

## Effect of herbal preparation, brahma rasayana, in amelioration of radiation induced damage

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Oral administration of brahma rasayana (BR; 10 and 50 mg/dose/animal) for 15 days increased significantly total leukocyte count and percentage of polymorphonuclear cells in irradiated mice. Bone marrow cellularity and  $\alpha$ -esterase positive cells also increased significantly in radiation-treated animals after BR administration. Number of nodular colonies on the surface of spleen on day seven increased significantly in lethally irradiated recipients receiving bone marrow cells from animals treated with BR. Oral administration of BR also enhanced in serum level of interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-2 (IL-2), and granulocyte macrophage-colony stimulating factor (GM-CSF) in normal and irradiated mice. These results indicated that proliferation of stem cells induced by BR in irradiated mice may be related to its stimulation of cytokine production.

Cancer treatment with chemotherapeutic agents and ionizing radiation have considerable effect on haemopoietic system. In fact, myelosuppression which is a major side effect of these treatments can at times produce even life threatening situation. Although growth factors could reduce these side effects, the treatment is costly and may not be immediately useful to patients in several developing countries.

Herbal drugs often contain constituents which are mitogenic and immunomodulatory. Indigenous medical practice in India (Ayurveda) often makes use of these medicines to boost immunity in normal and sick people. Rasayana a polyherbal preparation stimulates both humoral<sup>1</sup> and cell-mediated response in mice<sup>2,3</sup>. Rasayana also reduces the side effects of cyclophosphamide<sup>4</sup>. Brahma rasayana (BR) containing nearly 60 herbal plants inhibits metastasis induced by B16F-10 melanoma cells in mice<sup>5</sup> and reduces carcinogenesis induced by 20-methylcholanthrene<sup>6</sup>. In a pilot study of cancer patients undergoing chemotherapy and radiation therapy administration of BR significantly improves the total white blood cell count, especially neutrophils<sup>1</sup>. Maharishi amrith kalash, a modified indigenous herbal preparation, possesses many of activities produced by BR<sup>7</sup>.

In the present paper, it has been tried to study the mechanism of radioprotecting and immunostimulating activity of BR in mice.

### Materials and Methods

Brahma rasayana (BR) was purchased from Vaidyaratnam Oushadhasala, Ollur. Aqueous suspension of BR was used for all experiments. 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*.

p-Rosaniline hydrochloride and  $\alpha$ -naphthyl acetate were obtained from Loba Chemie, Bombay. Harris haematoxylin was purchased from Glaxo India Ltd., Bombay. Cytokine mouse Elisa kits (IFN- $\gamma$ , IL-2 and GM-CSF) were obtained from Endogen, USA. All other chemicals and reagents were of analytical grade.

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).

*Effect of BR on haematological parameters*— Male Swiss albino mice (4-5 weeks, 20g) were divided into three groups (6 animals/group). Group I received single whole body radiation (6 Gy/animal) and served as control. Group II and III received whole body radiation and daily oral administration of BR (10 and 50 mg/dose/animal) which was started 3 days prior to radiation and continued for 15 days. Blood was collected from caudal vein, and total leukocyte count (Haemocytometer<sup>8</sup>) and differential count were

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recorded prior to drug administration, 24 hr after radiation and continued on every third day for 30 days.

**Effect of BR on body weight, organ weight, bone marrow cellularity and  $\alpha$ -esterase activity**—Four groups of male Swiss albino mice (4-5 weeks old, 12 mice/group) were used for the experiment. Group I was kept as normal control. Other groups (Group II-IV) received single exposure of whole body radiation (6 Gy/animal). Group II served as radiation treated control. Group III and IV were treated orally with 10 and 50 mg/dose/animal respectively. Administration of BR was started 3 days prior to radiation and continued for 15 days. Three mice from each group were sacrificed on day 3, 9, 16 and 21 for analysis of bone marrow cellularity and  $\gamma$ -esterase activity.

Bone marrow cellularity was done according to the method of Sredni *et al*<sup>9,10</sup>. 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 (trypan blue exclusion) per femur. Bone marrow cells from the above preparations were smeared on clear glass slides and stained with p-rosaniline and Harris haematoxylin to determine the non-specific  $\alpha$ -esterase activity by simultaneous azo dye coupling method<sup>11</sup>.

