CHAPTER 8

EFFECT OF 10-METHOXYCANTHIN-6-ONE ON THE REVERSAL OF TOXICITY OF CURRENT THERAPIES – RADIATION THERAPY AND CHEMOTHERAPY
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8.4. DISCUSSION
8.1. INTRODUCTION

Radiotherapy is an important modality for cancer cure, and about one half of cancer patients derive benefit from it. However, non-specificity and severe toxic effect associated contribute limited use in therapeutics (Grdina et al., 2002). Myelosuppression, gastrointestinal, renal and pulmonary disorders are the major adverse effects of radiation (Augustine et al., 2005).

Ionizing radiation can induce death by three different modes, depending on the dose of radiation. At very high doses (above 20 Gy), death occurs in mice from neurological and cardiovascular breakdown and is called cerebrovascular syndrome. Intermediate doses (usually exceeding 15 Gy) cause gastrovascular system failure. Death due to such failure, known as gastrointestinal syndrome, occurs within 4 to 7 days after radiation. At lower doses of radiation (ranging from 8 to 15 Gy), death occurs within 1 to 4 weeks in mice due to hematopoietic failure and, therefore, known as hematopoietic syndrome (Singh and Yadav, 2005). One of the major complications of ionizing radiations used a therapeutic doses is lymphopenia, which results from the marked radiosensitivity of the lymphocyte, and lymphopenia leads to severe immunosuppression (Manori et al., 1986).

Cyclophosphamide (CTX) is a cytotoxic alkylating drug with a high therapeutic index and broad spectrum of activity against lymphoproliferative, neoplastic and non-neoplastic diseases (Calabresi and Chabner, 1992). Use of cyclophosphamide as an effective chemotherapeutic agent is often restricted because of its wide spread adverse side effects and toxicity that includes nausea, vomiting, alopecia, hemapoietic suppression, nephrotoxicity, immunotoxicity and urotoxicity (Fleming, 1997).

It has been recognized that many agents capable of non- specifically enhancing immunologic and hematopoietic responses can also function to reduce the toxicities of radiation therapy. An ideal radio protectant should protect normal tissue while preserving therapeutic efficacy without moderate or severe toxicity. The present chapter evaluate the protective role of 10-MC against radiation as well as chemotherapy induced immune system suppression.
8.2. MATERIALS AND METHODS

8.2.1. Animals
Male Swiss albino and C57BL/6 mice (6-8 weeks old) were used.

8.2.2. Cell lines
B16F-10 melanoma cells, a highly metastatic cell line was used.

8.2.3. Chemicals
Highly specific quantitative sandwich ELISA Kit for mouse IL-1β, IL-6, TNF-α, IL-2 and VEGF were used. All other chemicals and reagents were of analytical grade.

8.2.4. Administration of 10-MC
10-Methoxycanthin-6-one (10-MC) was dissolved in minimum volume of ethanol and suspended in 1% gum acacia. 10-MC was administered at a dose of 0.5mg/Kg body weight, intraperitoneally. Control group of animals were given 1% gum acacia intraperitoneally. For in vitro studies, the test materials was dissolved in DMSO and resuspended in medium to obtain required concentrations with less than 0.1% DMSO content. 10-MC was used at 2μg/ml, 1μg/ml and 0.5μg/ml concentrations.

8.2.5. Irradiation protocol
Animals were exposed to a single acute dose of gamma-radiation of 6 Gy, 4Gy and 2Gy using the 60Co-Theratron-Phoneix teletherapy unit (Atomic Energy Ltd., Mississauga, Ontario, Canada). The animals were kept immobilized in a specially designed, well-ventilated cage without any anesthesia and exposed to whole-body radiation at a rate of 1.41 Gy/min. PTW unidos dosimeter, 0.6 cc Thimble ionization chamber was used for measuring the exposure rate. The radiation field size was 25 × 25 cm², and the cage housed 10 animals at a time. It was at a distance of 80cm from the source.

