

# Validation of DNA Damage Progression with Days after Single Exposure of Sublethal Dosage of Electron Beam Radiation

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## ABSTRACT

The aim of this study was to assess the DNA damage progression with days after single exposure of 6Gy electron beam radiation (EBR). Molecular DNA damage in lymphocytes of mice was assessed using alkaline comet assay and in bone marrow cells by micronucleus assay. In comet assay %DNA in tail, Comet length, Olive Tail Moment (OTM) served as a measure of DNA damage and in micronucleus test, frequency of micronucleus formation in bone marrow cells was measured to evaluate the DNA damage. The experiment was performed by taking 24 healthy Swiss albino mice with body weight  $30 \pm 5$ g. The animals were grouped into four. Group 1 served as control, Group 2, 3 and 4 were irradiated by 6Gy EBR. Animals of Group 2, 3 and 4 were sacrificed on 5<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> day post irradiation respectively. The comet assay procedure was carried out for all these groups by using lymphocytes separated from EDTA treated blood. The micronucleus test was performed in bone marrow cells (PCE-Poly chromatic erythrocytes, NCE-normochromatic erythrocytes). The slides prepared for this were scored for the measure of DNA damage. The results showed all comet parameter were significantly ( $P < 0.05$ ) affected by prolonging the post irradiation days from zero (control) to 5, 10 and 15. There was an alteration found in the PCE/PCE+NCE ratio in irradiated mice. A linear increase in the micronucleus formation was observed in post irradiated days. These results conclude a progression in DNA damage with days, post irradiation.

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## INTRODUCTION

A long-standing paradigm in radiation biology has been that many effects induced by ionizing radiation, including its carcinogenic effects and ability to kill cancer cells are the result of DNA damage arising from the actions of ionizing radiation in cell nuclei, especially interactions of ionizing radiation and its products with nuclear DNA (Goodhead, 1994). Consistent with this view, ionizing radiation undoubtedly can damage DNA by directly ionizing DNA itself and by indirect processes in which DNA reacts with numerous radiolytic reactive products including  $\bullet\text{OH}$ ,  $\text{H}\bullet$ ,  $\text{O}_2\bullet^-$ , and  $\text{H}_2\text{O}_2$ , that are generated in aqueous fluid surrounding DNA (O'Neill *et al.*, 1993). Electron beam radiation is a form of ionizing energy that is generally characterized by its low penetration and high dosage rates. It is a concentrated, highly charged stream of electrons,

generated by particle accelerators which are capable of producing beams that are either pulsed or continuous. This high energy electron are used for various purposes in the field of biology, it is also used in the radiation therapy (Loevinger *et al.*, 1961). A single ionization causes a break in one of the strands of the DNA molecule. The break can occur either at the bond between the sugar and phosphate or between the sugar and base (Uma Devi *et al.*, 2000). Lethal and mutagenic effects of ionizing radiation result principally from incompletely or incorrectly repaired DNA lesions (Ward, 1985, 1994). A two or more single strand break on opposing strands within about 10-12 bp has been postulated to produce other clustered damages (Ward, 1981). Due to the DNA repair mechanism the relative break frequency found to be decreases with initial time after exposure (Schwartz, 1998), but it may increase as the increase in the time after exposure. There are no studies reported till today on Electron beam radiation induced DNA damage on mice lymphocytes. So the present study was carried out to know the

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effect of EBR on lymphocytes and also to validate the progression of DNA damage at post irradiation days after a single sub-lethal electron beam dosage.

## MATERIALS AND METHODS

### Animal care and handling

Animal care and handling was carried out according to the guidelines set by WHO (World Health Organization; Geneva, Switzerland). The institutional animal ethical committee had approved this study. Swiss albino mice aged 6 -8 weeks and weighing  $30 \pm 5$  g, taken from an inbred colony, was used for this study. The mice were maintained under controlled conditions of temperature and light (light: 10 h; dark: 14 h). The animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed and water *ad libitum*.

### Irradiation

The irradiation work was carried out at Microtron centre, Mangalore University, Mangalore, Karnataka, India. The animals were restrained in well-ventilated perspex boxes and exposed to whole-body electron beam at a distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

### Experimental protocol

The experiment was performed by taking 24 healthy Swiss albino mice with body weight  $30 \pm 5$ g. The animals were grouped into four. Group 1 served as control, Group 2, 3 and 4 were irradiated to 6Gy EBR. Animals of Group 2, 3 and 4 were sacrificed on 5<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> day post irradiation respectively. The comet assay procedure was carried out for all these groups by using lymphocytes separated from EDTA blood. And the Micronucleus assay was performed with the bone marrow cells.

