Chapter 6

Role of Brahma Rasayana in Immune Responses of Normal and Irradiated Mice
6.1. Introduction

Cancer treatment with chemotherapeutic agents and ionizing radiation have considerable effect on haemopoietic system. In fact, profound myelosuppression and the resulting leukocytopenia is a major side effect of ionizing radiations (Manch et al., 1995). A variety of immunopotentiating agents administered to mice before irradiation increased the survival of animals after lethal irradiation (Thatte and Dhanukar, 1989; Sakagami et al., 1991). The radioprotective effect conferred by immunomodulators could reduce these side effects (Mihich, 1982). Immunotherapy is costly and may not be immediately useful to the patients in several developing countries.

Traditional use of herbal medicine is usually an integral part of culture, which was developed within an ethnic group before the development and spread of modern science. There are several herbal preparations used in indigenous medical therapy, which can enhance the body’s immune status. Rasayanas are a group of drug preparations made of several plant products used in Ayurvedic system of medicine to improve body’s immune system (Singh, 1990). Brahma Rasayana (BR) is a non-toxic polyherbal preparation. According to Ayurvedic texts Rasayana therapy arrests aging, increases intelligence, vigour and resistance to diseases. However no concrete studies have done to substantiate these claims. Mahasarishi Amruth Kalash (MAK), a modified indigenous herbal preparation, possesses many of activities produced by BR (Sharma et al., 1992). The biological products obtained from plant sources such as polysaccharides, lectins, peptides etc. have been shown to stimulate the immune system (Hajto et al., 1989; Kuttan and Kuttan, 1992). Immunomodulatory and immunorestorative properties of Rasayanas find their use in diseases like cancer, AIDS, Tuberculosis etc. Chemoprotective effects of Rasayanas have been shown to stimulate stem cell proliferation and possibly its differentiation (Praveenkumar et al., 1994) Present chapter has been designed to establish the effect of Brahma Rasayana on immune response in normal and irradiated mice.
6.2. Materials and Methods

Methanolic extract of BR was prepared by dissolving 10gm of material in 100ml of 70% methanol twice, by stirring overnight, filtered and centrifuged. The supernatant was collected and evaporated to dryness. The dried methanolic extract (70% yield) was thoroughly mixed with double distilled water and was used for culture experiments. Water suspension of BR was used for all in vivo studies.

Tissue culture medium- RPMI 1640 (Rose Well Park Memorial Institute), Hank’s balanced salt solution (HBSS), Bovine serum albumin (BSA) and Minimum Essential Medium (MEM) were obtained from Hi-media Laboratories, Mumbai. Foetal calf serum (FCS) was obtained from Biological Industries, Kibbutz Beit Haemek, Israel. Phytohaemagglutinin (PHA-M) was obtained from Difio, USA. [3H]-thymidine was obtained from BRIT, Babha Atomic Research Centre (BARC), Mumbai, MTT was obtained from sigma Aldrich. Dimethyl sulfoxide (DMSO) and perchloric acid were obtained from Sisco Research Laboratories, Mumbai. Trypanblue was obtained from E-Merck (India). All other reagents were of analytical grade. Sheep red blood cells (SRBC) was collected in Alsever’s solution from local slaughter house.

6.2.a. Cell lines

Dalton’s lymphoma ascites cells (DLA) were obtained from Cancer Institute, Adyar and cells were maintained as ascites tumour in Swiss albino and Balb/c mice. L-929 (lung fibroblast) cells were obtained from National Centre for Cell Science, Pune. The cells were maintained in culture using minimum essential medium (MEM) containing 10% heated inactivated goat serum and antibiotics, subcultured by standard procedure.

6.2.b. Animals

Inbred strains of Balb/c mice (4-5 weeks old, 20-25g) were reared from our animal house and were housed in ventilated cages in air-controlled rooms and fed with normal mouse chow and water ad libitum.
6.2.c. Radiation treatment

Whole body radiation (400 rads) was given using Cobalt -60 teletherapy unit (Theratron 780, Canada) Animals were kept in capacity of holding ten mice and irradiated using gamma rays at a dose level of 100 rads per minute.