8.2.6. Chemotherapy protocol
Cyclophosphamide (CTX) was administered, intraperitoneally (0.1ml) at a concentration of 25mg/kg body weight for 10 consecutive days.
8.2.7. Determination of the effect of 10-MC on chemotherapy and radiation therapy induced immunosuppression

a) Effect on haematological parameters and body weight

Swiss albino mice were divided into five groups (n=8/group). Group I served as normal control, group II and III were exposed to radiation (6Gy/animal). Group II were kept as irradiated control while group III animals were treated with 10-MC, started simultaneously with radiation exposure and continued for ten consecutive days. Group IV and V animals received ten doses of CTX of which group IV was kept as CTX treated control while group V animals were treated with ten doses of 10-MC, starting from the day of CTX administration. Blood was collected from all the animals by tail vein bleeding, one day prior to therapy (irradiation/CTX administration) and every third day thereafter and used for the estimation of total WBC count and differential count as explained in Chapter 2. Body weights of all groups of animals were monitored on every third day for a period of one month.

b) Effect on lymphoid organ weight, bone marrow cellularity, number of α-esterase positive cells and serum cytokine levels

Male Swiss albino mice were used for the experiment, and grouped as explained above. Six mice from each group were sacrificed at different time points such as 48h, 7th day and 15th day after the irradiation/CTX treatment for the analysis of organ weight, bone marrow cellularity and α-esterase positive cells.

Lymphoid organs such as thymus and spleen were excised, weighed and expressed as relative organ weight. Bone marrow cellularity was determined using haemocytometer according to the method of Sredni et al., (1992). Bone marrow cells were counted and the cell preparation was smeared on glass slides and stained with pararosaniline and Harris haematoxylin to determine the α-esterase positive cells (Chapter 2). Serum used for the estimation of IL-1β, IL-6, TNF-α and IL-2 levels by ELISA.
8.2.8. Augmentation of therapeutic benefits of radiation by 10-MC

a) Effect on solid tumour reduction

C57BL/6 mice (6-8 weeks old) were divided into eight groups (n=8/group). All the animals were injected with B16F-10 cells (10^6 cells per animal) subcutaneously on the right hind limb. Group I was kept as untreated tumour control. Group II was treated with 10 dose of 10-MC. Group III, IV and V animals were exposed to a single dose of 6Gy, 4Gy and 2Gy radiation respectively and kept as radiation control. Group VI, VII and VIII animals were exposed to 6Gy, 4Gy and 2Gy radiation respectively and all the animals were treated with ten dose of 10-MC, one hour after the radiation exposure. The tumour volume was measured at 24th hour, 7th day and 15th day after radiation exposure.

b) Effect on the serum VEGF levels

Blood was collected from the above group of animals by tail vein bleeding at 24th hour, 7th day and 15th day after radiation exposure, serum separated and used for the estimation of VEGF levels by ELISA.

c) Gene expression of VEGF, HIF-1α and COX-2

Total RNA was isolated from the tumour, and cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase. Amplification was performed using specific primers of VEGF, HIF-1α and COX-2. The amplified products were electrophoresed on agarose gel, stained with ethidium bromide, and photographed under ultraviolet light.

d) In vitro proliferation assay

B16F-10 melanoma cells (5000 cells/well) were plated in a 96-well culture plate and incubated at 37°C in 5% CO_2 atmosphere. After 24h, the cells were exposed to different doses of radiation (0.5, 1, 2, 3, 4, and 5 Gy) and were further incubated for 48h in the presence and absence of 10-MC (2μg/ml). Rate of proliferation determined using ^3H-thymidine as explained in Chapter 2 and expressed as radioactive counts per minute (CPM).

e) Soft agar clonogenic survival assay

B16F-10 melanoma cells were exposed to different doses of radiation (0.5, 1, 2, 3, 4, and 5 Gy). Cells were cultured in soft agar (0.3%) in a 24-well
culture plate (500 cells/well) in the presence and absence of 10-MC (2μg/ml) and were incubated for 6 days. Colonies were then fixed, stained with crystal violet, and counted. The surviving fraction (SF) was estimated as follows; SF= (Number of colonies formed after treatment)/(Number of cells seeded X plating efficiency)

8.3. RESULTS

8.3.1. Effect of 10-MC on chemotherapy and radiation therapy induced immunosuppression

a) Effect on body weight and haematological parameters

The effect of 10-MC on change in body weight in irradiated animals is shown in Figure 8.1.A. There was also significant reduction in body weight when animals were irradiated/treated with CTX when compared to normal animals. The change in body weight was resorted to near normal levels when animals received 10-MC. The radiation exposed control animals showed 76.61% reduction the total WBC count (Figure 8.1.A) on 6th day after radiation exposure (1757±236 cells/cmm³). Administration of 10-MC enhanced the total WBC count to 3247±372 cells/cmm³ on 6th day and normalized by 24th day (6852±348 cells/cmm³). Cyclophosphamide treated animals have 73.80% reduction in total WBS count on 12th day which was brought near to normal levels by day 30. When these animals received ten doses of 10-MC, the maximum reduction in total WBC count was only 56.91% by 12th day and this was restored to normal levels (7036±427 cells/cmm³) by 27th day itself. Differential count did not show any significant variation in both treated and untreated animals (Data not shown).