### Alkaline comet assay

The alkaline comet assay was performed basically as described by Tice *et al.* 1991. Electrophoresis, which allowed for fragmented DNA migration was carried out for 20 min at 25 V and 300 mA. After electrophoresis, the slides were neutralized with 0.4 M Tris, pH 7.4, stained with 50  $\mu$ L of ethidium bromide (20  $\mu$ g/mL) and analyzed with a fluorescence microscope (Olympus. 40x objective). The extent of DNA damage was assessed from the DNA migration distance, which was derived by subtracting the diameter of the nucleus from the total length of the comet. Fifty randomly selected cells were examined for each replicate, for each sample or subject. The quantification of the DNA strand breaks of the stored images was performed using Comet score software by which the percentage of DNA in the tail, tail length and OTM could be obtained directly.

### Micronucleus assay

The mouse bone marrow micronucleus test was carried out according to the method described by Schmidt (1975), by

evaluation of chromosomal damage in experimental animals. The bone marrow from femur was flushed in the form of a suspension into a centrifuge tube containing 5% BSA. The cells were dispersed by gentle pipetting and collected by centrifuge at 2000 rpm for 5 min at 4°C. The cell pellet was resuspended in a drop of BSA and bone marrow smear were prepared. After air drying the smear were stained with May-Grunwald/Giemsa. Micronucleated polychromatic erythrocytes and Non chromatic erythrocytes were observed under Microscope. The percentage of micronucleated polychromatic erythrocytes (MnPCEs), micronucleated normochromatic erythrocytes (MnNCEs) and ratio of PCE to (PCE + NCE) was calculated.

### Statistical analysis

All values were expressed as Mean  $\pm$  SD. Comparison between the control and treated groups were performed by analysis of variance (ANOVA) with Tukey's test. In all these test criterion for statistical significance was  $P < 0.05$ .

## RESULTS

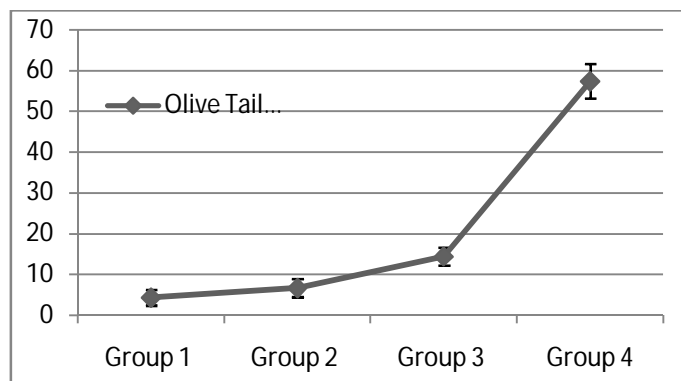
### Alkaline comet assay

To investigate the effect of EBR induced DNA damage in mice lymphocyte, single cell gel electrophoresis was performed. Table 1 shows the results of comet parameter determination in lymphocytes from mice exposed to 6Gy EBR. Post irradiation days after a single dosage of 6Gy EBR showed a linear increase in the tail length, percentage of tail DNA (Table 1). The DNA migration (%DNA in tail) and OTM (Figure 1) significantly ( $P < 0.05$ ) affected by prolonging the post irradiation days from zero (control) to 5, 10 and 15 (Figure 2).

**Table 1:** Alteration in radiation-induced DNA damage in mice lymphocytes at different post irradiation days.

	Tail Length	% Tail DNA
Group 1	105.5 $\pm$ 11.23	1.69 $\pm$ 0.55
Group 2	193.75 $\pm$ 13.75	6.78 $\pm$ 1.11
Group 3	252.75 $\pm$ 24.08	8.34 $\pm$ 1.23
Group 4	392.50 $\pm$ 20.21*	22.89 $\pm$ 3.23*

\* $P < 0.05$ ,  $P < 0.05$  considered as statistically significant. Results are expressed as Mean $\pm$ standard deviation. Group 1: Control, Group 2: 5<sup>th</sup> post irradiation day, Group 3: 10<sup>th</sup> post irradiation day, Group 4: 15<sup>th</sup> post irradiation day.

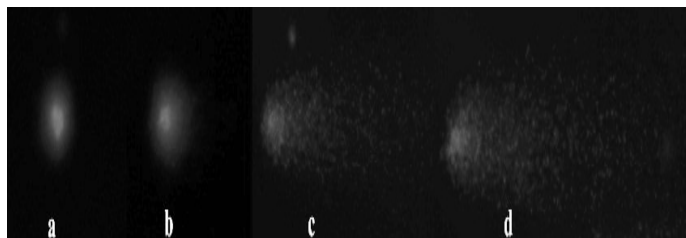


**Group. 1:** Control, Group 2: 5<sup>th</sup> post irradiation day, Group 3: 10<sup>th</sup> post irradiation day, Group 4: 15<sup>th</sup> post irradiation day.