**Determination of effect of BR on spleen colony assay**—Inbred strains of female Balb/c mice (4-5 weeks) were divided into three groups (6 animals/group). All groups were exposed to single whole body radiation (4 Gy/animal). At this dose the radiation related mortality could be significantly reduced ( $LD_{50}$  6Gy) and there was a significant depletion of bone marrow cells<sup>12</sup>. Group I received bone marrow cells ( $1 \times 10^6$  cells/mouse) from normal mice through caudal vein (iv) which served as normal control. Groups II and III received bone marrow cells from BR treated mice (daily dose of 50mg/dose/mouse for 10 days, po). Group III continued receiving BR for five more days (50mg/dose/mouse, po). Maximum number of spleen colonies are seen by 7-9days<sup>13</sup>. Hence all the animals were sacrificed on day 7 and the number of nodular colonies on the surface of spleens<sup>14</sup> were counted. Each colony formed was derived from a single precursor stem cell designated as colony forming units/spleen (CFU-S).

**Effect of BR on cytokine production**—Four groups of female Balb/c mice (4-5 weeks old) were used to carry out this study. Group I was treated as normal.

Group II was treated with BR(daily dose of 50 mg/dose/mouse for 10 days, po). Group III and IV were exposed to whole body radiation (6 Gy/animal). Group III was kept as radiation treated control. Group IV was treated with daily dose of BR (50 mg/dose/mouse for 10 days, po). All the animals were sacrificed on day 11. 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.

**Statistical analysis**—Data was expressed as mean  $\pm$  standard deviation (SD). Significance levels for comparison of differences were determined using Student's *t* test.

## Results

**Effect of BR on total white blood cells and per cent of polymorphonuclear (PMN) cells in irradiated mice**—Irradiation significantly reduced total WBC in mice within 24hr and continued to be low (<3500) up to 12th day and thereafter increased. It did not reach the normal value even on 30th day (Fig.1A). BR

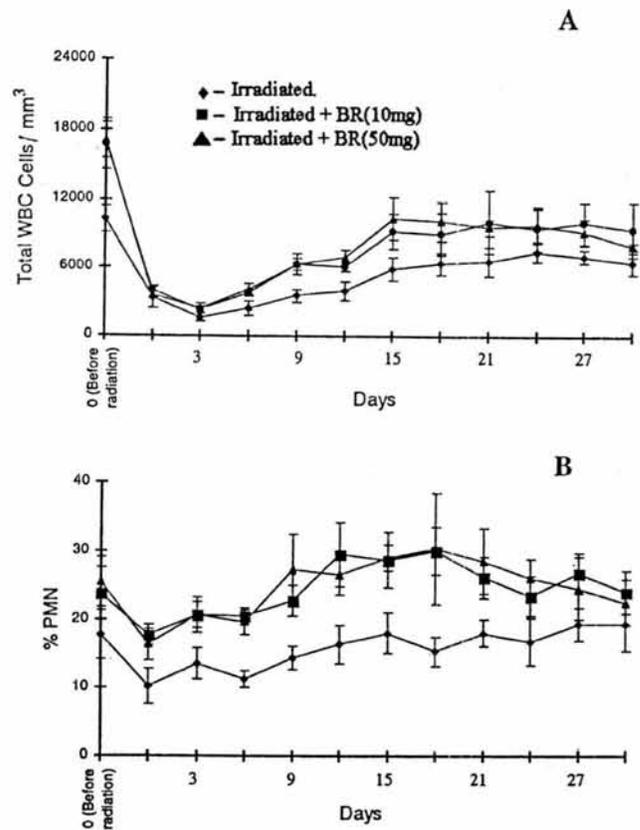


Fig. 1 — Effect of brahma rasayana on total leukocyte count and differential count in irradiated mice -- (A) total WBC count/mm<sup>3</sup>; and (B) percentage PMN.

treated animals had lower WBC initially, however the values increased significantly thereafter. Values were higher than 3500 cells/mm<sup>3</sup> on 6th day and continued to be higher than untreated controls. On 30th day while total WBC in control was 6513 cells/mm<sup>3</sup>, BR treated animals had a total WBC between 8000-9400 cells/mm<sup>3</sup>.

Polymorphonuclear cells (Fig. 1B) were also low in radiation treated animals (13.5%) on 3rd day and values increased thereafter and on 30th day it was 19.3%. In BR treated animals PMN values were 20-22% on 3rd day and thereafter increased and reached maximum(30%) on 18th day. These experiments indicated that BR treatment increased the total count and PMN cells in radiation treated animals.

*Effect of BR on bone marrow cellularity and  $\alpha$ -esterase activity*—There was also a significant reduction in bone marrow cellularity which reduced significantly in radiation treated animals ( $3.8 \times 10^6$  cells/femur) as compared with normal ( $12.7 \times 10^6$  cells/femur; Table 1). BR treatment significantly increased bone marrow cellularity which was comparable to normal animal or higher on various days.

Effect of BR on  $\alpha$ -esterase positive cells is given in Table 2.  $\alpha$ -Esterase positive cells in bone marrow of radiation treated animals 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 increase in  $\alpha$ -esterase positive cells (Table 2).