b) Effect on lymphoid organ weight, bone marrow cellularity and α-esterase positive cells in immunosuppressed animals

Effect of 10-MC on lymphoid organ weight is given in Table 8.1. Radiation exposure/CTX administration caused a drastic reduction in the weight of lymphoid organs such as thymus (0.062±0.003g/100g body wt; 0.071±0.008g/100g body wt) and spleen (0.156±0.02g/100g body wt; 0.176±0.01g/100g body wt) after 48-hour/7th day. Treatment of animals with 10-MC in radiation exposed animals showed increase in the weight of thymus
Figure 8.1

Effect of 10-MC on immunosupression

a) Change in body weight

b) Total WBC count

Five groups of Swiss albino mice were used. Mice received whole body radiation (6Gy)/CTX administration. Treated group received 10 doses of 10-MC. Body weight monitored before treatment and then every third day for a month. Blood was collected on every third day by tail vein bleeding for a month. Values are mean ± SD.
### Table 8.1

**Effect of 10-MC on lymphoid organ weight during irradiation/CTX induced immunosupression**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thymus</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48h</td>
<td>Day 7</td>
</tr>
<tr>
<td>Normal</td>
<td>0.112 ± 0.073</td>
<td></td>
</tr>
<tr>
<td>Radiation alone</td>
<td>0.062 ± 0.003</td>
<td>0.094 ± 0.005</td>
</tr>
<tr>
<td>Radiation +10-MC</td>
<td>0.085±0.005*</td>
<td>0.117±0.007*</td>
</tr>
<tr>
<td>CTX alone</td>
<td>0.096 ± 0.006</td>
<td>0.071 ± 0.008</td>
</tr>
<tr>
<td>CTX + 10-MC</td>
<td>0.110 ± 0.009</td>
<td>0.089 ± 0.006*</td>
</tr>
</tbody>
</table>

Five groups of Swiss albino mice were used. Mice received single dose of whole body radiation (6Gy)/CTX administration for 10 days. Treated group received 10 doses of 10-MC at a dose of 0.5mg/Kg body weight. Six animals from each group were sacrificed on different time points (48h, 7th day and 15th day). Thymus and spleen was excised and weight was recorded as g/100g body weight. Values are mean ± SD. *P < 0.05 compared with radiation/CTX control.
(0.085±0.005g/100g body wt) and spleen (0.171±0.03g/100g body wt) after 48h of irradiation and further increased and reached to normal range on 15\textsuperscript{th} day (thymus 0.146±0.011g/100g body wt; spleen 0.369±0.03g/100g body wt). Administration of 10-MC to CTX administered animals restored the weight of thymus (0.131±0.011g/100g body wt) and spleen (0.352±0.02g/100g body wt) by 15\textsuperscript{th} day.

Change in bone marrow cellularity after 10-MC administration is presented in Table 8.2. In radiation exposed control mice bone marrow cellularity was drastically reduced to 6.24±0.57 x10\textsuperscript{6} cells/femur after 48h of radiation exposure while 10-MC treatment restored the bone marrow cellularity to normal levels (15.78±1.14 x10\textsuperscript{6} cells/femur) by 15\textsuperscript{th} day. In CTX administered mice there was a similar reduction in the bone marrow cells on 7\textsuperscript{th} day (7.39±0.81 x10\textsuperscript{6} cells/femur), which was increased to normal range of 15.34±1.27 x10\textsuperscript{6} cells/femur by 15\textsuperscript{th} day.

In radiation exposed animals there was drastic reduction in the number of α-esterase positive cells (253.71±18.46 cells/4000 bone marrow cells) after 48h (Table 8.2). CTX treatment also resulted in suppression of α-esterase positive cells (526.52±44.69 cells/4000 bone marrow cells) by 7\textsuperscript{th} day. Treatment of irradiated/CTX treated animals with 10-MC for 10 days increased the number of α-esterase positive cells to 904.19±72.53 and 816.36±53.60 cells/4000 bone marrow cells respectively by day 15\textsuperscript{th} day.

c) Effect on serum cytokine levels in immunosuppressed animals.