**Table 2:** Measure of EBR-induced micronucleus in bone marrow cells at different post irradiation days.

	Post irradiation days			
	Group 1	Group 2	Group 3	Group 4
MnPCE/PCE (%)	0.0	2.35±1.09	12.22±2.06	28.4±4.44*
MnNCE/NCE (%)	0.0	1.43±0.22	3.54±1.12	12.67±7.3*
PCE/PCE+NCE (%)	48.72±2.32	46.32±1.35	39.43±1.77	26.5±8.32*

\*P<0.05. PCE: Polychromatic erythrocytes, NCE: Normochromatic erythrocytes, MnPCE: Micronucleated Polychromatic erythrocytes, MnNCE: Micronucleated Non chromatic erythrocytes. Group 1: Control, Group 2: 5<sup>th</sup> post irradiation day, Group 3: 10<sup>th</sup> post irradiation day, Group 4: 15<sup>th</sup> post irradiation day.



**Fig. 2:** Comet images a) control mice b) slightly damaged lymphocyte DNA on 5<sup>th</sup> post irradiation day c) Highly damaged lymphocyte DNA on 10<sup>th</sup> post irradiation day d) Complete DNA fragmentation on 15<sup>th</sup> post irradiation day.

### Micronucleus Assay

The whole body electron beam exposed mice showed the formation of micronucleus in the bone marrow cells. The frequency of micronuclei was significantly ( $P<0.05$ ) increases in post irradiation days. The effect of electron beam irradiation on the induction micronucleus in PCE, NCE and alteration in PCE/PCE+NCE ratio in bone marrow cells is shown in Table 2.

### DISCUSSION

Ionizing radiation is a potent DNA-damaging agent, which interacts with cellular DNA by producing free radicals through the direct and/or indirect effect to induce lesions in the irradiated cells. It is well known that some background level of DNA breaks is always present in mammalian cells due to normal metabolism. These breaks can be divided into two classes. Breaks resulted from DNA damage by free radicals produced by cellular oxygen metabolism and breaks resulted from DNA replication, repair, transcription processes, as well as chromatin condensation and decondensation (Osipov *et al.*, 2006). An ionizing radiation induces approximately 1000 DNA single strand break per cell (Billen, 1990). 4Gy gamma irradiation showed an average DNA tail length up to 22 $\mu$ m per cell (Tiwari *et al.*, 2009). The observation based on the data obtained in the present study showed that irradiation with 6Gy EBR will induce about 200 $\mu$ m DNA tail length per cell on 15<sup>th</sup> post irradiation day.

The DNA damage was also evaluated as a measure of DNA tail fragmentation, i.e. Olive motive moment (OTM) immediately after irradiation. The irradiation of HeLa cells to gamma radiation showed an elevation in the migration of DNA. The DNA damage was maximum at 4 hour post irradiation time. Further increase in time up to 24h showed a decrease in the DNA tail fragmentation (Jagetia *et al.*, 2001). This is due to the DNA repair mechanism. But as the time progresses the DNA tail

fragmentation increases. The present study results showed a linear increase in the OTM from zero to 5, 10 and 15<sup>th</sup> post irradiation days. The increase in the olive tail moment can be due to direct induction of DNA strand breaks and/or generation of such modifications in DNA, which can be transformed into strand-breaks. The DNA strand breaks may also follow from various modifications of the DNA bases and by the products of reactive oxygen species (Yang *et al.*, 1996). Single exposure of mice to EBR induces lipid peroxidation (Suchetha Kumari *et al.*, 2010), this generates the production of lipid peroxides. Lipid peroxidation and lipid peroxides produce excessive damage to the cellular DNA (Wang *et al.*, 2004). Since lipid peroxidation is a chain reaction process, as days progresses after radiation exposure more lipid peroxides will be generated. There by lipid peroxidation is directly responsible for progression in the DNA damage in post irradiated days.

Similar results obtained in micronucleus assay, the frequency of MnPCE/PCE and MnNCE/NCE was significantly increased in irradiated groups when compare to the control group. The percentage of PCE/PCE+NCE ratio declined in irradiated mice. 24 hour after 2Gy gamma radiation exposed mice bone marrow cells showed increase in the MnPCE/PCE ratio upto 14.29%, the same study showed 0.23% of MnPCE/PCE ratio in the control group (Hosseinimehr *et al.*, 2006). The present study showed 5.2 fold increase in MnPCE/PCE from post irradiation day 5 to day 10 and 2.32 fold increase in the ratio from day 10 to 15. Decline in PCE/PCE+NCE in the electron beam irradiated mice showed a pronounced cytotoxic effect of radiation on bone marrow proliferation. The cells irradiated at G<sub>0</sub>/G<sub>1</sub> phase show an accumulation of chromosomal damage (Uma Devi *et al.*, 2000). These cells show a formation of micronucleus in the cell division. The repeated attack of reactive oxygen species and lipid peroxides induce micronucleus in each cell cycle. This shows increased frequency of micronucleus in the post irradiation days.

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