Ionizing radiation induces a large number of different types of molecular damage in mammalian cells which can subsequently lead to diversity in cellular response, such as inactivation, chromosomal rearrangement and mutation. It is well established that the efficiency of producing biological damage varies with quality of radiation. Ionization density influences the damage distribution in the genome. On the cellular level, low LET radiation such as X-rays and gamma radiation, induces sparsely ionizations which are typically randomly distributed within the nucleus. For such radiation, the predominant indirect action via free radicals can cause cluster of ionization that lead to complex DSB critical for cell survival. However, for most of the DNA damage induced by low LET radiation consists of SSB and base damages that are relatively easily repaired. In contrast, high LET radiation leads to induction of more complex and highly localised DNA damage along particle tracks. Thus, high LET radiation induces DNA damage in a clustered lesion consisting of multiple strand breaks, base alteration etc. produced by direct interaction between DNA and charged particle, which deposits its energy densely along its path.

The Pelletron at the Inter University Accelerator Centre (IUAC), New Delhi, provided the accelerated ions. Two different ions $^{12}$C and $^7$Li were extracted from the ion source and accelerated to energies of 85 and 50 MeV respectively. Beam properties are listed in the Table.
Beam properties:

<table>
<thead>
<tr>
<th>Ion Species ((^{12}\text{C}))</th>
<th>Energy (MeV)</th>
<th>LET (KeV/(\mu\text{m}))</th>
<th>Fluence (particles/cm(^2))</th>
<th>Dose Equivalent (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Ion</td>
<td>85</td>
<td>287</td>
<td>2.3\times10^6</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.9\times10^6</td>
<td>3.17</td>
</tr>
<tr>
<td>Lithium Ion ((^{7}\text{Li}))</td>
<td>50</td>
<td>60</td>
<td>1.1\times10^7</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.2\times10^7</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Sulfhydryl agents such as cysteine, glutathione, cysteamine and other antioxidants have been seen to protect against lethal effects of radiation. Glutathione (GSH), the major non-protein thiol in mammalian cells, is involved in many cellular functions. This tripeptide plays a role in protection against tissue damage produced by oxidative stress, radiation and chemotherapy. Modification of GSH metabolism has been postulated as being useful in cancer therapy. Indeed, the introduction of agents that can either increase or decrease GSH concentrations in cells opened up the possibility of modulating the cellular response to different anticancer treatments. GSH has been suggested as potential regulator of protein synthesis, DNA synthesis and cell proliferation.

It has been found that endogenous GSH is one of the factors playing an important role for cellular sensitivity towards radiation and chemicals. However, fragmentary report exists in literature that could explain the
role of GSH as radioprotector against high LET radiation. Studies have shown that chemical agents that bring change in the low-LET radiation sensitivity of mammalian cells are less effective against high-LET radiations. The extent of DNA damage and delay induced in cell cycle proliferation by low LET radiation with respect to GSH status of the cell has been explored much extensively. There is no such information available on the role of GSH on high LET radiation induced CA and cell cycle delay in mammalian cell system. Therefore, it will be interesting to compare the influence of GSH on the effect of low and high LET radiation induced DNA damage and delay in cell proliferation. To examine the mechanistic basis for the LET-specific cytogenetic changes we determined the frequency and type of CA induced by $^7$Li ion, $^{12}$C ion and X-radiation.

We made an attempt to modulate the level of GSH and to evaluate its role in low and high LET radiation induced chromosome damage and cell cycle proliferation.

The important aspects of these investigations are:

- CA and delay in cell cycle is LET and dose dependent. $^{12}$C beam (LET 287 KeV/µm) is inducing higher percentage of CA and delay in cell proliferation than $^7$Li beam (LET 60 KeV/ µm) in CHO cell line. Low LET X-radiation induces comparatively lesser percentage of CA and cell cycle delay than high LET $^{12}$C and $^7$Li beam. Due to clustering of ionisation by high LET particles the DSB induced by
such radiation are believed to be more severe and less repairable, thus producing more chromosome DNA damage and division delay.

- It is observed that the frequency of CA increases with increase in sampling time. Such increase is more with high LET radiation. This is due to the inhomogenous energy deposition by the ion particle. The cell with less particle hit induces less damage, enter mitosis earlier than cells with more chromosome damage, thereby showing higher aberration at later harvest period. Unlike, X-radiation deposits energy uniformly leading to homogenous distribution of aberration and delay within the exposed cell population.

- Spectrum of aberration is dominated by deletion in both high and low LET radiation exposed cells. Chromosome as well as chromatid type of exchanges is also observed. On comparing the type of exchange aberration, it is observed that the frequency of chromatid exchange is higher in high LET irradiated cells than low LET X-radiation exposed cells, though the cells are being irradiated at G₁ phase. The SSB when unrepaired at G₁, can be converted into DSB by replication and repair thereby leading to chromatid type aberration in subsequent mitosis.

- From the present data, it seems that GSH showed mild protective effects against low-LET radiation. Protection of GSH depends on the ability to reduce the intracellular concentration of free radicals and
reactive oxygen species. However, GSH is unable to act as radioprotector against high LET radiation. Damage from high LET radiation is primarily due to direct interaction, and because the relative yields of water radiolytic products and reactive oxygen species decreases with increasing LET, protection against high LET radiation by GSH is more difficult to achieve.