6.2.1. Effect of BR on Dalton’s Lymphoma Ascites (DLA) cells - *in vitro* short term cytotoxicity assay

The tumour cells were aspirated from the peritoneal cavity of tumour bearing mice cells were washed thrice with phosphate buffered saline (PBS -pH 7.2) and checked for viability using trypan blue exclusion method (Kuttan et al; 1985). The cell suspension (1x10^6 cells/0.1mL) was added to tubes, containing various concentrations of extracts of BR and the volume was made upto 1ml using PBS. Control tubes contained only cell suspensions. The assay mixture was incubated for 3 hour at 37°C and percent of dead cells were evaluated by trypan blue exclusion method.

6.2.2. Effect of BR on lung fibroblast (L-929) cells in presence and absence of serum *in vitro* (MTT assay)

The MTT assay was conducted according to the procedure described by Carmichael et al (1987). The assay is dependent on the cellular reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. One hundred microliters (100μL) of RPMI-1640 containing antibiotics and 10% FCS with L-929 cells (1x10^4) were plated in 96 well flat bottom titre plates. Plates were incubated at 37°C for 24 hour. After incubation the medium was removed and washed with PBS. Different concentration of extracts of BR was added to the well to the medium with or without serum. Plates were again incubated at 37°C for 48 hour. Twenty microliters (20μL) of MTT solution was added 4 hour before the termination of incubation. After 4 hour incubation at 37°C, the plates were centrifuged at 450xg for 10min. in a plate holder, and remaining MTT solution was removed from each well. Care was taken not to disturb the formazan crystals at the bottom of wells. One hundred microliters (100μL) of dimethyl sulfoxide (DMSO) were added to
each well and plates were placed on a shaker at room temperature (RT) for 30 min. to ensure adequate dissolution. The optical density of each well was read immediately at 570 nm (test wavelength) and a reference wavelength (690 nm) using a multiwell scanning spectrophotometer (ELISA reader). All measurements were performed in triplicates percent viability was calculated by comparing the optical density of treated and untreated controls.

6.2.3. Effect of BR on \[^3\text{H}\]-thymidine uptake of PHA stimulated and non stimulated thymus, spleen and bone marrow cells of mice, in vitro

Thymus or spleen or bone marrow was collected from normal Balb/c mice. Single cell suspension of thymus or spleen or bonemarrow cells was prepared under sterile, cold conditions (0°C). Mice were killed by cervical dislocation, bone marrow from both femurs were collected into a sterile tube containing PBS (pH 7.2) and 2% heat inactivated goat serum, centrifuged washed thrice, made up to single cell suspension. After checking viability using typan blue solution and the cells number was adjusted to 1x10^6 cells/0.1 mL. Spleen and thymus were removed from mice, thoroughly cleaned and cut into small pieces for smashing the tissue. The smashed cells (single cells) were collected into a sterile tubes containing PBS and 2% goat serum. The tubes were kept at 4°C for 3 minutes. After removing the debris, the tubes were centrifuged, washed thrice, checked viability and adjusted the cells number to 1x10^6 cells/0.1 mL. Processed cells (1x10^6 cells) were cultured in 1 ml RPMI-1640 medium containing 10% FCS in presence and absence of mitogen (PHA-M, 6µg/mL) different concentrations of extract of BR (0, 0.5, 1,2,5,10 and 50µg/mL) and antibiotics. Culture vials were incubated in a humidified atmosphere of 5% CO₂ at 37°C. After 48 hour incubation, \[^3\text{H}\]-thymidine (1 µCi/vial) was added and was continued for incubation (16-18 hour) at 37°C. After incubation, vials were kept in ice buckets and centrifuged to remove the supernatant. To each vial, ice cold 2 mL Perchloric acid (0.8M PCA) and 100 µL of 1% BSA were added and kept at -20°C for 20 minute. After incubation, each vial was vortex mixed, centrifuged and supernatant was removed. The above process was again repeated for once. Each vial
containing the cell pellet was dissolved by adding 0.5mL of 0.5N sodium hydroxide. The dissolved solution (0.5ml) from each vial was taken into a 5mL scintillation fluid and kept overnight in dark and the radioactivity was measured in β-counter (Mustafa, 1992) Cell proliferation was measured by stimulation index (SI= cpm sample/cpm control).

