Chapter 5

Effect of Brahma Rasayana on Antioxidant Systems and Cytokine Level in Mice Treated with Cyclophosphamide
5.1. Introduction

Cancer chemotherapy generally produces non-selective cell killing as it effects in normal as well as cancerous tissues. The available chemotherapeutic drugs have distinct mechanism of action, which may vary at different drug concentrations and in their effects on different types of normal and cancer cells. While not selectively lethal to cancer cells, in many instances these drugs produce more extensive injury to cancer cells than to normal tissues, presumably because of quantitatively altered metabolic processes in the cancer cells or slower recovery of cancer cells than of normal cells.

Cyclophosphamide (CTX) is an alkylating agent is used as a chemotherapeutic agent Microsomal oxidase system in liver convert the CTX to 4-hydroxy cyclophosphamide, which re-enters the circulation and is subsequently taken up by peripheral tissues and tumour, where it undergoes tautomerisation to aldophosphamide. Spontaneous degradation of aldophosphamide to form a reactive reagent acrolein, which is capable of depleting cellular glutathione (GSH) and causing DNA alkylation (Mc Diarmid et al, 1991).

In recent years there has been an upsurge in the clinical use of indigenous drugs. Ayurveda, a science of life, has a great potency to face this challenge. Ayurvedic drugs enhance the immune power of the body which not only help to cure the disease but also avoids the recurrence. Adaptogenic agents produce complex of biochemical, neural and immunological mechanism and plays a role in the restoration of normal physiological condition and generalized increase in the resistant against infection. Rasayanas are a group of non-toxic polyherbal preparation commonly used in indigenous medical practice in India (Ayurveda) to improve the health and longevity. In the present chapter the effect of BR on the endogenous antioxidant enzymes in mice treated with cyclophosphamide has been explained.
5.2. Materials and Methods

Materials used for the present study is same as described in the chapter III cyclophosphamide (CTX) was obtained from Dabour pharmaceuticals, New Delhi, India. Cytokine Mouse Elisa Kits (IFN-γ, IL-2, GM-CSF) were obtained from Endogen, USA.

5.2.1. Animals

Inbred strains of Swiss albino mice and 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.

Swiss albino mice were divided into five groups of six mice each as given below.

- **Group I** Normal control
- **Group II** Cyclophosphamide (CTX) control -25 mg/kg b.wt. mouse daily for 1-10 days intraperitoneally (ip)
- **Group III** CTX control (25mg/kg b.wt./mouse daily for 1-10 days, ip) was kept untreated for 30 days
- **Group IV** CTX (25mg/kg b.wt./mouse daily for 1-10 days, ip) + BR (50mg/dose/mouse for 1-10 days per orally (po))
- **Group V** CTX (25mg/kg b.wt./mouse daily for 1-10 days (ip) + BR (50mg/dose/mouse for 1-30 days, po)

