pro-oxidant stress, thus performing as a free radical scavenger. Oral administration of DDW or RE did not significantly influence the endogenous GSH level in blood. In the present study, GSH levels were found to be lower in the blood of irradiated alone animals than that observed in the RE pre-treated mice. The levels of GSH were found to be elevated in the blood of mice after RE administration.

One of the mechanisms of RE protection against radiation can be an elevation in the glutathione level that is mediated through the modulation of cellular antioxidant level. Rosmarinic acid has been experimentally found to have a significant antioxidant role through free radical scavenging activity (1). Kilic *et al*. observed that lipid peroxidation starts as soon as the endogenous GSH gets exhausted, and the addition of GSH stops further peroxidation promptly (20). Increase in the GSH concentration, towards normal, could have resulted in reduced levels of LPx, thereby protecting against damage caused by radiation in the RE pre-treated irradiated group.

The mechanism of the radioprotective action of *Rosmarinus officinalis* leaf extract in this animal model may thus be its free radical scavenging activity and its ability to thus protect cellular molecules from oxidative damage. Furthermore, it inhibited lipid peroxidation and modulated GSH levels in blood of these Swiss albino mice. The activity of rosemary may also be attributed to stimulating or protecting hematopoiesis in bone marrow and a subsequent increase of hematological constituents in the peripheral blood. Since significant protection was obtained at a non-toxic low dose, RE may have an advantage over the known radioprotectors. Further investigations are in progress to study the exact mechanism of action and clinical applicability of *R. officinalis* in radioprotection.

Table 1: Variation in differential leucocyte counts (DLC) of 3 Gy irradiated Swiss albino mice at various autopsy intervals

Post-Irradiation Interval	Treatment Group	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Neutrophils (%)
Day 1	Irradiation Control	48.4±0.10[c]	2.2±0.50[b]	1.4±0.66	0.8±0.33	47.2±0.79[c]
	RE Experimental	54.8±3.16[c]	2.6±0.82	2.0±0.21	0.6±0.35	40.0±1.50[c]
Day 3	Irradiation Control	52.0±1.56[c]	2.0±0.44[c]	1.8±0.52	0.6±0.21	43.6±0.91[c]
	RE Experimental	57.8±1.28[b]	2.4±0.35	1.6±0.59	0.4±0.17	37.8±1.88[c]
Day 5	Irradiation Control	58.2±1.38[b]	1.6±0.92[b]	2.4±0.21	0.4±0.21	37.4±1.65[c]
	RE Experimental	59.6±2.16	1.8±1.01	2.6±0.40	0.8±0.17	35.2±1.80
Day 10	Irradiation Control	58.6±1.50[b]	1.8±0.40[b]	1.8±0.53	0.4±0.33	36.4±1.30[c]
	RE Experimental	61.6±1077	1.4±0.21	2.2±0.48	0.6±0.35	34.2±1.86[c]
Day 20	Irradiation Control	56.8±3.28[c]	1.6±0.45[c]	2.0±0.33	0.8±0.28	38.8±1.95
	RE Experimental	59.2±1.92	2.4±0.77	1.6±0.17	0.6±0.33	36.2±1.62
Day 30	Irradiation Control	58.8±2.42[a]	1.8±0.87[c]	2.0±0.48	0.6±0.21	36.0±1.08[c]
	RE Experimental	64.8±1.34	2.8±0.63	2.2±0.77	0.4±0.17	30.2±0.60
	Sham-irradiated	67.2±2.16	3.2±0.40	2.8±0.17	0.8±0.21	25.4±0.52
	RE alone	67.4±1.34	3.4±0.22	3.2±0.54	0.8±0.12	25.2±0.45

'**a**' indicates significant ($p < 0.05$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental
'**b**' indicates significant ($p<0.01$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental
'**c**' indicates significant ($p<0.001$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental

Table 2: Variation in differential leucocyte counts (DLC) of 6 Gy irradiated Swiss albino mice at various autopsy intervals

