Chapter -3

Role of Brahma Rasayana on Antioxidant Systems in Normal Mice and Mice Treated Radiation
3.1. Introduction

The hazards of exposure to ionizing radiation were recognized shortly after Roentgen's discovery of the X-ray in 1895. Ionizing radiation has been called a "Universal Caranogen" in that it will induce cancer in most species at all ages, including the fetus. This was clearly linked to the excess cancer cases in the populations of Hiroshima and Nagasaki. The data provided good evidence to suggest that various forms of cancer such as leukemia represent the most significant late effect when human populations are exposed to substantial doses of radiation. Mechanism of radiation carcinogenesis is either by direct formation of free radicals i.e. chemical change, which are highly unstable and extremely reactive oxygen species (ROS) or indirect change in DNA leads to mutations. ROS are likely to involve in many human diseases such as cancer (Dreher & Junod, 1996), cardiovascular diseases (Westhuyzen, 1997), neurodegenerative diseases (knight, 1997) and viral pathogenesis (Peterhans, 1997). Human body has an inherent mechanism to reduce the free radicals induced injury by enzymatic and non-enzymatic methods (Sun Y, 1990).

Under normal physiological conditions, the body can prevent free radical damage by protective mechanisms that include antioxidant defense systems comprising superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and other cellular antioxidants like reduced glutathione (GSH). This normal level of antioxidant effect may not always be sufficient for eradication of free radicals induced injury. Many antioxidants of plant origin are known to bring about effective protection against oxidative stress (Nishigaki et al, 1992).

Ayurveda, a medical system of Indian origin that is gaining importance nowadays, for its quality and unimaginable efficiency without any side effects. Brahma Rasayana (BR) is a non-toxic polyherbal preparation, used as a rejuvenating health tonic to vitalize the immune system. In the present chapter deals with the effect of BR on the endogenous antioxidant enzymes in normal mice and mice treated with radiation.
3.2. Materials And Methods

Nitroblue tetrazolium (NBT), Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPHNa4), reduced glutathione (GSH), 5,5'-dithiobis (2 nitrobenzoic acid) (DTNB), DL-alanine, 4-aminoantipyrine, α-Ketogularic acid and 1-chloro-2,4 dinitrobenzene (CDNB) were obtained from Sisco Reasearch Laboratories (SRL) Pvt. Ltd., Mumbai. 1,1,3,3-Tetramethoxypropane was obtained from MTM Reasearch Chemicals, Eastgate, England. Phenyl Phosphate disodium salt dihydrate was obtained from Lancaster Synthesis, Eastgate, England. Thiobarbituric acid (TBA) was obtained from BDH Laboratory Supplies, Poole, England. Glutathione peroxidase (GPX) kit was obtained from Randox Laboratories Ltd.Ardmore, U.K. All other chemicals and reagents used were of analytical grade.

3.2.a. Animals

Inbred strains of Swiss albino mice (4-5 weeks old, 20-25g) were purchased from our animal house and were housed in ventilated cages in air controlled rooms and fed with normal mouse chow and water ad libitum.

3.2.b. Radiation Treatment

Whole body radiation (600 rads) was given using Cobalt-60 Teletherapy unit (Theraton 780, Canda) Animals were kept in specially constructed restraining boxes with a capacity of holding ten mice and irradiated by gamma rays (100 rads/min). The mice were divided into 7 groups of 6 mice each as given below.

- **Group I** Normal control.
- **Group II** BR (50 mg/dose/mouse) for 1-10 days per orally (po).
- **Group III** BR (50 mg/dose/mouse) for 1-30 days po.
- **Group IV&V** Radiation control (600 rads/mouse)
- **Group VI** Radiation (1st day) + BR (50mg/dose/mouse) for 1-10 days po
- **Group VII** Radiation (1st day) + BR (50 mg/dose/mouse) for 1-30 days po

The animals of group I, II, IV, VI 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-cold Tris-Hcl buffer (pH 7.4) and cytosolic sample of liver homogenate was prepared by centrifuging at 10,000 rpm for 30 min. at 4°C. The blood, serum, liver homogenate and cystosol were used for the biochemical analysis. The following enzymatic and the non-enzymatic assays were preformed.