The animal of group I, II and IV were sacrificed on 11th day and other groups on 31st day by cervical dislocation. Blood was collected by heart puncture immediately and liver was excised and thoroughly washed in ice-cold saline (0.9%). Liver homogenate was prepared in ice-old Tris-HCl buffer (pH 7.4) and cystosolic sample of liver homogenate was prepared by centrifuging at 10,000 rpm for 30min. at 4°C. The blood, serum, liver homogenate and cytosol were used for the biochemical, analysis as given below.
<table>
<thead>
<tr>
<th>Protein</th>
<th>Reduction of Folin-Ciocalteau phenol reagent</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>Superoxide dimutase (SOD)</td>
<td>NBT reduction, method</td>
<td>Mc Cord and Fridovich, 1969</td>
</tr>
<tr>
<td>Catalase (CAT)</td>
<td>Rate of decomposition of H$_2$O$_2$ at 240 nm Reaction with 5-5' dithiobis (2-nitrobenzoic acid)</td>
<td>Aebi, 1983</td>
</tr>
<tr>
<td>Glutathione (GSH)</td>
<td>Oxidation of GSH by cumene hydroperoxidase</td>
<td>Moron et al, 1979</td>
</tr>
<tr>
<td>Glutathione peroxidase (GPX)</td>
<td>Rate of increase in conjugate formation between GSH and 1-Chloro-2,4-dinitrobenzene</td>
<td>Habig et al, 1974</td>
</tr>
<tr>
<td>Glutathione-S-transferase</td>
<td>NADPH consumed during the conversion of GSSG to GSH</td>
<td>Racker, 1955</td>
</tr>
<tr>
<td>Glutathione reductase (GR)</td>
<td>Thiobarbituric acid (TBA)</td>
<td>Ohkawa et al, 1979</td>
</tr>
<tr>
<td>Lipid peroxidation (LP)</td>
<td>Procedure given in appendix</td>
<td>King and Armstrong, 1934</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>Procedure given in appendix</td>
<td>Bergmeryer and Bernt, 1980</td>
</tr>
<tr>
<td>Glutamate pyruvate transaminase (GPT)</td>
<td>Procedure given in appendix</td>
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</table>
5.2.2 Determination of effect of BR on cytokine production

Inbred strains of Balb/c mice were used for the experiment. The animals were divided into four groups. Group I was treated as normal. Group II was treated with 10 doses of BR (50mg/dose/mouse, for 10 days po) . Group III animals were treated with 10 doses of CTX (25mg/kg b.w.t for 10 days, ip) . Group IV was treated with same dose of CTX as above as well as BR (50mg/dose/mouse for 10 days po). All animals were sacrificed on 11th day. Blood was collected by heart puncture and serum was separated, cytokine levels, of interferon-γ (IFN-γ), interleukin-2 (IL-2) and granulocyte macrophage – colony stimulating factor (GM-CSF) were determined by mouse Elisa kits, Endogen, USA.

5.3. Statistical analysis

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

5.4. Results

5.4.1. Effect of BR on liver superoxide dismutase (SOD) and catalase (CAT) activity in cyclophosphamide (CTX) treated mice.

As shown in Figure 28 , CTX administration was found to decrease the SOD activity in normal mice on 11th day (3.8±0.3) and 31st day (3.9±1.0) when compared to normal mice (4.5±1.1). Administration of BR significantly (p<0.005) increased the SOD activity in CTX treated mice on 11th day (4.9±0.7) . On 31st day SOD activity in BR treated CTX treated mice were found to be 5.5±0.8 (p<0.01).

There was slight decrease in liver CAT activity ( Fig.29) in CTX treated mice on 11th day (48.4±3.1) when compared to normal mice (53.1±4.5) which got normalized on 31st day. BR treatment significantly (p<0.001) increased the CAT activity in CTX treated mice on 11th day (69.6±6.4) and 31st day (86.3±5.4).
Figure 28
Effect of Brahma Rasayana (BR) on Liver Superoxide Dismutase (SOD) Activity in Cyclophosphamide (CTX) Treated Mice

Day 11
Day 31

Normal □ CTX alone □ CTX + BR (50 mg)
Figure 29
Effect of Brahma Rasayana (BR) on Liver Catalase (CAT) Activity in Cyclophosphamide (CTX) Treated Mice

Day 11

Day 31

U/mg protein

Normal □ CTX alone □ CTX + BR (50 mg)
5.4.2. Effect of BR on serum and liver glutathione (GSH) level in CTX treated mice

Figure 30 represents the effect of BR on serum GSH level in CTX treated mice. Administration of CTX was found to decrease the serum GSH level in normal mice on 11th day (56±18) and 31st day (29.7±11) when compared to normal mice (77.5±10). BR administration was found to significantly increase the serum GSH level in CTX treated mice on 11th day (94±22) and 31st day (108±17).

Liver GSH level was also found to decrease in CTX treated normal mice on 11th day (1.8±1.1) and 31st day (0.9±0.1) when compared to normal mice (2.3±0.4) (Fig.31). Administration of BR was found to significantly (p<0.001) increase the liver GSH level in CTX treated mice on 11th day (6.3±0.9) and 31st day (6.6±1.3).

