Chapter 4

Effect of Brahma Rasayana in Amelioration of Radiation Induced Damage
4.1. Introduction

Radiation has been known to induce undesired side effect of which myelosuppression is a major one. The inhibition of cellular proliferation is the mechanism by which radiation kills most cells (Little, 1968). Radiation can kill cells by two mechanisms. The first is apoptosis (programmed cell death or interphase death) (Lowe et al., 1993). Cells undergoing apoptosis usually die in interphase within a few hours of irradiation, irrespective of and without intervening mitosis. The second mechanism is radiation induced mitotic failure. Radiation in sufficient doses can inhibit mitosis. The co-ordination of the stem cell system is maintained partly by local and humoral regulations. Even under physiologic conditions, both pluripotent and committed stem cells migrate from one haemopoietic site to the other. At any given time the circulating stem cells form an insignificant portion of the total stem cell compartment (0.25%) (Hellman et al., 1968). However the rapid clearance of circulating colony forming units (CFUs) from blood suggests that 20-30% of the total CFU content of the organism may migrate through the circulation everyday (Metcalf et al., 1971). These factors undoubtedly play an important role in the regeneration of the hemopoietic system under certain pathological conditions, especially in regeneration after partial-body irradiation. Partial shielding of hemopoietic tissues during exposure protects the organism from the lethal effects of ionizing radiation (Jacobson et al., 1949). Lord and Schofield (1973) showed that liver, thymus cells can augment spleen colony formation in a specific manner under conditions in which the bone marrow populations have been damaged by sublethal irradiation. In the present chapter, it has been tried to study the mechanism of radioprotecting and immunostimulating activity of BR in mice.

4.2. Materials and Methods

Aqueous suspension of BR was used for all experiments. p-Rosaniline hydrochloride and α-naphthyl acetate were obtained from Loba Chemie, Bombay. Harris haematoxylin was purchased from Glaxo India Ltd., Mumbai.
Figure 26

Effect of Brahma Rasayana (BR) on Total Leukocyte Count and Differential Count in Irradiated Mice

A

B

A - Total Leukocyte Count

B - Differential Count
Cytokine mouse Elisa kits (IFN-γ, IL-2 and GM-CSF) were obtained from Endogen, USA. All other chemicals and reagents were of analytical grade.

Inbred strains of Balb/c and Swiss albino mice (4-5 weeks old, 20-25g) were purchased from National Centre for Laboratory Animal Sciences, Hyderabad. They were housed in Ventilated cages in air controlled rooms and fed with normal mouse chow (Lipton, India) and water ad libitum. Whole body radiation was given using Cobalt -60 teletherapy unit (Theratron 780, Canada). Animals were kept in specially constructed restraining boxes with a capacity of holding ten mice and irradiated by Gamma rays (1Gy/min).

4.2.1. Effect of BR on haematological parameters in normal and irradiated mice.

4.2.1.a. Normal animals.

Inbred strains of male Balb/c mice (4-5 weeks old, 20-25g) were used to carry out this study. Mice were divided into two groups (6 animals/group). Group I mice were treated orally with 15 daily doses of BRR (10mg/dose/mouse for 15 days). Group II mice were also treated orally with 15 daily doses of BR (50mg/dose/mouse for 15 days). Initial value without treatment was considered as normal control value. Blood was collected from the caudal vein of mouse and parameters such as total, leukocyte count (TC-haemocytometer) and differential count (Leishman’s stain) (Chesbrough and Mac Arthur, 1976) were recorded prior to the drug administration and continued on every 3rd day for 30 days.

4.2.1.b. Irradiated animals.

Inbred strains of male Swiss albino mice were used for the experiment. Mice were divided into three groups of 6 animals per group. Group I received single exposure of whole body radiation (600 rads /mouse) and served as control. Group II animals were received whole body radiation and daily oral administration of BR (10 mg /dose/mouse). Group III animals were treated with whole body radiation and daily oral administration of BR (50 mg/dose/mouse). BR administration was started three days prior to radiation and continued for fifteen days. Blood was collected from the caudal vein, total leukocyte count (TC) and differential count.
were recorded prior to drug administration, 24 hour after radiation and continued on every 3rd day for 30 days.

