MODULATION OF RADIATION INDUCED ALTERATIONS IN SWISS ALBINO MICE BY *BRASSICA COMPESTRIS* (VAR SARASON)

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Summary

The present study reports the protective effect of *Brassica compestris* seed extract on radiation induced hematological and biochemical changes in Swiss albino mice. *Brassica compestris* seed extract was given orally for seven consecutive days prior to radiation exposure. The dose reduction factor (DRF=1.59) for *Brassica compestris* seed extract was calculated from $LD_{50/30}$ values. The hematological parameters were assessed at different intervals of post-irradiation from day 1 to 30. The average hemoglobin (Hb) level, total erythrocyte count (TEC) and total leucocyte count (TLC) in experimental group were significantly elevated as compared to the control group of animals. Also, *Brassica compestris* seed extract treatment significantly elevated reduced glutathione (GSH) level in liver against radiation-induced depletion. Treatment with *Brassica* seed extract caused a significant decrease in malondialdehyde (MDA) formation in the liver, suggesting its role in protection against radiation induced membrane and cellular damage. The results of the present study suggest that *Brassica compestris* seed extract modulate the radiation induced hematological and biochemical alterations in Swiss albino mice.

Key words: *Radioprotection, Brassica compestris, Swiss albino mice, hemoglobin, reduced glutathione, lipid peroxidation.*

Ionizing radiations are widely used for the treatment of Cancer. However, one of the limitations for using radiation is its toxic effects on normal tissues. Radiation induced damage to normal tissues can be partially reduced by the use of radioprotectors that scavenge free radicals produced during irradiation, sparing cancer tissues(1) . The potential application of radioprotective chemicals in the event of planned exposures or radiation accidents/incidents has been investigated(2,3) . It has been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissues.

Some antioxidant nutrients and phytochemicals have the advantage of low toxicity and are protective when administered at pharmacological doses. Naturally occurring antioxidants provide protection against low-dose and low-dose-rate irradiation, including therapeutic potential when administered after irradiation. A number of phytochemicals,

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including caffeine, genistein and melatonin, have multiple physiological effects as well as antioxidant activity that result in radioprotection *in vivo* (4).

Recently interest has increased in the development of potential drugs of plant origin for the modification of radiation effects. Plant extract such as *garlic(5), ginseng(6) , aloe vera(7) , podophyllum* (8), *ocimum(9), spirulina(10) , menthas(11,12) , amaranthus* (13,14) and *Adhatoda(15)* have been found to have an advantage over the synthetic compounds in terms of low/no toxicity at the effective dose with minimum side effects.

Brassica compestris (var sarason) is one of the most popular species of mustard of the family Cruciferae. Mustard seed is widely utilized in the preparation of varieties of edible sauces, pastes and pickles. Scientific investigations into the nature, origin and composition of the mustard oils have been described for over 300 years and by the end of the last century it was known that the volatile oils were isothiocyanates which were not present in the plant as such but as volatile precursors and that they were only obtained after the plant or seed was crushed in water (16). In mustard seed, allyl isothiocyanate are present accompanied by large quantities of their cognate glucosinolates. Isothiocyanates have been implicated in various pharmacological and toxic activities antibacterial, antifungal, antiprotozoal, ability to attract and repel insects, cytotoxicity, chromosomal abnormalities and neoplasia and blocking of carcinogenesis (17, 18). It has been reported that the ethanolic extract of seeds of *Brassica compestris* significantly inhibit the induction of skin papillomas in Swiss albino mice (19). The present study has been undertaken to evaluate the effect of *Brassica compestris* seed extract on radiation induced hematological and biochemical alterations in Swiss albino mice.

Materials and Methods

Animals:

Adult male Swiss albino mice (8-weeks old) were obtained from the animal facility (JNU, New Delhi). The animals were provided with standard mice feed (Hindustan Lever Ltd., India) and tap water *ad libitum*. The colony was maintained at room temperature of $25 \pm 2\ ^0$C and the light: dark exposure of 12 hr: 12 hr.

Source of irradiation:

The animals were whole body exposed to gamma radiation by a Co^{60} source (dose rate = 0.98 Gy/min), at a distance of 101.98 cm from the source at Department of Radiotherapy, SMS Medical College & Hospital, Jaipur, India.