5.4.3. Effect of BR on blood and liver glutathione peroxidase (GPX) activity in CTX treated mice

Blood GPX activity (Fig.32) was found to significantly (p<0.001) decrease in CTX treated mice on 11th (457±107) and 31st (196±12) day when compared to normal mice (2160±151). Administration of BR increased blood GPX activity in CTX treated mice on 11th day (1068±95) and 31st day (1434±214).

In CTX treated normal mice, liver GPX significantly increased on 11th day (41.6±2.5) and 31st (38.9±3.7) day when compared to normal mice (30.5±6.9) (Fig.33). BR treatment was found to increase (p<0.01) tissue GPX in CTX treated mice on 11th (46.3±3.2) and 31st (42.4±2.4) When compared to CTX treated control mice.

5.4.4. Effect of BR on liver cytosolic glutathione S transferase (GST) and glutathione reductase (GR) in CTX treated mice.

Figure 34 represents the effect of BR on liver GST activity. There was no change in liver GST activity in CTX treated normal mice on 11th (653±163) and 31st (615±160) day when compared to normal mice (638±248). Administration.
Figure 30
Effect of Brahma Rasayana (BR) on Serum Glutathione (GSH) Level in Cyclophosphamide (CTX) Treated Mice
Figure 31
Effect of Brahma Rasayana (BR) on Liver Glutathione (GSH) Level in Cyclophosphamide (CTX) Treated Mice

Day 11
Day 31

Normal □ CTX alone □ CTX + BR (50 mg)
Figure 32
Effect of Brahma Rasayana (BR) on Blood Glutathione Peroxidase (GPX) Activity in Cyclophosphamide (CTX) Treated Mice

![Graph showing the effect of Brahma Rasayana (BR) on blood glutathione peroxidase (GPX) activity in cyclophosphamide (CTX) treated mice. The graph compares the activity on Day 11 and Day 31 for normal, CTX alone, and CTX + BR (50 mg) groups. The y-axis represents the units of hemolysate, and the x-axis represents the days (Day 11 and Day 31).]
Figure 33
Effect of Brahma Rasayana (BR) on Liver Glutathione Peroxidase (GPX) Activity in Cyclophosphamide (CTX) Treated Mice

[Bar chart showing the effect of Brahma Rasayana (BR) on liver glutathione peroxidase (GPX) activity in cyclophosphamide (CTX) treated mice. The chart compares normal, CTX alone, and CTX + BR (50 mg) groups at Day 11 and Day 31.]
Figure 34
Effect of Brahma Rasayana (BR) on Liver Cytosolic Glutathione - S - Transferase (GST) Activity in Cyclophosphamide (CTX) Treated Mice

Day 11
Day 31

Normal □ CTX alone □ CTX + BR (50 mg)
of BR was found to significantly \((p<0.001)\) increase liver GST level in CTX treated mice on 11th \((1259\pm136)\) and 31st \((1010\pm203)\) day when compared to CTX treated mice. CTX treated normal mice showed no change in liver GR activity on 11th day \((127\pm33)\) and 31st day \((104\pm50)\) when compared to normal mice \((124\pm19)\) Fig.35. There was no significant change in GR activity in BR treated CTX treated mice on 11th \((156\pm26)\) and 31st \((121\pm29)\) day.

5.4.5. Effect of BR on serum and liver lipid peroxidation in CTX treated mice.

Figure 36 represents the effect of BR on serum and liver MDA level in CTX treated mice. Serum MDA level in CTX treated normal mice was found to be significantly \((PC 0.0001)\) increased on 11th day \((4.3\pm0.6)\) when compared to normal mice \((1.8\pm0.3)\) BR treatment significantly \((p<0.001)\) reduced serum MDA level in CTX treated mice on 11th \((2.3\pm0.3)\) and 31st \((1.6\pm0.3)\) day.