Serum IL-1β, IL-6 and TNF-α levels were elevated to 64.58±5.37pg/ml, 41.79±2.53pg/ml and 213.63±13.57pg/ml respectively on the 48h of radiation. In CTX treated animals, serum IL-1β, IL-6 and TNF-α levels on 7\textsuperscript{th} day were 49.84±3.68pg/ml, 49.84±3.68pg/ml and 284.69±23.55pg/ml respectively. Treatment of both groups of animals with 10-MC for ten days resulted in significant reduction in the levels of these pro inflammatory cytokines by 15\textsuperscript{th} day (Table 8.3).

Serum IL-2 levels on 48 hours after irradiation was reduced to 284.69±23.55pg/ml when compared to normal levels of 284.69±23.55pg/ml. CTX treatment also resulted in a similar reduction in IL-2 levels to 17.54±0.92pg/ml on 7\textsuperscript{th} day. Treatment of irradiated animals with 10-MC
**Table 8.2**

**Effect on bone marrow cellularity and α-esterase positive cells during irradiation/CTX induced immunosupression**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bone marrow cellularity (x10⁶/femur)</th>
<th>α-esterase positive cells/4000 bone marrow cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48h</td>
<td>Day 7</td>
</tr>
<tr>
<td>Normal</td>
<td>15.62 ± 0.93</td>
<td>874.57 ± 53.64</td>
</tr>
<tr>
<td>Radiation alone</td>
<td>6.24 ± 0.57</td>
<td>8.06 ± 0.92</td>
</tr>
<tr>
<td>Radiation +10-MC</td>
<td>6.89±0.73</td>
<td>10.27±0.82*</td>
</tr>
<tr>
<td>CTX alone</td>
<td>12.83 ± 0.89</td>
<td>7.39 ± 0.81</td>
</tr>
<tr>
<td>CTX + 10-MC</td>
<td>13.48 ± 1.16</td>
<td>9.72 ± 0.64*</td>
</tr>
</tbody>
</table>

Five groups of Swiss albino mice were used. Mice received single dose of whole body radiation (6Gy)/CTX administration for 10 days. Treated group received 10 doses of 10-MC at a dose of 0.5mg/Kg body weight. Six animals from each group were sacrificed on different time points (48h, 7th day and 15th day) and bone marrow cellularity and α-esterase activity determined. Values are mean ± SD. *P < 0.05 compared with radiation/CTX control.
**Table 8.3**

Effect of 10-MC on serum cytokine levels in irradiation/CTX induced immunosuppression

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Radiation alone</th>
<th>Radiation + 10-MC</th>
<th>CTX alone</th>
<th>CTX + 10-MC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-1β</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td>14.93±1.27</td>
<td>64.58±5.37</td>
<td>57.84±4.68</td>
<td>22.57±1.33</td>
<td>18.61±0.76*</td>
</tr>
<tr>
<td>Day 7</td>
<td>37.09±2.88</td>
<td>24.76±1.83</td>
<td>49.84±3.68</td>
<td>39.58±2.49</td>
<td></td>
</tr>
<tr>
<td>Day 15</td>
<td>21.83±1.51</td>
<td>16.23±1.26</td>
<td>47.16±2.42</td>
<td>31.83±1.35</td>
<td></td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>48h</td>
<td>36.46±2.15</td>
<td>56.36±4.28</td>
<td>51.68±3.72</td>
<td>43.65±3.59</td>
<td>42.70±3.17</td>
</tr>
<tr>
<td>Day 7</td>
<td>41.79±2.53</td>
<td>38.76±3.09</td>
<td>50.72±2.61</td>
<td>46.05±1.95</td>
<td></td>
</tr>
<tr>
<td>Day 15</td>
<td>38.41±2.96</td>
<td>35.25±2.47</td>
<td>46.32±3.40</td>
<td>39.92±2.70</td>
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<tr>
<td><strong>TNF-α</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>48h</td>
<td>23.57±1.86</td>
<td>362.86±24.60</td>
<td>316.94±18.52</td>
<td>96.71±7.26</td>
<td>76.85±5.22*</td>
</tr>
<tr>
<td>Day 7</td>
<td>213.63±13.57</td>
<td>146.77±10.16</td>
<td>284.69±23.55</td>
<td>191.37±12.48</td>
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</tr>
<tr>
<td>Day 15</td>
<td>57.81±4.61</td>
<td>31.42±2.58</td>
<td>183.70±7.34</td>
<td>124.22±9.53</td>
<td></td>
</tr>
<tr>
<td><strong>IL-2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td>23.96±1.62</td>
<td>16.64±1.47</td>
<td>19.63±0.69</td>
<td>20.36±1.61</td>
<td>21.18±1.67</td>
</tr>
<tr>
<td>Day 7</td>
<td>18.43±0.81</td>
<td>22.47±1.93</td>
<td>17.54±0.92</td>
<td>20.26±1.32</td>
<td></td>
</tr>
<tr>
<td>Day 15</td>
<td>22.38±1.76</td>
<td>24.68±0.74</td>
<td>19.72±1.35</td>
<td>22.83±1.68</td>
<td></td>
</tr>
</tbody>
</table>