Preparation of extract:

The extract was prepared as described earlier (19). Briefly the extract was prepared by distilling the dried seed powder of *Brassica compestris* in round bottom flask using 95% ethanol at 60 ^{0}C. The procedure was repeated thrice for 12 hr duration. The left over residual after the third distillation was filtered and the remaining alcohol was allowed to evaporate. For the dose level of 800 mg/kg body weight, the alcoholic extract was dissolved in vehicle, double distilled water (DDW), accordingly 0.05 ml of *Brassica compestris* seed extract was given orally to each animal by oral gavage daily.

Experimental Design:

Survival Assay (DRF)

The mice were divided in two groups, Group-I animals were fed orally (using 22-gauge oral feeding needle; Popper and Sons Inc., New York, U.S.A.) with 0.1 ml of double distilled water once a day for 7 days before radiation and served as the control group, while Group-II received 800mg/kg body weight of *Brassica compestris* seed extract in 0.1 ml double distilled water in a similar fashion. One hour after administration on day 7, the animals of both groups were exposed to 4, 6, 8 and 10 Gy of gamma radiation. The survival percentage of mice up to 30 days of exposure against each radiation dose was used to construct survival dose-response curves. Regression analysis was done to obtain $LD_{50/30}$ values and to determine dose reduction factor (DRF).

Modification of radiation response

The mice were divided in two groups, Group-I animals were fed orally with 0.1 ml of double distilled water once a day for 7 days before radiation and served as the control group, while animals of Group-II received 800mg/kg body weight of *Brassica compestris* seed extract in 0.1 ml double distilled water in a similar fashion. One hour after administration on day 7, the animals of both groups were exposed to 8 Gy of gamma radiation.

Hematological Study

Puncturing with the tip of capillary tube collected the blood samples from each group at various post-exposure intervals. The blood was collected in a vial containing 2% ethylenediamine tetra acetic acid (EDTA) as anticoagulant. Hematological parameters such as total leucocyte count, total erythrocyte count and hemoglobin level were determined at 1, 3, 5, 9 and 14 days after radiation exposure by adopting standard procedures.

Biochemical Study:

Animals were sacrificed by cervical dislocation and liver was perfused *in situ* immediately with cold 0.9% NaCl and thereafter carefully removed and rinsed in chilled 0.15 M Tris KCl buffer (pH 7.4) to yield a 10% (W/V) homogenate. Aliquots (0.5 ml) of this homogenate were used for assaying reduced glutathione and lipid peroxidation.

Reduced Glutathione (GSH):

Reduced glutathione (GSH) level was determined by method as described by Moron et al, (20). Homogenates were immediately precipitated with 0.1 ml of 25% TCA and the precipitate was removed after centrifugation. Free-SH groups were assayed in a total 3 ml volume by the addition of 2 ml of 0.6 mM DTNB and 0.9 ml 0.2Mm sodium phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and the absorbance was read at 412 nm using a UV-VIS Systronics spectrophotometer. Glutathione was used as a standard to calculate μmole GSH/gm tissue.

Lipid Peroxidation (LPO):

Lipid peroxidation in liver was estimated spectrophotometrically by Thiobarbituric acid reactive substances (TBARS) by method of Ohkawa et al, (21) and is expressed in terms of malondialdehyde (MDA) formed per mg protein. In brief, 0.4 ml of microsomal sample was mixed with 1.6 ml of 0.15 M Tris KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 52 mM TBA was added and placed in a water bath for 25 min 80 ^{0}C, cooled in ice and centrifuged at room temperature for 10 min at 3,000 rpm. The absorbance of the clear supernatant was measured against reference blank of distilled water at 531.8 nm.

Statistical Analysis:

The results obtained were expressed as mean ± SE. Student's't' test was used to make a statistical comparison between the groups. A statistical comparison was completed with the irradiation alone group Vs the normal and irradiation alone group Vs the *Brassica* and radiation combined group. The significance levels were set at $P<0.05$, 0.005 and 0.001. Regression analysis was done to obtain $LD_{50/30}$ values and to determine dose reduction factor (DRF).