Liver MDA level was found to be significantly \((p<0.001)\) increased in CTX treated normal mice on 11th \((3.7\pm0.6)\) and 31st \((51.6\pm0.9)\) day when compared to normal mice. Administration of BR decreased \((p<0.001)\) liver MDA level in CTX treated mice on 11th \((2.9\pm0.4)\) and 31st \((3.8\pm0.4)\) day.

5.4.6. Effect of BR on serum and liver alkaline phosphatase (ALP) activity in CTX treated mice

Figure 37 represents the effect of BR on serum and liver ALP activity. There was no change in serum ALP level in CTX treated normal mice on 11th day \((21\pm2)\) and 31st day \((26.6\pm2)\) when compared to normal mice \((25.4\pm2.6)\). BR treatment significantly \((p<0.005)\) decreased serum ALP level in CTX treated mice on 11th \((13\pm2.8)\) and 31st \((14\pm1.2)\). Administration of CTX significantly \((p<0.001)\) increased liver ALP level in normal mice on 31st day \((41.2x10^3\pm4.7)\). When compared to normal mice \((29.4x10^3\pm5.4)\). BR administration was found to significantly decrease the liver ALP level in CTX treated mice on 11th \((24.9x10^3\pm3.8)\) and 31st \((32.7x10^3\pm9.2)\) day.
Figure 35
Effect of Brahma Rasayana (BR) on Liver Cytosolic Glutathione Reductase (GR) Activity in Cyclophosphamide (CTX) Treated Mice

Day 11     Day 31

Normal  □  CTX alone  □  CTX + BR (50 mg)
Figure 36
Effect of Brahma Rasayana (BR) on Serum and Liver Lipid Peroxidation in Cyclophosphamide (CTX) Treated Mice.

<table>
<thead>
<tr>
<th>Days</th>
<th>Serum (nmol/mL)</th>
<th>Liver (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 11</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Day 31</td>
<td></td>
<td>CTX alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTX + BR (50 mg)</td>
</tr>
</tbody>
</table>
Figure 37
Effect of Brahma Rasayana (BR) on Serum and Liver Alkaline Phosphatase (ALP) Activity in Cyclophosphamide (CTX) Treated Mice

![Bar chart showing the effect of Brahma Rasayana (BR) on Serum and Liver Alkaline Phosphatase (ALP) Activity in Cyclophosphamide (CTX) Treated Mice. The chart displays the activity levels on Day 11 and Day 31 for serum and liver, with groups compared to Normal, CTX alone, and CTX + BR (50 mg).]
5.4.7. Effect of BR on serum and liver glutamate pyruvate transaminase (GPT) activity in CTX treated mice.

As shown in figure 38, there was no change in serum GPT level in CTX treated control mice on 11th (121 ± 21.5) and 31st (151 ± 48) day when compared to normal mice (112 ± 7.7). BR treatment significantly (p < 0.001) decreased serum GPT level in CTX treated mice on 11th (84 ± 11.5) and 31st (101 ± 13.6) day. In CTX treated mice, tissue GPT level was not altered in CTX treated normal mice on 11th (286 ± 36) and 31st (260 ± 10) day when compared to normal mice (306 ± 123). There was no change in liver GPT level in BR treated CTX treated mice on 11th (307 ± 22) and 31st (258 ± 13) day.

5.4.8. Effect of BR on cytokine production in CTX treated mice

CTX administration decreased interferon -γ (IFN-γ) in normal mice on 11th day and was found to be 2130 pg/mL. IFN-γ level in normal mice was 2980 pg/mL (Table 13). Administration of BR was found to increase IFN – γ level in CTX treated mice (3070 pg/mL). There was a decrease in interleukin-2 (IL-2) level in CTX treated normal mice (5.1 pg/mL) when compared to normal mice (7.5 pg/mL). BR treatment increased IL-2 level in CTX treated mice on 11th day and was found to be 25.4 pg/mL. CTX treatment was also decreased granulocyte macrophage-colony stimulating factor (GM-CSF) in normal mice (19.2 pg/mL) when compared to normal mice (32 pg/mL). Administration of BR increased GM-CSF level in CTX treated mice and was found to be 45 pg/mL.
Figure 38
Effect of Brahma Rasayana (BR) on Serum and Liver Glutamate-Pyruvate Transaminase (GPT) Activity in Cyclophosphamide (CTX) Treated Mice