Five groups of Swiss albino mice were used. Mice received single dose of whole body radiation (6Gy)/CTX administration for 10 days. Treated group received 10 doses of 10-MC at a dose of 0.5mg/Kg body weight. Six animals from each group were sacrificed on different time points (48h, 7th day and 15th day). Serum used to analyse cytokine levels by ELISA. Values are mean ± SD. *P < 0.05 compared with radiation/CTX control.
resulted in restoration of IL-2 levels (24.68±0.74pg/ml) by 15th day after radiation exposure. In CTX treated animals, administration of 10-MC increased IL-2 levels to 22.83±1.68pg/ml on 15th day.

8.3.2. Augmentation of therapeutic benefits of radiation by 10-MC

a) Solid tumour reduction in irradiated mice

Effect 10-MC on solid tumour volume in irradiated animals is given in Figure 8.2.A. Tumour volume was significantly (p<0.05) reduced by the administration of 10-MC in all the groups. Mice carrying B16F10 cells alone (Tumour control) had the tumour volume 1.53±0.16cm³ on 15th day, which was significantly reduced by the administration of 10-MC (0.82±0.07cm³). Radiation treatment at 6G, 4G and 2G also significantly decreased the tumour volume in a dose dependent manner. Maximum reduction in the tumour volume was observed when the animals treated with 10-MC along with 6G radiation. Tumour bearing mice exposed to 6 Gy radiation alone had a tumour volume of 0.56±0.03cm³ on 15th day, which was further reduced to 0.51±0.02cm³ by the administration of 10-MC.

b) Effect of 10-MC on VEGF levels in irradiated mice

Changes in serum VEGF level during tumour development and radiation treatment in presence and absence of 10-MC is given in the Figure 8.2.B. Serum VEGF level was elevated during tumour progression (563.24±38.47pg/ml on 15th day) which was drastically increased in radiation exposed animals (1947.36pg/ml in 6G, 1756.14pg/ml in 4G and 1169.72pg/ml in 2G). Administration of 10-MC significantly reduced the serum VEGF compared to untreated tumour control (1253.51pg/ml in 6G, 946.74pg/ml in 4G and 576.38pg/ml in 2G).

c) Effect on the expression of VEGF, HIF-1α and COX-2 genes

Gene expression of VEGF, HIF-1α and COX-2 is given in Figure 8.3. The expression levels of these genes were upregulated in the untreated tumour control. Tumour bearing mice exposed 6Gy radiation showed more significant expression, compared to other control groups. Animals treated with 10-MC
A) Effect of 10-MC on solid tumour volume in irradiated mice

B) Effect of 10-MC on serum VEGF levels in irradiated mice

C57BL/6 mice were injected with 10⁶ B16F-10 cells subcutaneously on right hind limb. Animals were given 6G, 4G and 2G of whole body radiation and/or 10-MC for 10 days. The tumour volume was measured at 24th hour, 7th day and 15th day after radiation exposure. Blood collected by tail vein bleeding at 24th hour, 7th day and 15th day and used for VEGF estimation. All values are mean ± SD.
Figure 8.3

Effect on the expression of VEGF, HIF-1α and COX-2

VEGF

HIF-1α

COX-2

Line 1: Molecular weight marker
Line 2: Tumour control
Line 3: Tumour + Radiation 2G
Line 4: Tumour + Radiation 4G
Line 5: Tumour + Radiation 6G
Line 6: Tumour + Radiation 2G + 10-MC
Line 7: Tumour + Radiation 4G + 10-MC
Line 8: Tumour + Radiation 6G + 10-MC
A) Effect of 10-MC on proliferation of radiation exposed B16F-10 melanoma cells

B16F-10 melanoma cells (5x10^3 cells) exposed to different doses of radiation. Cells were incubated for 48h in the presence and absence of 10-MC (2µg/ml). Rate of proliferation determined using radioactive thymidine incorporation assay.