3.2.1. Estimation of liver superoxide dismutase (SOD) activity (Mc Cord and Fridovich, 1969)

Principle

The assay is based on the ability of the enzyme to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide (O₂⁻) which is generated by the reaction of photoreduced riboflavin with oxygen.

Reagents

M/15 Phosphate buffer, pH 7.8
EDTA (0.1M) containing 1.5 mg of sodium cyanide per 100 mL.
Riboflavin (0.12mM) stored in a dark bottle at 4°C.
NBT (1.5mM) Prepared in phosphate buffer, pH 7.8

Procedure

Enzyme activity was calculated from the inhibition of reduction of NBT using standard curve constructed by varying amount of tissue homogenate. Incubation medium contained 0.2mL EDTA, NaCN, 0.1mL NBT, 0.05mL riboflavin and phosphate buffer to give a total volume of 3mL. The tubes containing no enzyme was kept as control. Riboflavin was added last after the tubes were brought to room temperature, subsequent exposure of bright light was avoided until the initial reading time. Then the tubes were placed to receive uniform illumination for 15min. and optical density was measured at 560nm. One unit of enzyme activity is defined as amount of enzyme giving 50% inhibition of reduction of NBT and expressed as units per milligram (mg) protein. For each sample to be assayed the amount of enzyme added to the incubation mixture was kept below one unit of enzyme activity.
3.2.2. Estimation of liver catalase (CAT) activity (Aebi, 1983)

Principle

The decomposition of hydrogen peroxide \( (\text{H}_2\text{O}_2) \) catalysed by CAT can be readed by UV spectrum, due to the absorbance of \( \text{H}_2\text{O}_2 \) in this region. At 240nm, the molar extinction coefficient for \( \text{H}_2\text{O}_2 \) is 43.6/min.

Reagents

0.5M phosphate buffer, pH 7
Diluted stock of hydrogen peroxide solution (8.8 to 9.1M) in phosphate buffer.

Procedure

At 240 nm, the absorbance of the diluted \( \text{H}_2\text{O}_2 \) sample was recorded against a quartz cuvette containing 0.5M phosphate buffer as blank. Initial optical density of the sample was noted (adjusted between 0.5-0.6 absorbance units) at 240nm. The decrease in absorbance was measured for 3 min at 25°C after addition of 0.1mL (100 mg protein) of enzyme source. Specific activity of the enzyme at 25°C is defined in terms of micromoles of \( \text{H}_2\text{O}_2 \) consumed per minute per milligram of protein sample and expressed as unit of enzyme

\[
\text{Specific activity} = \frac{\Delta A}{\text{min}} \times 1000 \text{ activity per milligram of protein (U/mg protein)}
\]

\[
43.6 \times (\text{mg Protein/mL of reaction mixture})
\]

3.2.3. Estimation of liver and serum glutathione (GSH) activity (Moron et al, 1979)

Principle

Glutathione (GSH) is measured by its reaction with 5,5’ dithiobis (2 nitrobenzoic acid) (DTNB)s to give a yellow coloured complex with absorption maximum at 412 nm.

Reagents

0.2 M phosphate buffer, pH 8
25% Trichloroacetic acid (TCA)
5% TCA
0.6mM DTNB
Procedure

0.125 mL of 25% TCA was added to 0.5 mL of 10% tissue homogenate or serum for precipitation of proteins. The tubes were cooled on ice for 5 min. and the mixture was further diluted with 0.6 mL of 5% TCA, centrifuged for 5 min. and 0.3 mL of resulting supernatant was taken for GSH estimation. The volume of aliquot was made up to 1 mL with 0.2 M phosphate buffer (pH 8). 2 mL freshly prepared 0.6 mM DTNB was added to the tubes and intensity of yellow colour formed was read at 412 nm.

Standard curve of GSH was prepared using concentrations varying from 50 – 100 nanomoles in 5% TCA for each assay. Value was expressed as nanomole/mg. protein.