**4.2.2. Effect of BR on body weight, organ weight, bone marrow cellularity and \( \alpha \) - esterase activity in normal and irradiated mice.**

**4.2.2.a. Normal animals.**

Male balb/c mice were used for this study and were divided into three groups of 6 animals per group. Group I was kept as normal. Group II was treated orally with daily doses of BR(10 mg /dose/mouse) for 15 days. Group III was treated orally with daily doses of BR(50 mg/dose/mouse) for 15 days. Body weight of mice were recorded prior and after the drug administration. Three mice from each group were sacrificed on 16th day and 21st day of treatment and bone marrow cellularity, \( \alpha \) - esterase activity and weight of vital organs (liver, spleen, thymus, kidney and lungs expressed as relative organ weight) were recorded.

Bone marrow cellularity was done according to the method of Sredni et al 1992 as given in Mehta and Vaidya (1984). Bone marrow was collected from femur into the medium containing 2% goat serum and made into a single cell suspension. The number of cells were determined using a haemocytometer, and expressed as total live cells per femur. Bone marrow cells from the above preparation were smeared on clear glass slides and stained with para-rosaniline and Harris haematoxylin to determine the non-specific \( \alpha \)-esterase activity by simultaneous azodye coupling method (Bancroft and Cook, 1984)

**4.2.2.b. Irradiated animals.**

Four groups of Swiss albino mice (12 mice/group) were used for this experiment. Group I was kept as normal control. Other groups II-IV received single exposure of whole body radiation (600 rads/mouse). Group II served as radiation treated control. Group III and IV were treated orally with 10 and 50 mg/dose/mouse respectively. Administration and BR was started 3 days prior to radiation and continued for 15 days. On 3rd, 9th, 16th and 21st day after irradiation, body weight of animals (3 mice from each group) were recorded and
animals were sacrificed the animals for the analysis of organ weight, bone marrow cellularity and \( \alpha \) - esterase activity (Bancroft and Cook, 1984) as given above.

### 4.2.3. Determination of effect of BR on spleen colony assay

Inbred strains of Balb/c mice were divided into 3 groups (6 mice/group). All groups were exposed to single whole body radiation (400 rads/mouse). At this dose the radiation related mortality was significantly low but produced significant depletion of bone marrow cells (Praveen Kumar et al, 1996). Group I received bone marrow cells (1x10^6 cells/mouse) from normal mice through caudal vein (intravenously) which served as normal control. Group II and III received bone marrow cells from BR treated mice (daily dose of 50mg/mouse for 10 days, orally). Group III continued receiving BR for 5 more days (50mg/dose/mouse, po). Maximum number of spleen colonies are seen by 7-9 days (Robert et al, 1989). All the animals were sacrificed on 7th day and the number of nodular colonies on the surface of spleens (Till and McCulloch, 1961) were counted. Each colony formed was derived from a single precursor stem cell designated as colony forming unit-spleen (CFU-S).

### 4.2.4. Effect of BR on cytokine production in normal and irradiated mice.

Four groups of Balb/c mice were used to carry out this study. Group I was treated as normal. Group II was treated with BR (daily dose of 50mg/mouse for 10 days, po). Group III and IV were exposed to whole body irradiation (600 rads/mouse). Group III was kept as radiation treated control. Group IV was treated with daily dose of BR (50mg/mouse for 10 days, po). All the animals were sacrificed on 11th day. Blood was collected and serum was separated, levels of interferon-\( \gamma \) (IFN-\( \gamma \)), interleukin-2 (IL-2) and granulocyte macrophage-colony stimulating factor (GM-CSF) were determined by Mouse Elisa kits Endogen, USA.