B) Effect of 10-MC on colony formation by radiation exposed B16F10- melanoma cells

B16F-10 melanoma cells exposed to different doses of radiation. Cells cultured in soft agar in a 24-well culture plate (500 cells/well) in presence and absence of 10-MC for 6 days. Colonies were stained with crystal violet, counted and the surviving fraction was estimated.
showed down regulation in the expression of VEGF, HIF-1α and COX-2 in tumour bearing mice.

d) Effect of 10-MC on proliferation of radiation exposed B16F-10 melanoma cells

The effect of radiation and 10-MC treatment on the inhibition of B16F-10 cell proliferation is shown in Figure 8.4.A. Cell proliferation was markedly decreased with increase in radiation dose. Proliferation of tumour cells was further decreased when the cells were treated with 10-MC along with radiation.

e) Soft agar clonogenic survival assay of radiation exposed B16F-10 melanoma cells

Soft agar assay for colony formation shows the effect of radiation on the cell survival (Figure 8.4.B). The number of tumour cell colonies was reduced by the radiation exposure. Survival fraction was decreased with the increase of radiation dose. B16F-10 cells treated with 10-MC along with radiation further decreased the survival fraction, which is in accordance with rate of cell proliferation data.

8.4. DISCUSSION

Cytotoxic drugs are commonly used in the treatment of various human cancers. Chemotherapy has been used to cure most patients with malignancies such as paediatric acute lymphocytic leukaemia, Hodgkin’s disease and testicular cancer. However, for many other malignancies chemotherapy has had only marginal effects.

Radiation exposure leads to the development of cascade of changes including injury to the lymphoid and hemopoietic system, which results in septicemia and death (Jagetia et al., 2003). CTX administration also induces acute and transient myelosuppression which primarily damage the rapidly proliferating hematopoietic progenitors and their mature progeny leading to decline in the number of peripheral blood cells (Fishman et al., 2001). It is evident from the present study that administration of 10-MC in radiation or CTX exposed Swiss albino mice could enhance the total WBC, α-esterase positive cells and this revealed the hematopoietic stem cell regeneration to resist radiation and CTX induced depletion of hematopoietic stem cells.
Therapeutic doses of radiation triggers cells to release inflammatory cytokines such as TNF-α, IL-1β, IL-6, and various other chemokines that help recruit macrophages and lymphocytes to the damaged area, which further enhances the production of those cytokines (Willey and Hallahan, 2008). The increase in the levels of pro inflammatory cytokines followed by radiation/cyclophosphamide therapy was efficiently managed by the administration of 10-MC to mice. The levels of IL-2, a major cytokine involved in stimulation of immune system, was found to be resorted back to normal levels when the mice were treated with 10-MC.

During radiation therapy, initial tissue damage from radiation is generated by the direct action of reactive oxygen species on DNA. This tissue injury is followed by an inflammatory response including macrophage accumulation and activation. These macrophages are able to release a number of cytokines and ROS. Both vascular changes as well as an increase in oxygen consumption, due to macrophage activation, contribute to the development of hypoxia. Hypoxia further stimulates production of ROS, and profibrogenic and proangiogenic cytokines. This response to hypoxia perpetuates tissue damage and stimulates angiogenesis through VEGF production.

Tumour bearing mice receiving different doses of radiation both in presence and absence of 10-MC treatment showed significant reduction in tumour volume. Mice receiving 10-MC and 4G of radiation had comparable reduction in tumour volume to that of mice treated with 6G of radiation alone. The production of VEGF in animals treated with 10-MC and 4G of radiation was much lesser when compared to animals receiving 6G of radiation alone. So co administration of 10-MC was found to produce the similar therapeutic effects of 6G of radiation by using a smaller dose of radiation (6G) while reducing the tumour spreading risk associated with radiation.

In summary, this study clearly demonstrates that 10-MC offer protection against the radiation and chemotherapy associated toxic side effects to immune system. This may be due to the stimulation of immune system and it could be used as an adjuvant during radiation treatment. This was found to produce same beneficial effects at lower radiation dose, with reduced tumour volume and levels of VEGF.