3.2.4. Estimation of liver glutathione peroxidase (GPX) activity (Hafemann et al, 1974)

Principle

Glutathione peroxidase degrades H$_2$O$_2$ in presence of glutathione (GSH) thereby by depletion of GSH occurs. GSH remaining is measured using DTNB which gives a coloured complex.

Reagents

1M phosphate buffer, pH 7.0
5mM GSH
25mM Sodium azide
1.2mM H$_2$O$_2$
0.4M disodium hydrogen phosphate (Na$_2$HPO$_4$)
1mM DTNB
1.67% metaphosphoric acid

Procedure

One unit of enzyme activity was defined as a decrease in the log GSH by 0.001/min. after substraction of the decrease in log GSH/min. for the non-enzymatic reaction. The value was expressed as units/mg. protein.
Reaction mixture containing 0.2mM GSH, 0.4M phosphate buffer (pH 7), 1mM sodium azide, 500 µg protein sample and 0.25 mM H₂O₂ in a total volume of 2.5mL was incubated at 37°C for 6 minute. After addition of 2mL of 1.67% metaphosphoric acid, this mixture was centrifuged at 800xg for 15 minute. Supernatant 2mL was added to a mixture of 2mL of 0.4M Na₂HPO₄ and 1mL of 1mM DTNB. After 10 min. of incubation at 37°C the absorbance of the reaction mixture was measured at 412nm.

3.2.5. Estimation of blood glutathione peroxidase (GPX) activity (Paglia and Valentine, 1967)

Principle

GPX catalyses the oxidation of GSH by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340nm is measured.

\[
2\text{GSH} + \text{ROOH} \rightarrow \text{GPX} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O}
\]

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow \text{GR} \rightarrow \text{NADP}^+ + 2\text{GSH}.
\]

Randox Kit (Ransel) was used for the assay. It consists of the following reagents.

1. Reagent : Glutathione 4 mmol/L, glutathione reductase ≥ 0.5 U/L, NADPH 0.34 mmol/L
2. Buffer : Phosphate buffer 0.05 mol/L (pH 7.2)
3. Cumene hydroperoxide 0.18mmol/L
4. Diluting agent

Procedure

Heparinised blood sample diluted with diluting agent (0.05mL + 2mL diluting agent) Reagent 1 was reconstituted with appropriate volume of buffer 2 (6.5mL). Cumene hydroperoxide 10 µL was diluted with 10mL of redistilled water and mixed thoroughly by shaking vigorously. Prepare fresh daily.

Reaction mixture containing 0.02mL diluted sample, 1mL reagent and 0.04 mL, cumene hydroperox ide. The total volume of reaction mixture 1.06mL was
read against a reagent blank at 340 nm for every 1 min. for 3 min. at 37°C. Glutathione peroxidase (GPX) concentration was calculated from the following formula:

\[ \text{U/L of heamolysate} = 8412 \times \Delta A \text{ 340 nm/minute. } \Delta A \text{ is change in optical density/minute.} \]

**3.2.6. Estimation of cytosolic glutathione reductase (GR) activity (Racker, 1955)**

**Principle**

Activity of GR is determined by the amount of NADPH consumed in the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH). The reaction is catalysed by glutathione reductase.

**Reagents**

- 1M phosphate buffer, pH 7
- 10 mM EDTA
- 10 mM GSSG
- 2 mM NADPH

**Procedure**

1 mL of reaction mixture containing 0.1M phosphate buffer, pH 7, 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH. The reaction was started with the addition of liver cytosol (10%) and decrease in the absorbance per minute was noted and followed at every 1 min. interval for 5 min at 340 nm.

\[ \Delta \text{OD / min.} \times 1000 \]

GR activity was calculated using the formula:

\[ 6.22 \times \text{mg. protein in cytosolic sample} \]

and expressed as nanomoles of NADPH consumed/min./mg. protein. Where 6.22 mM\(^{-1}\)cm\(^{-1}\) is the extinction coefficient.