### 4.3. Statistical Analysis

Data was expressed as mean \( \pm \) standard deviation. Significance levels for comparison of differences were determined using student’s t test.
4.4. Results

4.4.1. Effect of BR on total white blood cells (WBC) and percent of polymorphonuclear (PMN) cells in normal and irradiated mice.

Figure 25 represents the effect of BR on total WBC and percent PMN in normal mice. There was a significant \( p < 0.001 \) increase in the value of WBC count on 15\(^{th}\) day in BR treated mice (20800 cells/mm\(^3\)) as compared to the value of pretreatment (6625 cells/mm\(^3\)). PMN were also significantly \( p < 0.001 \) increased after BR treatment (29%) when compared to pretreatment value (14%).

Single exposure of whole body irradiation significantly reduced the total WBC count in mice within 24 hr and continued to be low (<3500) upto 12\(^{th}\) day and thereafter increased. It did not reach the normal value on 30\(^{th}\) day (Fig. 26A). BR treated animals had lower WBC initially, but the values increased significantly thereafter. Values were higher than 3500 cells/mm\(^3\) on 6\(^{th}\) day and continued to be higher than untreated controls. On 30\(^{th}\) day while total WBC in irradiated control animals was 6513 cells/mm\(^3\), BR treated animals had a total WBC between 8000-9400 cells/mm\(^3\).

Figure 26B represents the effect of BR on percent polymorphonuclear cells in irradiated mice. PMN cells were low in irradiated animals on 3\(^{rd}\) day (13.5%) and increased thereafter. On 30\(^{th}\) day it was (9.3%). In BR treated animals PMN values were 20-22% on 3\(^{rd}\) day and thereafter increased and reached maximum on 18\(^{th}\) day (30%). These experiments indicated that BR treatment increased the total count and PMN cells in normal and irradiated animals.

4.4.2. Effect of BR on body weight, organ weight, bone-marrow cellularity and \( \alpha \)-esterase activity in normal and irradiated mice

Change in body weight of normal mice after treated with BR (10 and 50 mg dose/mouse, po for 15 days) was slightly increased when compared to normal mice (0.03 ± 0.05), the value was 0.45 ± 0.14 and 0.6 ± 0.36 respectively (Table 8). There was no change in relative organ weight of BR treated mice when compared to untreated normal mice (Fig. 27). Administration
Figure 25

Effect of Brahma Rasayana (BR) on Total Leukocyte Count and Differential Count in Normal Mice
Table 8
Effect of Brahma Rasayana (BR) on body weight of normal and irradiated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>BR (10 mg)</td>
<td>-</td>
</tr>
<tr>
<td>BR (50 mg)</td>
<td>-</td>
</tr>
<tr>
<td>Radiation alone</td>
<td>-6.8±1.1</td>
</tr>
<tr>
<td>Radiation + BR (10 mg)</td>
<td>-4.9±0.8</td>
</tr>
<tr>
<td>Radiation + BR (50 mg)</td>
<td>-4±1.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 3 observations.
Figure 27
Effect of Brahma Rasayana (BR) on Relative Organ Weight of Normal and Irradiated Mice

- Normal
- BR 10 mg
- BR 50 mg
- Radiation alone
- Radiation + BR 10 mg
- Radiation + BR 50 mg
- Radiation alone
- Radiation + BR 10 mg
- Radiation + BR 50 mg
- Radiation alone
- Radiation + BR 10 mg
- Radiation + BR 50 mg

Days:
16th Day, 3rd Day, 16th Day, 21st Day

Relative organ wt. (g)

Legend:
- Spleen
- Thymus
- Liver
- Kidney
- Lung

Notations:
a p<0.001, b p<0.02, c p<0.05
of BR (50mg/dose/mouse for 15 days) significantly (p<0.001) increased the viability of bonemarrow cells (20.89x10^6/femur) when compared to untreated normal mice (12.7x10^6 cells/femur) (Table 9). The number of α-esterase positive cells were also increased significantly (p<0.001) in the 10mg (1499/4000 cells) and 50mg (1703/4000 cells) BR treated group when compared to untreated normal mice (1046/4000 cells) (Table 10).