Post-Irradiation Interval	Treatment Group	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Neutrophils (%)
Day 1	Irradiation Control	51.8±1.68[c]	2.4±0.66	2.2±0.35[a]	0.2±0.33	43.4±1.03[c]
	RE Experimental	54.4±2.18	2.2±0.35	2.6±0.66	0.6±0.17	40.2±1.80[b]
Day 3	Irradiation Control	53.8±2.77[b]	2.4±0.17[a]	1.8±0.63	0.4±0.21[a]	41.6±1.63[c]
	RE Experimental	51.6±1.46	2.2±0.44	1.4±0.95	0.2±0.35	44.6±1.85
Day 5	Irradiation Control	58.2±0.92	1.2±0.21[c]	2.6±0.96	0.4±0.33	39.6±1.10[c]
	RE Experimental	56.4±1.94	1.6±0.33	2.2±1.06	0.4±0.28	39.4±1.79
Day 10	Irradiation Control	50.0±3.35[c]	1.4±0.78[a]	2.2±0.33[a]	0.6±0.21	45.8±1.51[c]
	RE Experimental	57.2±2.60	1.0±0.25	1.2±0.56[a]	0.4±0.17	40.2±0.93[b]
Day 20	Irradiation Control	54.6±2.14[c]	1.2±0.48[b]	1.8±0.45[a]	0.2±0.35	42.2±0.40[c]
	RE Experimental	57.8±2.60	2.2±0.46[a]	2.0±0.48	0.8±0.28	37.2±0.79[c]
Day 30	Irradiation Control	52.2±1.66[c]	1.6±0.60[a]	2.0±0.53	0.6±0.10	43.6±0.80[c]
	RE Experimental	63.8±1.37	1.8±0.43	2.0±1.16	0.4±0.28	31.8±1.03
	Sham-irradiated	67.2±2.16	3.2±0.40	2.8±0.17	0.8±0.21	25.4±0.52
	RE alone	67.4±1.34	3.4±0.22	3.2±0.54	0.8±0.12	25.2±0.45

'**a**' indicates significant ($p < 0.05$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental
'**b**' indicates significant ($p < 0.01$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental
'**c**' indicates significant ($p < 0.001$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental

Table 3: Variation in differential leucocyte counts (DLC) of 9 Gy irradiated Swiss albino mice at various autopsy intervals

Post-Irradiation Interval	Treatment Group	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Neutrophils (%)
Day 1	Irradiation Control	52.0±2.38	3.0±0.44	2.8±0.21	0.2±0.21	42.0±0.92
	RE Experimental	54.0±1.32	2.0±0.52	2.6±0.40	0.2±0.17	41.2±1.61
Day 3	Irradiation Control	51.8±1.44	2.8±0.21	2.6±0.17	0.2±0.21	42.6±2.95
	RE Experimental	53.8±0.96	2.0±0.63	2.0±0.25	0.4±0.43	41.8±1.13
Day 5	Irradiation Control	56.0±2.24	2.4±0.48	3.0±0.35	0.4±0.17	38.2±2.51
	RE Experimental	58.2±1.86	2.6±0.95	1.8±0.84	0.2±0.35	37.2±1.08
Day 10	Irradiation Control	55.8±1.45	2.4±0.71	2.8±0.65	0.8±0.28	38.2±2.57
	RE Experimental	66.4±2.56	2.2±0.45	1.4±1.12	0.6±0.33	29.4±1.68
Day 20	Irradiation Control	N.S.	N.S.	N.S.	N.S.	N.S.
	RE Experimental	65.6±1.73	2.2±0.77	1.4±1.08	0.2±0.21	30.4±3.68
Day 30	Irradiation Control	N.S.	N.S.	N.S.	N.S.	N.S.
	RE Experimental	64.2±1.16	2.0±0.71	1.2±0.82	0.4±0.17	31.4±3.83
	Sham-irradiated	67.2±2.16	3.2±0.40	2.8±0.17	0.8±0.21	25.4±0.52
	RE alone	67.4±1.34	3.4±0.22	3.2±0.54	0.8±0.12	25.2±0.45

N.S. = No Survival
'**a**' indicates significant ($p < 0.05$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental
'**b**' indicates significant ($p < 0.01$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental
'**c**' indicates significant ($p < 0.001$) difference with respect to Sham-irradiated v/s Irradiation control, as well as Irradiation control v/s RE experimental

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