**3.2.7. Estimation of cytosolic glutathione-S-transferase (GST) activity (Habig et al, 1974)**

**Principle**

GST activity is based on the rate of increase in conjugate formation between reduced glutathione and 1-chloro 2,4 dinitrobenzene (CDNB). The conjugate have maximum absorbance at 340 nm.
Reagents
0.1M phosphate buffer pH 6.5
30mM 1-chloro - 2,4, dinitrobenzene (CDNB) in ethanol
30mM GSH

Procedure
3mL reaction mixture contained 0.1M phosphate buffer, 1mM CDNB and 1mM GSH. Reaction was started by addition of diluted 10% cytosolic sample. The initial reading was taken at 340nm with a reference cuvette containing the complete assay mixture without cytosol and continued for 5 min. with 1min. interval. GST Activity was calculated from the following formula and is expressed as nanomoles of CDNB-GSH.

\[
\text{Conjugate formed/min./mg. protein.} = \frac{\text{OD/\text{min.} \times 1000 \times 3}}{9.6 \times \text{mg. protein in cytosolic sample}}
\]

Where OD/\text{min.} is the increased in OD/\text{min} and 9.6mM⁻¹ cm⁻¹ is the extinction coefficient between CDNB GSH conjugate and CDNB.

3.2.8. Estimation of serum lipid peroxidation (Yagi, 1984)

Principle

The method is to isolate lipid peroxide in serum by precipitating them with phosphotungstic acid – sulphuric acid system and to determine their amount by TBA reaction in acetic acid solution. The assay was calibrated using 1,1,3,3 tetramethoxy propane. The results are expressed in terms of the amount of malonaldehyde (MDA) as nanomol per milliliter.

Reagents
N/12 sulphuric acid (H₂SO₄)
10% phosphotungstic acid
TBA reagent (a mixture of equal volumes of 0.67% TBA in aqueous solution and glacial acetic acid)
n-butanol
Procedure

0.5 mL of serum was mixed with 4 mL of N/12 H₂SO₄ and 0.5 mL of 10% phosphotungstic acid. After incubation at room temperature for 5 min, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the sediment was mixed with 2 mL of N/12 H₂SO₄ and 0.3 mL of 10% phosphotungstic acid. After incubation at room temperature for 5 min, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was discarded, the sediment was mixed with 2 mL of N/12 H₂SO₄ and 0.3 mL of 10% phosphotungstic and the mixture was centrifuged again at 3000 rpm for 10 min. The sediment was suspended in 4 mL of distilled water and 1 mL of TBA reagent. The reaction mixture was heated for 60 min at 95°C in a boiling bath. After under tap water, 5 mL of n-butanol was added and the mixture was shaken vigorously. After centrifugation at 3000 rpm for 15 min, the n-butanol layer was taken and absorbance was read at 532 nm. The amount of MDA was calculated from the standard curve.

3.2.9. Estimation of liver lipid peroxidation (Ohkawa et al, 1979)

Principle

Small amounts of malonaldehyde (MDA) are produced during peroxidation can react with TBA to form a pink coloured product, which has an absorption maximum at 532 nm. Since MDA in unstable must prepare immediately before use for constructing a standard curve. TBA assay was calibrated with 1,1,3,3-tetramethoxypropane which on hydrolysis produced MDA. The results are expressed in terms of amount of MDA as nanomol per mg protein. Procedure was described in chapter II.

3.3. Statistical Analysis

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

3.4.1. Effect of BR on liver superoxide dismutase (SOD) and catalase (CAT) activity in normal and irradiated mice.

As shown in figure 14, BR treated normal mice showed an increase \((p<0.02)\) in SOD activity on 11th \((5.4\pm0.4)\) and 31st \((5.9\pm0.6)\) day when compared to SOD activity of normal mice \((4.5\pm1.1)\). SOD activity in the liver was found to be reduced by irradiation and reached the value \(1.0\pm0.8\) on 31st day. Administration of BR increased the SOD activity in irradiated mice and the value was \(4.8\pm1.0\) and \(2.3\pm0.5\) on 11th and 31st day respectively when compared to irradiated control mice.