*Effect of BR on spleen colony assay*—Irradiated animals which received bone marrow cells from BR treated animals showed significant increase in the number of nodular colonies on the surface of spleens ( $6.2 \pm 0.75$ ) compared to those animals received bone marrow cells from normal animals ( $3.83 \pm 1.07$ ). Group of animals with continued BR treatment for five days after irradiation showed a significantly higher number of nodular colonies on spleens ( $9.83 \pm 1.07$ ).

*Effect of BR on cytokine production*—The concentration of IFN- $\gamma$  in serum increased significantly in BR treated normal animals and irradiated animals treated with BR (Table 3). IL-2 and GM-CSF levels also enhanced in BR treated normal and irradiated animals.

## Discussion

Administration of BR significantly increases total WBC cells and per cent of polymorphonuclear cells in both normal<sup>15</sup> and irradiated mice, indicating that BR stimulates the haemopoietic system. In normal and irradiated animals, BR treatment significantly increased the bone marrow cellularity and  $\alpha$ -esterase positive cells which indicated proliferation of stem cells and its differentiation.

Administration of bone marrow cells from BR treated animals to irradiated recipients increased the number of nodular colonies on the surface of spleens. As we have used the radiation dosage of 4Gy for depleting the stem cells from recipient mice, part of

Table 1 — Effect of brahma rasayana (BR) on bone marrow cellularity in mice treated with radiation  
[Values are mean  $\pm$  SD of 3 observations]

Treatment	Bone marrow cellularity/femur			
	day3	day9	day16	day21
Radiation alone	$3.8 \times 10^6 \pm 0.8$	$11.8 \times 10^6 \pm 1.2$	$8.0 \times 10^6 \pm 1.2$	$11.8 \times 10^6 \pm 1.5$
Radiation + BR (10mg)	<sup>a</sup> $28.8 \times 10^6 \pm 6.4$	$21.8 \times 10^6 \pm 3.0$	<sup>c</sup> $17.2 \times 10^6 \pm 2.6$	$19.3 \times 10^6 \pm 1.1$
Radiation + BR (50mg)	<sup>a</sup> $22.6 \times 10^6 \pm 0.8$	<sup>c</sup> $18.8 \times 10^6 \pm 2.0$	<sup>a</sup> $17.8 \times 10^6 \pm 0.4$	<sup>b</sup> $15.0 \times 10^6 \pm 0.8$

Normal bone marrow cellularity/femur was  $12.7 \times 10^6 \pm 0.7$   
Significant at <sup>a</sup><0.001, <sup>b</sup><0.005 and <sup>c</sup><0.01

Table 2 — Effect of brahma rasayana (BR) on  $\alpha$ -esterase activity in irradiated mice  
[Values are mean  $\pm$  SD of 3 observations]

Treatment	Number of $\alpha$ -esterase positive cells/4000			
	day3	day9	day16	day21
Radiation alone	242 $\pm$ 23	212 $\pm$ 14	405 $\pm$ 41	393 $\pm$ 7
Radiation + BR (10mg)	<sup>a</sup> 887 $\pm$ 104	<sup>a</sup> 930 $\pm$ 25	<sup>a</sup> 1136 $\pm$ 36	<sup>a</sup> 1262 $\pm$ 105
Radiation + BR (50mg)	<sup>a</sup> 778 $\pm$ 24	<sup>a</sup> 965 $\pm$ 30	<sup>a</sup> 1263 $\pm$ 26	<sup>a</sup> 1298 $\pm$ 46

Number of  $\alpha$ -esterase positive cells in normal animal was 1046 $\pm$ 79/4000 cells  
Significant at <sup>a</sup><0.001

Table 3—Effect of brahma rasayana (BR) on cytokine levels in normal and irradiated mice

[Values are mean of 3 observations]

Treatment	Cytokines (pg/mL serum)		
	IFN- $\gamma$	IL-2	GM-CSF
Normal	2980	7.5	32.0
BR (50mg)	3205	22.5	56.3
Radiation alone	690	7.2	26.3
Radiation + BR (50mg)	1770	24.3	37.1

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 cytokine, such as IFN- $\gamma$ , 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- $\gamma$  enhances the immune response by increasing T<sub>H</sub> cell function which can promote expression of IL-2 on T-cells. GM-CSF promotes differentiation of activated B-cells that secrete IgM, IgG<sub>2a</sub> and IgG<sub>3</sub> (Ref. 16).

These results indicated 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 the immune system<sup>17</sup>. 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 combined effect of several plant derived compounds. Active principle involved in it, is yet to be confirmed.

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