6.2.4. Effect of BR on [³H] thymidine uptake of thymus, spleen and bonemarrow cells of normal and irradiated mice (in vivo)

Inbred strains of Balb/c mice (4-5 weeks old) were used for this study. All mice were divided into two groups. Group I kept as normal control and group II was treated with BR (50mg/dose/mouse,po) for five days. All mice were sacrificed on 5th day. Thymus, spleen and bone marrow were collected from each animal. The processed cell (1x10⁶cells/mL) were cultured as described above (Mustafa, 1992).

Inbred strains of Balb/c mice were divided into two groups. Group I was kept as irradiated control. Group II was treated with BR (50mg/dose/mouse,po) for 5 days. 24 hour after radiation, all mice were killed by cervical dislocation. All mice were treated with single exposure of whole body radiation (400 rads/mouse) on 4th day. Thymus, spleen and bonemarrow were collected from each animal. The processed cells (1x10⁶cells/mL) were cultured (Mustafa, 1992) and [³H]-thymidine uptake in presence and absence of mitogen was measured as given above.

6.2.5. Effect of BR on circulating antibody titre in irradiated mice.

Inbred strains of Balb/c mice (4-5weeks old) were used for this experiment. All mice were immunized with 0.1ml of 20% SRBC intraperitoneally (ip) on 5th day. Group I (6 mice/group) was kept as normal. Group II and III received single exposure of whole body radiation (400 rads/mouse) on 4th day. Group II was kept as irradiated control. Group III was treated with BR (50mg/dose/mouse, po) for five days. Blood was collected from caudal vein every 3rd day after 5th dose of drug administration and continued for a period of thirty days. Antibody titre was estimated by the method of Singh et al (1984) using SRBC as antigen. Sera
samples of each group were pooled and heat inactivated at 56°C for 30 minutes. Two fold dilutions of sera samples were made in PBS (PH 7.2) in microtitre plates and mixed (1:1) with 1% trypsinized suspension of SRBC in PBS. Agglutination was noted after incubation for 3 hour at room temperature (Nelson and Davey, 1992).

6.2.6. Effect of BR on antibody forming cells in normal and irradiated mice.

Jerne's plaque assay (Jerne and Nordia, 1963) using a modified slide technique was employed (Mehrotra, 1992). Inbred strains of Balb/c mice (4-5 weeks old, 20-25g) were divided into 5 groups (8 mice/group). Group I was kept as normal. Group II was treated with BR (50mg/ dose/mouse, po) for five days. Group III and IV were treated with single exposure of whole body radiation (400 rads/mouse) on 4th day. Group III was kept as irradiated control. Group IV was treated with BR (50mg/dose/mouse, po) for five days. All mice were immunized with SRBC (2.5x10^8 cells/mouse) intraperitoneally (ip) on 5th day. The animals were sacrificed on various days (3 to 9) and spleens were processed into single cell suspension (8x10^6 cells/mL) in Hank's balanced salt solution (HBSS). To 0.5mL of 5% agarose prepared in HBSS, 50 µL of 7% SRBC and 50 µL of spleen cell suspension were added, mixed well and poured over a glass slide. The slides were allowed to solidify and incubated with fresh rabbit serum as a source of complement for one hour at 37°C. The plaques formed were counted using a colony counter and represented as plaque forming cells (PFCs) per million spleen cells.