Figure 15 represents the effect of BR on CAT activity in normal and irradiated mice. BR treated normal mice showed a significant \((P<0.001)\) increase in CAT activity on 11th \((64.0\pm3.1)\) and 31st \((80.8\pm6.7)\) day when compared to normal mice \((53.1\pm4.5)\). Irradiation was found to significantly reduce the CAT activity in normal mice on 11th \((37.6\pm6.1)\) day and reached the normal value on 31st day \((62.2\pm5.8)\). BR treatment significantly \((P<0.005)\) increased CAT activity in irradiated mice on 11th \((48.3\pm5.6)\) and 30th \((77.6\pm5.8)\) day.

3.4.2. Effect of BR on serum and liver glutathione (GSH) level in normal and irradiated mice.

Figure 16 represents the effect of BR on serum GSH level in normal and irradiated mice. BR treatment significantly \((P<0.001)\) increased the serum GSH on 11th \((158\pm21)\) and 31st \((146.8\pm12)\) day in normal mice when compared to untreated control mice \((77.5\pm10)\). Irradiation was found to significantly reduce the serum glutathione level in normal mice \((11th=30\pm7.0, \ 31st=25.6\pm3.1)\). Oral administration of BR was found to significantly \((p<0.001)\) increase the serum GSH level on 11th \((115\pm12.6)\) and 31st \((85\pm23)\) day when compared to irradiated control mice.

Figure 17 represents the effect of BR on liver GSH level in normal and irradiated mice. There was a significant increase in liver GSH level in BR treated normal mice and were found to be \(6.6\pm0.9\) and \(4.2\pm1.0\) on 11th and 31st day.
Figure 14
Effect of Brahma Rasayana (BR) on Liver Superoxide Dismutase (SOD) Activity in Normal and Irradiated Mice
Figure 15
Effect of Brahma Rasayana (BR) on Liver Catalase (CAT) Activity in Normal and Irradiated Mice
Figure 16
Effect of Brahma Rasayana (BR) on Serum Glutathione (GSH) Level in Normal and Irradiated Mice

Day 11 Day 31
Normal BR(50mg) Radiation alone Radiation + BR (50mg)
Figure 17
Effect of Brahma Rasayana (BR) Liver Glutathione (GSH) Level in Normal and Irradiated Mice

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<th>nmol/mg protein</th>
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Day 11
- Normal
- BR (50mg)
- Radiation alone
- Radiation + BR (50mg)

Day 31
respectively when compared to normal mice (2.3±0.4). Irradiation was found to significantly reduce the liver glutathione level in normal mice (11th=1.6±0.2, 31st=0.9±0.4). Oral administration of BR was found to significantly (p<0.001) increase the liver GSH level on 11th (4.6±0.6) and 31st (2.2±0.8) day when compared to irradiated control mice.

3.4.3. Effect of BR on blood and liver glutathione peroxidase (GPX) activity in normal and irradiated mice

Effect of BR on GPX activity in blood is shown in figure 18. BR treated normal mice showed a significant (p<0.001) decrease in blood GPX on day 11 (1467±50) and reached normal level on day 31 (2427±301). Blood GPX was significantly reduced on 11th day in irradiated control (1490±169) and BR treated irradiated mice (1756±95) when compared to normal mice (2160±151) and reached the normal level on day 31.

Figure 19 represents the effect of BR on liver GPX activity in normal and irradiated mice. There was no significant change in liver GPX in BR treated normal mice (31.9±4.0) and irradiated control mice (34.6±6.6) on 11th and 31st day when compared to normal mice (30.5±6.9). Administration of BR in irradiated mice showed a slight change (p<0.05) in liver GPX on 11th day (40.6±6.1) and reached the normal value on 31st day (29.6±3.7) when compared to irradiated control mice.