Results

Survival assay:

Brassica compestris seed extract treatment for seven consecutive days prior to whole body irradiation with different doses of gamma radiation showed increased survival percentage (30 days). When survival data were fit on regression line equation, the LD50/30 values for control (radiation alone) and experimental (Brassica + Radiation) were found as 6.75 Gy and 10.76 Gy, respectively. These data were used for determination of dose reduction factor (DRF) and it was calculated as 1.59.

Table 1: Radiomodulatory influence of *Brassica compestris* seed extract on 30 day survival of Swiss albino mice

Group	$LD_{50/30}$	DRF
Group I	6.75 Gy	1.59
Group II	10.76 Gy	

Group I = Radiation alone; Group II = *Brassica* + Radiation

Hematological parameters:

Brassica compestris seed extract treatment for seven consecutive days prior to radiation exposure (8 Gy) showed significant increase in hematological parameters as compared to control (radiation alone) group of animals. The hemoglobin levels of control and *Brassica compestris* treated groups were considerably decreased after radiation exposure however; the maximum decrease was observed at day 5 post-irradiation (Table

2). The total erythrocyte count (TEC) decreased continuously till day 5 in both control and *Brassica compestris* seed extract treated group.

Total erythrocyte count decreased to 31.2% in control group of animals while it was 24.5% in animals of *Brassica compestris* seed extract treated group day 5 post-irradiation (Table 2). Thereafter, the erythrocyte count was slightly increased; the percentage decrease was 16.6% in control and 7.1% in the *Brassica compestris* seed extract treated group on day 14 post-irradiation.

The average total leucocyte counts of mice are shown in Table 2. The decrease in number of leucocytes was observed till day 5. A decrease in the total leucocyte count of 61.9% was noticed in the control animals, whereas, decrease of 58.3% was observed in the *Brassica compestris* seed extract treated group on day 5. The leucocyte counts increased after day 5 but remained below normal in both control and experimental group. However, the total leucocyte counts in the *Brassica* treated group were significantly higher at all the studied intervals.

Table 2: Effect of *Brassica compestris* seed extract on hematological parameters of Swiss albino mice at various post-irradiation time intervals

Hematological parameters	**Group**	**Post-irradiation time intervals (in days)**				
		1	**3**	**5**	**9**	**14**
Hb (g/100ml) 14.10±0.36g/100ml	Group-I	11.20 ± 0.03[c] (20.5)	10.10 ± 0.03 (28.3)	8.81 ± 0.01[c] (37.5)	10.85 ± 0.05[c] (23.0)	11.06 ± 0.05[c] (21.5)
	Group-II	12.48 ± 0.04[b] (11.5)	11.27 ± 0.04[b] (20.0)	10.30 ± 0.03[b] (26.9)	11.80 ± 0.03[b] (16.3)	12.30 ± 0.09[b] (12.7)
TEC ($11.10 \pm 0.365/mm^3$)	Group-I	9.50 ± 0.41[a] (14.4)	8.46 ± 0.34[a] (23.7)	7.63 ± 0.44[a] (31.2)	8.51 ± 0.35[a] (23.3)	9.25 ± 0.38[a] (16.6)
	Group-II	10.40 ± 0.40[a] (6.3)	9.33 ± 0.27 (15.9)	8.38 ± 0.41[a] (24.5)	9.43 ± 0.28 (15.0)	10.31 ± 0.39[a] (7.1)
TLC ($5233 \pm 55.77/mm^3$)	Goup I	2275 ± 21.41[c] (56.5)	20.40 ± 23.94[c] 61.0	1991 ± 19.00[c] (61.9)	2716 ± 23.76[c] (48.0)	3950 ± 48.30[c] (24.5)
	Group-II	2350 ± 3.65[b] (55.00)	2150 ± 9.67[b] (58.9)	2180 ± 7.30[b] (58.3)	2975 ± 9.12 (43.1)	4233 ± 8.63[b] (19.1)

a = P<0.05; b = P<0.001; c = P<0.005;
Group I = Radiation alone (8 Gy)
Group II = *Brassica* + Radiation

Biochemical parameters:

There was significant decrease in the hepatic glutathione level (GSH) of mice exposed to gamma radiation whereas, *Brassica* seed extract treated group showed significant increase in the hepatic GSH level. The lipid peroxidation level (MDA formation) in liver was found to be significantly decreased after exposure to gamma radiation. In *Brassica* treated group the MDA level was observed significantly higher than the control group.