6.3. Statistical Analysis

Data was expressed as mean ± standard deviation. Significant levels for comparison of differences were determined using student's t test.
6.4. Results

6.4.1. Effect of BR on Dalton’s lymphoma ascites (DLA) cells – *in vitro* short term cytotoxicity assay

Figure 39 represents the effect of BR on DLA cells by trypan blue exclusion method. When concentration of BR extract was increased up to 100mg/mL, the percent of dead cells were increased to 78%. At 80mg/mL of BR concentration, the percent cytotoxicity was found to be 56%. Percent cytotoxicity was decreased at 50mg/mL of BR concentration and was found to be 21%. This study concluded that BR produced cytotoxicity only at higher concentration and percent of dead cells decreased with lowering of the concentration of BR extract.

6.4.2. Effect of BR on lung fibroblast (L-929) cells in presence and absence of serum, *in vitro* (MTT assay)

Figure 40 represents the effect of BR on L-929 cells. BR was found to be more cytotoxic to culture cells. Percent of cell viability was decreased with increased concentration of BR at higher concentration (10mg/mL), percent of cell viability was found to be 49% in presence of serum and 10% in absence of serum. At 5 mg/mL concentration, the percent viability was found to be 58% in both conditions. When concentration of BR was decreased to 2mg/mL, percent of viable cells was found to be 88% in presence of serum and 73 in absence of serum. Percent of cell viability was found to be 90% (presence of serum) and 84% (absence of serum) at 1mg/mL of BR extract.

6.4.3 Effect of BR on *[^3]H*- thymidine uptake of PHA stimulated and non-stimulated thymus, spleen and bonemarrow cells of mice, *in vitro*

6.4.3.a. Thymus proliferation.

Effect of BR on *[^3]H*-thymidine uptake of thymus cells is shown in table 14. Uptake of *[^3]H*-thymidine (proliferation capacity) was found to be increased initially up to 2µg/mL of BR extract and thereafter decreased. At zero concentration of BR maximum thymidine uptake was 611 ± 71.2 in PHA non stimulated cells and 2124 ± 144 in PHA stimulated cells. *[^3]H* - thymidine uptake was significantly *(p<0.001)* increased with increasing concentration up to 2µg/mL.
Figure 39
Effect of Brahma Rasayana (BR) on Dalton's Lymphoma Ascites (DLA) Cells -
In Vitro Short Term Cytotoxicity Assay

Concentration of BR extract (mg/mL)

Percent cytotoxicity

- With BR
Figure 40
Effect of Brahma Rasayana (BR) on Lung Fibroblast (L-929) Cells in Presence and Absence of Serum *In Vitro* (MTT Assay)
### Table - 14

**Effect of Brahma Rasayana (BR) on [³H]- thymidine uptake of PHA stimulated and non-stimulated thymus cells of mice, in vitro**

<table>
<thead>
<tr>
<th>Concentration of BR (µg/10⁶cells/mL)</th>
<th>Without PHA</th>
<th>With PHA (6µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cpm</td>
<td>SI</td>
</tr>
<tr>
<td>0</td>
<td>611±72.1</td>
<td>0</td>
</tr>
<tr>
<td>0.05</td>
<td>b2407±368</td>
<td>3.93±0.6</td>
</tr>
<tr>
<td>1.0</td>
<td>a3341±74</td>
<td>5.5±0.12</td>
</tr>
<tr>
<td>2.0</td>
<td>a3859±359</td>
<td>6.32±0.59</td>
</tr>
<tr>
<td>5.0</td>
<td>a1561±159</td>
<td>2.55±0.27</td>
</tr>
<tr>
<td>10</td>
<td>a1168±237</td>
<td>1.91±0.39</td>
</tr>
<tr>
<td>50</td>
<td>a824±150</td>
<td>1.35±0.24</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicates

aP>0.001, bP<0.001, cP<0.005, dP<0.01, eP<0.05
in PHA non-stimulated cells (3859 ± 359), PHA stimulated cells (4979 ± 402) and gradually decreased. At 50μg/mL, [³H]-thymidine uptake in PHA non-stimulated and PHA stimulated cells were found to be 824 ± 150 and 1360 ± 539 respectively (Fig.41 and 42).