3.4.4. Effect of BR on cytosolic liver glutathione-S-transferase (GST) and glutathione reductase (GR) activity in normal and irradiated mice

Figure 20 represents the effect of BR on cytosolic liver GST activity in normal and irradiated mice. BR treated normal mice showed a significant (p<0.001) increase in liver GST on 11th day (1580±377) when compared to normal mice (638±248) and reached the normal value on 31st day (780±216). Irradiation was found to be significantly (p<0.001) increased the GST activity on day 11 (1807±321) and attained the normal value on day 31 (464±341). BR treated irradiated mice showed a significant (p<0.001) increase in liver GST on 11th day (3140±331) and reached the normal value on 31st day (553±331).
Figure 18
Effect of Brahma Rasayana (BR) on Blood Glutathione Peroxidase (GPX) Activity in Normal and Irradiated Mice

Day 11
- Normal
- BR (50mg)
- Radiation alone
- Radiation + BR (50mg)

Day 31
- Normal
- BR (50mg)
- Radiation alone
- Radiation + BR (50mg)
Figure 19
Effect of Brahma Rasayana (BR) on Liver Glutathione Peroxidase (GPX) Activity in Normal and Irradiated Mice

Day 11
- Normal
- BR (50mg)
- Radiation alone
- Radiation + BR (50mg)

Day 31
- Normal
- BR (50mg)
- Radiation alone
- Radiation + BR (50mg)
Figure 20
Effect of Brahma Rasayana (BR) on Liver Cytosolic Glutathione S-Transferase (GST) Activity in Normal and Irradiated Mice
Cytosolic liver GR activity (Fig.21) was found to be significantly increased in BR treated normal mice on 11\textsuperscript{th} day (233±62) compared to normal mice (124±19) and attained the normal level on 31\textsuperscript{st} day (139±25). There was a slight increase (p<0.02) in cytosolic GR activity in irradiated mice on 11\textsuperscript{th} day (204±69) and reached the normal level on 31\textsuperscript{st} day (93±26). BR treatment increased cytosolic liver GR activity in irradiated mice on 11\textsuperscript{th} (308±107) and 31\textsuperscript{st} (176±63) day when compared normal mice.

3.4.5. Effect of BR on serum and liver lipid peroxidation (LP) in normal and irradiated mice.

Serum and liver lipid peroxide level were significantly (p<0.001) increased in irradiated control mice when compared to normal mice (Fig.22). In irradiated mice, malonaldehyde (MDA) level in serum was found to be 5.5±0.5 on 11\textsuperscript{th} day and 4.4±1.4 on 31\textsuperscript{st} day. Liver MDA level in irradiated mice was found to be 2.8±0.4 on 11\textsuperscript{th} day and 4.6±0.6 on 31\textsuperscript{st} day. Serum and liver MDA level in normal mice was found to be 1.8±0.3 and 2.2±0.4 respectively. Administration of BR significantly (p<0.001) reduced the serum and liver MDA level in irradiated mice on 11\textsuperscript{th} (3.0±0.3 and 0.5±0.1 respectively) and on 31\textsuperscript{st} (2.0±0.3 and 2.0±0.4 respectively) day when compared to irradiated control mice.

3.4.6. Effect of BR on serum and liver alkaline phosphatase (ALP) level in normal and irradiated mice.

As shown in Figure 23, BR treated normal mice showed a significant (p<0.001) decrease in the serum ALP level on 31\textsuperscript{st} day (14±2.3) when compared to normal mice. Irradiation was found to significantly (p<0.005) increase the serum ALP level in normal mice on 11\textsuperscript{th} day (39.2±6.8). Administration of BR significantly (p<0.001) decreased the serum ALP level on 11\textsuperscript{th} day (26.1±4.7) in irradiated mice and reached the normal level on 31\textsuperscript{st} day.

There was a significant (p<0.001) decrease in liver ALP level in BR treated normal mice on 11\textsuperscript{th} day (17.5 x 10\textsuperscript{3}±1.6) and reached the normal value on 31\textsuperscript{st} day (23 x 10\textsuperscript{3}±9.9). Irradiation significantly (p<0.005) increased the liver ALP level on 11\textsuperscript{th} day (44.1x10\textsuperscript{3}±8.6) and 31\textsuperscript{st} day (36.3x10\textsuperscript{3}±10). BR treated fig 21
Figure 21
Effect of Brahma Rasayana (BR) on Liver Cytosolic Glutathione Reductase (GR) Activity in Normal and Irradiated Mice