Table 3: Radiomodulatory influence of *Brassica compestris* seed extract on GSH and LPO levels in liver of Swiss albino mice

Group	Biochemical parameters	
	GSH* (38.6±1.60)	LPO** (0.521±0.037)
Group I	28.6±1.80	0.731±0.087
Group II	34.4±2.10	0.415±0.035

*GSH level was measured as μmole of GSH/g tissue.
**LPO level was measured as nmole MDA formed/mg.
Group I = Radiation alone; Group II = *Brassica* + Radiation

Discussion

The results from the present study indicate that the pretreatment of *Brassica compestris* seed extract protects from radiation induced hematological and biochemical alterations in Swiss albino mice. The radioprotective effect of *Brassica compestris* seed extract was demonstrated by evaluating the hematological parameters such as hemoglobin concentration (Hb), total erythrocyte counts (TEC) and total leucocyte counts (TLC) on various post-irradiation time intervals i.e. from day 1 to 30. Also biochemical parameters such as reduced glutathione (GSH) and lipid peroxidation (LPO) were assessed. A significant radioprotection was achieved when *Brassica compestris* seed extract was given orally (800 mg/kg body weight/day) for seven consecutive days before radiation exposure (8 Gy gamma radiation).

In the present study, a significant decrease in the hematologiocal constituents of peripheral blood in animals of the irradiation alone group was observed. The decline in hematological constituents may be attributed to a direct damage by radiation. Although, 3 Gy total body dose is required to produce detectable depletion in total erythrocyte cells, the whole body irradiation of the moderate dose range (5-10 Gy) leads to a decreased concentration of all the cellular elements in the blood. This can be due to a direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and loss of production of cells (22). Mitotically active precursor cells are sterilized by radiation, and the subsequent supply of RBCs, WBCs and platelets is thereby diminished.

The time at which the number of circulating cells in the blood reaches minimum value since, mature circulating cells begins to die off and the supply of new cells from the depleted precursor population is inadequate to replace them so that the full effect of radiation becomes apparent (23).

In the present study, it was observed that *Brassica* seed extract treatment significantly elevated GSH level and decreased MDA formation in the liver of Swiss albino mice. GSH is present in all mammalian cells in substantial concentrations. It represents an important defense against oxygen derived free radicals and cellular lethality from exposure to anticancer drugs or ionizing radiation (24,25). Thus radiomodulatory effect observed in the present study may be due to the significant elevation in GSH level.

Several pathways of radioprotection have been suggested for the mechanism of protective action in mammalian cells against the damaging effects of ionizing radiation. The mechanisms implicated in the protection of cells by radioprotectors include free radical scavenging that protects against reactive oxygen species (ROS) generated by ionizing radiation or chemotherapeutic agents, and hydrogen atom donation to facilitate direct chemical repair at sites of DNA damage. ROS generated by ionizing radiation are scavenged by radioprotectors before they can interact with biochemical molecules, thus reducing the harmful effects of radiation.

The active phytochemicals present in several members of the Brassicaceae family include indole, glucosinolates, aromatic isothiocyanates, dithiolthiones and phenols that may be responsible for the alterations in the level of enzymes, which protects the animals from free radicals (26). Administration of isothiocyanates to rodents has been shown to produce either increase or decrease of microsomal cytochrome P450 but is known to induce phase II enzymes like GST and quinone reductase (27, 28). Qiblawi et al (29) reported the modulatory influence of 95% ethanolic extract from seeds of *Brassica compestris* on the activity of phase II enzymes such as GST, DTD and GSH level in skin, lung, kidney and fore stomach of mouse.

Thus results of the present study suggest that *Brassica compestris* seed extract modulates the radiation induced hematological and biochemical alterations probably by inducing the phase II enzymes in the liver of mice.

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