6.4.3.b. Spleen proliferation

Table 15 represents the effect of BR on [³H]-thymidine uptake of PHA stimulated and non-stimulated spleen cells of mice. Uptake of [³H]-thymidine (proliferation capacity) was found to be increased initially upto 2μg/mL of BR extract and thereafter decreased. At zero concentration of BR maximum thymidine uptake was 435 ± 51.5 in PHA non-stimulated cells and 1544 ± 534 in PHA stimulated cells. [³H]-thymidine uptake was significantly (p<0.001) increased with increasing concentration upto 2μg/mL in PHA non-stimulated cells (4277 ± 1002), PHA stimulated cells (5563 ± 1611) and gradually decreased. At 50μg/mL, [³H]-thymidine uptake in PHA non-stimulated and PHA stimulated cells were found to be 1163 ± 404 and 1799 ± 248 respectively (Fig.41 and 42).

6.4.3.c. Bonemarrow proliferation

Effect of BR on [³H]-thymidine uptake of bone marrow cells of mice is shown in table 16. Uptake of [³H]-thymidine (proliferation capacity) was found to be increased initially upto 5μg/mL of BR extract and thereafter decreased. At zero concentration of BR maximum thymidine uptake was 490 ± 98 in PHA non-stimulated cells and 1150 ± 152 in PHA stimulated cells. [³H]-thymidine uptake was significantly (p<0.001) increased with increasing concentration upto 5μg/mL in PHA non-stimulated cells (1822 ± 201), PHA stimulated cells (2299 ± 661) and gradually decreased. At 50μg/mL, [³H]-thymidine uptake in PHA non-stimulated and PHA stimulated cells were found to be 625 ± 188 and 930 ± 361 respectively (Fig. 41 and 42).
Figure 41
Effect of Brahma Rasayana (BR) on $[^3\text{H}]$ - Thymidine Uptake of PHA Non-Stimulated Thymus, Spleen and Bone marrow Cells of Mice - *In Vitro*.
Figure 42
Effect of Brahma Rasayana (BR) on $[^3\text{H}]$ - Thymidine Uptake of PHA Stimulated Thymus, Spleen and Bonemarrow Cells of Mice - *In Vitro*

![Graph showing the effect of Brahma Rasayana (BR) on $[^3\text{H}]$ - Thymidine Uptake of PHA Stimulated Thymus, Spleen and Bonemarrow Cells of Mice - *In Vitro*]
Table - 15

Effect of Brahma Rasayana (BR) on [³H] -thymidine uptake of PHA stimulated and non-stimulated spleen cells of mice, *in vitro*

<table>
<thead>
<tr>
<th>Concentration of BR (µg/10⁶cells/mL)</th>
<th>Without PHA</th>
<th>With PHA (6µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cpm</td>
<td>SI</td>
</tr>
<tr>
<td>0</td>
<td>435±51.5</td>
<td>0</td>
</tr>
<tr>
<td>0.05</td>
<td>b²789±564</td>
<td>6.4±1.3</td>
</tr>
<tr>
<td>1.0</td>
<td>b³955±1048</td>
<td>9.1±2.3</td>
</tr>
<tr>
<td>2.0</td>
<td>b⁴277±1002</td>
<td>9.8±2.2</td>
</tr>
<tr>
<td>5.0</td>
<td>b²285±113</td>
<td>5.3±0.24</td>
</tr>
<tr>
<td>10</td>
<td>b¹269±420</td>
<td>2.91±0.9</td>
</tr>
<tr>
<td>50</td>
<td>b¹163±404</td>
<td>2.7±0.9</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicates

*P<0.001, bP<0.001, cP<0.01, dP<0.02, eP<0.05
### Table – 16

**Effect of Brahma Rasayana (BR) on \[^{3}H\] -thymidine uptake of PHA stimulated and non-stimulated bone marrow cells of mice, in vitro**

<table>
<thead>
<tr>
<th>Concentration of BR (µg/10^6 cells/mL)</th>
<th>Without PHA</th>
<th>With PHA (6µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cpm</td>
<td>SI</td>
</tr>
<tr>
<td>0</td>
<td>490±98</td>
<td>0</td>
</tr>
<tr>
<td>0.05</td>
<td>^1605±421</td>
<td>3.28±0.86</td>
</tr>
<tr>
<td>1.0</td>
<td>^1387±654</td>
<td>2.83±1.3</td>
</tr>
<tr>
<td>2.0</td>
<td>^1432±292</td>
<td>2.92±1.2</td>
</tr>
<tr>
<td>5.0</td>
<td>^1822±201</td>
<td>3.72±0.4</td>
</tr>
<tr>
<td>10</td>
<td>^643±44.2</td>
<td>1.3±0.09</td>
</tr>
<tr>
<td>50</td>
<td>625±188</td>
<td>1.28±0.38</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicates

^p>0.001, ^b p<0.005, ^c p<0.01, ^d p<0.02, ^" p<0.05

Figure 43 represents the effect of BR on $[^3]H$-thymidine uptake of cells of normal mice (*in vivo*) in non-stimulated condition, tritiated thymidine uptake of spleen and thymus was found to be increased two times in BR treated mice (717±155cpm, 814±371cpm respectively) when compared to normal mice (352±55.6cpm, 432±36cpm respectively). When the cells were stimulated with PHA, $[^3]H$-thymidine uptake was found to increase in treated and untreated cells and the thymidine uptake in PHA stimulated thymus was (1617±91), spleen (1627±197) and bonemarrow (1600±92) cells of BR treated mice when compared to normal control.

There was no change in $[^3]H$-thymidine uptake of PHA stimulated and non-stimulated condition of thymus, spleen and bonemarrow cells of irradiated mice. Irradiation suppressed the mitogenic activity of PHA. In PHA stimulated and non-stimulated conditions, BR treated irradiated mice showed a slight increase in $[^3]H$-thymidine uptake when compared to irradiated control mice.

6.4.5. Effect of BR on circulating antibody titre in irradiated mice.

The effect of BR on circulating antibody titre is shown in figure 44. Normal mice were showed a maximum titre value 128 on 15$^{th}$ to 24$^{th}$ day. Radiation (400 rads/mouse, single dose) treated normal mice were showed no titre value on 3$^{rd}$ day titre value increased gradually on 12$^{th}$ to 21$^{st}$ day and on 24$^{th}$ to 30$^{th}$ day, the value reached to 256. In BR treated irradiated mice, on 3$^{rd}$ day titre value was 32 and increased on following days, the maximum titre was obtained on 18$^{th}$ to 21$^{st}$ day (512) and on 24$^{th}$ to 30$^{th}$ day, value reached to 256. From this experiment, it was concluded that BR treatment is effective to the enhance of antibody production in irradiated animals.
Figure 43
Effect of Brahma Rasayana (BR) on $[^3]H$ - Thymidine Uptake of Thymus, Spleen, and Bone marrow Cells of Normal and Irradiated Mice - In Vivo

Counts per minute (cpm)

Thymus
Without PHA
With PHA (6μg/mL)
Spleen
Without PHA
With PHA (6μg/mL)
Bone marrow
Without PHA
With PHA (6μg/mL)

Normal
BR (50mg)
Radiation alone
Radiation + BR (50mg)

$^a p<0.005$, $^b p<0.01$, $^c p<0.02$
Figure 44
Effect of BR on circulating antibody titre in irradiated mice
6.4.6. Effect of BR on antibody forming cells in normal and irradiated mice.