It is known that low LET and high LET radiations act differently on DNA because of the differing degrees of spatial clustering of ionisations and DSBs. In contrast to sparsely ionising radiation, high LET radiation leads to the induction of more complex and highly localized DNA damage along particle tracks. Thus, high LET radiation induced DNA damage is a clustered lesion consisting of multiple strand breaks, base alterations action produced by the direct interaction between DNA and the charged particle, which deposits its energy densely along its path. Both the localisation and complexity of high LET radiation induced DSB may influence the cellular capacity to repair such damage. The DNA damage induced by charged particles is associated with slower rejoining of DSBs (Lobrich et al. 1998). Till date, synergistic effect of BLM and high LET radiation on chromosome aberration is not known. Because of the difference in the molecular nature of the damage induced by low and high LET radiation, it is interesting to study and compare the pattern of interaction of the DNA lesion induced by BLM and heavy ion. It was demonstrated by Preston (1982) that if the DNA damage produced by
two agents is repaired at very different rates then the probability of producing a synergistic effect on aberration frequency is low. On the other hand, if the damage from both agents is repaired rapidly, then there is a high probability of producing a synergistic or interactive effect. Therefore, the present study considered these possibilities with an aim to investigate the pattern of interaction of DNA DSBs induced by high or low LET radiation with bleomycin (BLM) induced DNA lesions.

- Combined treatment of BLM and X-radiation induces more exchange aberration compared BLM and high LET ($^{12}$C and $^7$Li) radiation in CHO cells. Since DNA damage induced by BLM and X-radiation is repaired rapidly, it seems that in this synergistic process misrejoining and misrepair of DNA DSBs induced by both BLM and radiation may take place with a high frequency and thus increased the frequency of exchange aberrations. Due to the complex and clustered DNA damage produced by $^{12}$C and $^7$Li beam, the rate of interaction of DNA DSB lesions produced by BLM and high LET radiation is less than that of the interaction of DNA DSB lesions induced by BLM and X-radiation.

- Huge elevation in the frequency of exchange aberrations induced by combined treatment of BLM and X-rays but not with BLM and high LET radiation, to GSH-pretreated cells. It indicates that the better interaction of DNA lesions induced by BLM and X-rays is possible
in the presence of GSH which failed to improve such interaction between DNA lesions produced by BLM and high LET radiation.

From our data, we have seen that exogenous supply of GSH to the CHO cells, induces higher frequency of exchanges. Thus, to verify the role of GSH in interaction of DNA lesion in mammalian system, we have taken three approaches:

1. Allow the interaction of lesions induced by X-rays and BLM at 4°C in presence of GSH and compare it with the similar treatment at 37°C.
2. Allow the interaction of lesions induced by X-rays and BLM in presence of agent that selectively blocks DNA repair pathway.
3. Allow the interaction of lesions induced by X-rays and BLM in DNA-Repair deficient cell lines.

- Increased frequency of exchange aberrations and decreased frequency of deletion is observed in GSH post treated HPBL treated with BLM and X-radiation at RT and 4°C, suggesting involvement of GSH in NHEJ that is predominant repair pathway involved in G₀ lymphocytes. It can be said that NHEJ is involved in the production of higher exchanges under the presence of higher GSH level.

- On inhibiting the activity of DNA PKc by Vanillin (3-methoxy-4-hydroxybenzaldehyde) in HPBL treated with BLM and X-radiation, the frequency of exchange aberrations decreased insignificantly. Therefore, it seems that vanillin could be predominantly interfering the D-NHEJ
pathway and blocked the low level of DNA misjoining, not interfering with other components of NHEJ pathway. On GSH addition to the above treated cells, exchange aberration does not seem to increase significantly. Mere increase in the exchange frequency did not provide any strong evidence of involvement of GSH in B-NHEJ pathway.

An attempt was made to assess the role of GSH in NHEJ pathway, by using mammalian cells deficient in NHEJ pathway. The cell lines used in this study are as follows:

**Genotype and characteristics of CHO cell line used in this study**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Genotype</th>
<th>Defect</th>
<th>Human homologues</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA8</td>
<td>Wild-type</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V33</td>
<td>XRCC7</td>
<td>Deficient in NHEJ</td>
<td>DNA-PK</td>
</tr>
</tbody>
</table>

- A very high frequency of chromatid type of exchange is observed in DNA-PK deficient, V33 cell line. Besides this, a good number of chromosome exchanges are observed as well. This exchange aberration could be formed by B-NHEJ pathway which is involved in slow DSB repair component with high level of DSB misrejoining. Poor influence of BSO on chromosome-type exchanges observed in V33 cells indicate the less involvement of GSH in B-NHEJ pathway.
• Presence of GSH before radiation increased the frequency of chromosome type exchanges in AA8 and chromatid type than chromosome type exchanges in V33 cells. Whereas in the presence of BSO during irradiation, the frequency of chromosome type exchanges was decreased in AA8 cells but not in V33 cells where the frequency of chromatid type exchanges was reduced marginally. The present data indicate the involvement of GSH in DNA DSB joining irrespective of NHEJ or HR pathway.

• From cytogenetic study as well as through comet assay, it is observed that there is still higher damage even in the presence of GSH at dose beyond 3Gy of X-radiation. Increase in exchange aberration is noted on GSH pre-treatment to cells exposed to X-rays even at higher dose of 5Gy. It is also observed that GSH pre-treatment reduces the radiation-induced delay in cell cycle at all radiation doses irrespective of protection from CA. Thus, GSH is seen not be efficient radioprotector at higher dose of radiation but it acts as a modulator of DNA repair capacity.