Change in body weight of irradiated control mice were showed a 6.8 ± 1.1 gram per value on 3rd day which reaches to 2.6 ± 1.7 gram value on 21st day (Table 8). In BR treated irradiated mice, change in body weight on 3rd day was 4 ± 1.6 gram and it reached to 1.2 ± 0.83 gram on 21st day. On 3rd day, relative organ weight of spleen (p<0.02) and thymus (p<0.05) were increased in BR treated (50mg/mouse, for 15 days) irradiated mice when compared to irradiated control mice (Fig.27). There was a significant (p<0.001) increase in thymus and spleen weight on 16th day in BR treated irradiated mice. Relative organ weight of other organs were showed no change on 3rd, 16th and 21st day in BR treated irradiated mice and irradiated control mice. There was a significant reduction in bone marrow cellularity which reduced significantly in irradiated mice and reaches to 3.8x10^6 cells/femur when compared to normal (12.7x10^6 cell/femur) (Table 9). On 21st day, bone marrow cellularity of irradiated control mice were reached to normal value (11.8x 10^6 cells/femur). BR treatment significantly increased bonemarrow cellularity in irradiated mice. There was a significant increase (p<0.001) in immature bonemarrow cells on 3rd day (28x10^6±6.4) in BR treated irradiated mice and on other days the bonemarrow cells reached a stable number of cells may be due to the maturith of immature bonemarrow cells. On 21st day, the number of bonemarrow cells reached to 19.3x10^6±1.1 in BR treated irradiated mice. Table 10 represents the effect of BR on α-esterase positive cells in irradiated mice. α-esterase positive cells in bone marrow of radiation treated mice were low (242/4000 cells) and did not reach the normal level (1046/4000 cells) even after 21st day. In the case of BR treated irradiated animals, there was significant (p<0.001) increase in α-esterase positive cells on 3rd, 9th, 16th and 21st day.
Table 9

Effect of Brahma Rasayana (BR) on bone marrow cellularity in normal and irradiated mice

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Bonemarrow cellularity/femur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>Normal</td>
<td>–</td>
</tr>
<tr>
<td>BR (10mg)</td>
<td>–</td>
</tr>
<tr>
<td>BR (50 mg)</td>
<td>–</td>
</tr>
<tr>
<td>Radiation alone</td>
<td>3.8x10^6±0.8</td>
</tr>
<tr>
<td>Radiation + BR (10mg)</td>
<td>28.8x10^6±6.4</td>
</tr>
<tr>
<td>Radiation + BR (50 mg)</td>
<td>22.6x10^6±0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 3 observations.

*p<0.001,  *p<0.005,  *p<0.01,  *p<0.02
Table 10
Effect of Brahma Rasayana (BR) on α - esterase activity in normal and irradiated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of α - esterase positive cells/4000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>BR (10 mg)</td>
<td>-</td>
</tr>
<tr>
<td>BR (50 mg)</td>
<td>-</td>
</tr>
<tr>
<td>Radiation alone</td>
<td>242 ± 23</td>
</tr>
<tr>
<td>Radiation + BR (10 mg)</td>
<td>&quot;887±104</td>
</tr>
<tr>
<td>Radiation + BR (50 mg)</td>
<td>&quot;778±24</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 3 observations.  
\( ^{p} < 0.001, \quad ^{b} p < 0.005. \)
4.4.3. Effect of BR on spleen colony assay

Irradiated mice (Table 11) which received bone marrow cells from BR treated animals showed significant increase in the number of nodular colonies on the surface of spleens (6.2±0.75) compared to those animals received bone marrow cells from normal animals (3.83±1.07). Group of animals with continued BR treatment for 5 more days after irradiation showed a significantly higher number of nodular colonies on spleens (9.83±1.07).