Day 11  Day 31

Serum (U/mL)  Liver (U/mg protein)

Normal □ CTX alone □ CTX + BR (50 mg)
Table 13

Effect of Brahma Rasayana (BR) on cytokine levels in normal and cyclophosphamide (CTX) treated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokines (pg/mL serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN-γ</td>
</tr>
<tr>
<td>Normal</td>
<td>2980</td>
</tr>
<tr>
<td>BR (50mg)</td>
<td>3205</td>
</tr>
<tr>
<td>CTX alone</td>
<td>2130</td>
</tr>
<tr>
<td>CTX+BR (50mg)</td>
<td>3070</td>
</tr>
</tbody>
</table>

Values are mean of 3 observations.
5.5. Discussion

The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. GSH offers protection against oxygen-derived free radicals and cellular lethality (Biaglow et al., 1987). Non-enzymatic GSH possesses peroxidase activity and can directly attack the peroxides that may be generated via oxidative reduction recycling, resulting in decreased cytotoxicity (Prohaska, 1980).

In the present chapter demonstrated that CTX administration suppresses the level of glutathione (GSH) system in serum and liver tissue in normal mice. The decreased level of GSH may be due to the enhanced utilization of the antioxidant system in an attempt to detoxify the free radicals generated by electrophilic burden or oxidative stress induced by CTX. Treatment with CTX also decreased the activity of liver antioxidant enzymes such as SOD, CAT and blood GPX while GST and GR activity were unaltered. Function of SOD is to protect the cells against the high chemical reactivities of various oxygen derived free radicals (Fridovich, 1978). It catalyses conversion of superoxides (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$) which inturn either detoxified by CAT or GSH dependent reactions. Decreased SOD, CAT and GPX increases the oxidative stress already induced by CTX treatment. This was reflected in the increased serum and tissue peroxides produced after CTX treatment. Lipid peroxidation can be initiated by hydrogen abstraction from lipid molecules by hydroxyl and hydroperoxyl radicals (Raleigh, 1987). This leads to permeability changes, secondary alternations in membrane proteins. There is considerable evidence that lipid peroxidation may have detrimental consequences for the cell and DNA, and may lead to structural and functional damage to membrane proteins (Kappus, 1985). GPX plays an important role in reducing the potential for oxidative cell damage (Tappol, 1974). GPX can react with lipid peroxides to prevent lipid peroxidation (Charles et al., 1979).
Treatment with BR significantly increased the serum and liver GSH, liver SOD, CAT and cytosolic GST and GR activity making the cells more reactive against electrophilic drug metabolites and reactive oxygen species (ROS). Protection of DNA and membranes will preserve a larger number of healthy cells for synthesis and restoration of GSH and antioxidant enzymes. The significant reduction in serum and liver MDA level by BR treatment clearly demonstrates that BR protects the membranes against cyclophosphamide induced oxidative damage. One of the most critical side effects with use of cyclophosphamide is mylosuppression (Joseph et al, 1999). The duration of mylosuppression varies depending on a number of factors including general marrow reserve, but generally recovery is achieved in 3 to 7 days following the nadir level. BR treatment was also found to decrease the serum and liver lipid peroxide level, which was elevated by CTX after the defense mechanism becomes incapacitated. BR stimulated the production of cytokines levels such as IFN-γ, IL-2 and GM-CSF) in CTX treated mice. These experimental findings clearly reveals the possibility of reducing the oxidative stress associated side effects produced by CTX by treatment with BR.