Figure 45 represents the effect of BR on antibody forming cells. Administration of BR was found to increase antibody producing cells in both normal and irradiated mice. Irradiated control mice had decreased the number of antibody producing cells in spleen. Maximum number of plaques per $10^6$ spleen cells was $80\pm10.6$ on $4^{th}$ day and reached to $68 \pm 11.8$ on $9^{th}$ day. BR treated normal mice were showed an increase in number of plaque forming cells on $5^{th}$ day and the value was $442\pm20.9$. and on $9^{th}$ day the number of plaques were reached to $108 \pm 3.8$. In BR treated irradiated mice, the maximum number of plaque forming cells per $10^6$ spleen cells were found to be $293\pm20.7$ on $4^{th}$ day and on day 9, the maximum number of plaque forming cells per $10^6$ spleen cells were $122 \pm 15.6$.

6.5. Discussion

Present chapter highlights the effect of BR on cell proliferation in normal cells and cells treated with radiation as well as on antibody forming cells and antibody titre. When accidental exposure to radiation occurs in the normal body, one of the first observable effects of radiation on cells both in vitro and in vivo is the prevention of cells from entering mitosis, often referred to as G$_2$ block or mitotic delay (Elkind et al., 1963; Whitmore et al., 1967). Bone marrow and thymus are the primary lymphoid organs. Bone marrow is the major site of haemopoiesis, provides antigen independent differentiation of B-cells and antigen processing environment. Thymus is responsible for antigen-independent maturation and development of T-lymphocytes that effect cell-mediated immunity and regulate most humoral and cell-mediated immune responses. Spleen is the secondary lymphoid organs. It act as temporary reserve site for lymphocytes, provides antigen processing environment and is auxiliary site of hemopoiesis in extraordinary circumstances (Robert et al., 1989). Earlier studies reveals that BR enhanced both humoral and cell-mediated immunity (Praveen kumar et al., 1999).
Figure 45
Effect of Brahma Rasayana (BR) on Jerne's Plaque Forming Cells Assay in Normal and Irradiated Mice

Number of plaque forming cells/10^6 spleen cells

Days

- Normal
- BR (50mg)
- Radiation alone
- Radiation + BR (50mg)
BR Treatment had no cytotoxic effect on transformed (DLA or L-929) cell lines at lower concentration. In vitro [³H] thymidine uptake of normal cells (thymus, spleen and bone marrow) of mice reveals that BR treatment increased the proliferation level at lower concentration (1-5μg/10⁶cell/mL) in PHA stimulated and non-stimulated cells. BR treated normal mice (in vivo) showed an increase in the proliferation of theses cells in stimulated and non-stimulated conditions. Irradiation was found to suppress the proliferation level in PHA treated irradiated cells of normal mice. There was no change in the PHA non-stimulated irradiated cells when compared to PHA non-stimulated cells of normal mice. This effect may be due to cells which are in mitosis are not prevented from completing the division, but no new prophases are formed for a time that seems to be dependent upon the dose of radiation administered and the cell cycle time of un-irradiated cells (Doida and Okada, 1969). It was observed that, in vitro BR treatment enhanced proliferation of thymus, spleen and bone marrow cells in mitogen stimulated cells and non-stimulated conditions and it may be due to the ability of BR to enhance the stimulation of mitosis.

Administration of BR showed an increase in antibody titre on 18th and 21st day and plaque forming cells on 4th day in mice treated with radiation. Antibody titre is based on soluble antigen (Ag) is passively absorbed to red blood cells. Plaque forming cells is based on the ability of antibodies (Abs), secreted by a single plasma cell (mature B-lymphocyte), to bind to an antigenic determinant on an erythrocyte. These assays showing the stimulation of humoral immune responses during BR treated irradiated mice when compared to irradiated control mice. Present chapter concludes that, BR enhanced the in vitro and in vivo cell proliferation in normal cells and humoral immune responses in irradiated mice.