4.4.4. Effect of BR on cytokine levels in normal and irradiated mice.

Irradiation significantly reduced the serum cytokine levels such as IFN-γ (690), IL-2 (7.2) and GM-CSF (26.3) in irradiated mice when compared to normal mice (Table 12). Oral administration of BR (50mg dose/mouse, for 10 days) increased the IFN-γ (3205), IL-2 (22.5) and GM-CSF (56.3) in normal mice. The concentration of IFN-γ (1770), IL-2 (24.3) and GM-CSF (37.1) were found to be increased after BR treatment in irradiated mice when compared to irradiated control mice.

4.5. Discussion

In the present study the immunopotentiating activity of BR, an indigenous herbal preparation was determined. Administration of BR has been shown to significantly increase total leukocyte count and percent of polymorphonuclear cells compared to lymphocytes indicating that the BR could stimulate the haemopoietic system. BR treatment also significantly increased the bone marrow cellularity and α-esterase positive cells which indicated the proliferation of stem cells and its differentiation. Irradiation significantly reduced the total WBC count, percent of PMN, bone marrow cellularity and α-esterase positive cells and simultaneous administration of BR significantly increased the number of cells, indicating that BR stimulate the haemopoietic system, proliferation of stem cells and its differentiation.
Table 11

Effect of Brahma Rasayana (BR) on spleen colony assay in irradiated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average number of nodular colony / spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Normal spleen</td>
<td>3.83 ± 1.07</td>
</tr>
<tr>
<td>II. Treated with BR</td>
<td>6.20 ± 0.75</td>
</tr>
<tr>
<td>III. Treated with BR</td>
<td>9.83 ± 1.07</td>
</tr>
</tbody>
</table>

Group II and III received bonemarrow cells from BR treated mice (10 daily. Dose – 50 mg / dose / animal, p.o.).

Group III continued BR (50mg) five more days.

*p<0.001
**Table 12**

Effect of Brahma Rasayansa (BR) on cytokine levels in normal and irradiated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokines (pg/mL Serum)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN - γ</td>
<td>IL - 2</td>
<td>GM - CSF</td>
</tr>
<tr>
<td>Normal</td>
<td>2980</td>
<td>7.5</td>
<td>32.0</td>
</tr>
<tr>
<td>BR (50mg)</td>
<td>3205</td>
<td>22.5</td>
<td>56.3</td>
</tr>
<tr>
<td>Radiation alone</td>
<td>690</td>
<td>7.2</td>
<td>26.3</td>
</tr>
<tr>
<td>Radiation + BR (50 mg)</td>
<td>1770</td>
<td>24.3</td>
<td>37.1</td>
</tr>
</tbody>
</table>

Values are mean of 3 observations.
Administration of bone marrow cells from treated animals to irradiated recipients increased the number of nodular colonies on the surface of spleens. This indicates the ability of bone marrow cells to migrate and recirculate with the blood and eventually occupy the radiation damaged haemopoietic and lymphoid tissue. As we have used the radiation dosage of 4Gy for depleting the stem cells from recipient mice part of the colony may be derived from recipients' own stem cells. However, the data indicated a significant difference between bone marrow of BR treated animals and that of normal animals.

BR stimulated the production of cytokines, such as IFN-γ, IL-2 and GM-CSF in normal and irradiated mice. IL-2 stimulates specific receptors situated on the surface of T-lymphocytes and induce vigorous proliferation of T-cell clone in parallel with mitogenic stimulation. IFN-γ enhances the immune response by increasing T4 cells function, which can promote expression of IL-2 on T-cells. GM-CSF promotes differentiation of activated B-cells that secrete IgM, IgG2a and IgG3 (Robert et al, 1989).

These results indicate that BR, a nontoxic herbal drug preparation, has immunopotentiating activity in stem cell production, its differentiation and proliferation. The biological products obtained from plant sources such as polysaccharides, lectins, peptides etc. have been shown to stimulate immune system and useful in reducing the immunosuppression during irradiation (Landequist and Teuscher, 1985). Mechanism of action of many of the plant materials present in BR is largely unknown. As the preparation given a multitude of biological activity, it should be inferred that activity of BR is a combine effect of several plant derived compounds. Active principle involved it, is yet to be confirmed.