![Graph showing the effect of Brahma Rasayana (BR) on liver cytosolic glutathione reductase (GR) activity in normal and irradiated mice. The graph compares the activity on Day 11 and Day 31. The bars represent different treatment groups: Normal, BR (50 mg), Radiation alone, and Radiation + BR (50 mg).]
Figure 22
Effect of Brahma Rasayana (BR) on Serum and Liver Lipid Peroxidation in Normal and Irradiated Mice

Days

Serum (nmol/mL)

Liver (n mole/mg protein)

Day 11
Day 31
Day 11
Day 31

Malondialdehyde (MDA) formed

Normal BR (50 mg) Radiation alone Radiation + BR (50 mg)
Figure 23
Effect of Brahma Rasayana (BR) on Serum and Liver Alkaline Phosphatase (ALP) Level in Normal and Irradiated Mice
Figure 24
Effect of Brahma Rasayana (BR) on Serum and Liver Glutamate - Pyruvate Transaminase (GPT) Activity in Normal and Irradiated Mice

<table>
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<th>Days</th>
<th>Serum (U/mL)</th>
<th>Liver (U/mg protein)</th>
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<tr>
<td>Day 11</td>
<td>Normal</td>
<td>BR(50mg)</td>
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<tr>
<td>Day 31</td>
<td>Normal</td>
<td>BR(50mg)</td>
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irradiated mice was found to decrease the liver ALP level on 11th day (33x10^3±7.8) and 31st day (26.8x10^3±3.1) when compared to irradiated control mice.

3.4.7. Effect of BR on serum and liver glutamate pyruvate transaminase (GPT) activity in normal and irradiated mice.

Figure 24 represents the effects of BR on serum and liver GPT level. There was no change in serum GPT level in BR treated normal mice on 11th day (146±86) when compared to normal mice (112±7.7). Irradiation increased (p<0.02) the serum GPT activity in normal mice on 11th day (175±59) and significant (P<0.001) increase on 31st day (434±122). Administration of BR decreased the serum GPT activity on 11th day (166±64) in irradiated mice and increased (P<0.01) on 31st day (525±138).

BR treated normal mice showed no significant change in liver GPT level on 11th day (348±10) and 31st day (338±29) when compared to normal mice (306±12.3). There was a significant (P<0.001) increase in liver GPT level in irradiated normal mice on 11th day (437±65) and 31st day (356±28). BR treatment normalized the liver GPT level in irradiated mice on 11th day and 31st day when compared to normal mice.

3.5. Discussion

The interaction of cells with radiation produces generation of oxygen free radicals such as superoxide radical, hydroxyl radical, peroxide radical etc. and these radicals are scavenged by the endogenous antioxidant enzymes (Pryor, 1976). Superoxide dismutase (SOD) catalyses conversion of superoxides to hydrogen peroxide, which in turn either deboxified by catalase (CAT) or reduced by glutathione (GSH) dependent reactions. In the present study it was observed that animals exposed to radiation (600 rads) had suppressed endogenous antioxidant enzymes such as SOD, CAT and serum and tissue GSH. The decreased level of GSH may be due to effect of electrophilic burden and oxidative stress induced by radiation. Treatment with BR significantly increased the SOD and CAT levels making the cells more capable of scavenging the oxygen radicals.
Other than the direct activity of BR on the oxygen radicals *in vitro* and significant increase in the levels of antioxidant enzymes, modulation of the levels of GSH is perhaps the most important in reducing the oxidative stress produced by BR. GSH level in tissue and serum were found to be significantly reduced by radiation which was increased by BR administration. BR also produced an increase in glutathione reductase (GR), which reduces the oxidized glutathione (GSSG) and reduces the activity of blood glutathione peroxidase (GPX) conserving the glutathione (GSH) levels. In fact radiation alone also increased the GR in tissue and reduced the GPX in blood thus compensating the depletion of GSH in the cell. Similarly levels of GST was also enhanced after irradiation as well as after BR treatment. In summary oral administration of BR significantly enhanced the level of SOD, CAT, GSH, GST and GR and reduced GPX activity in normal and irradiated mice indicating that BR could be able to act as effective protector against exogenous oxidant insult (ie., radiation) by inducing the endogenous antioxidant enzymes level. Irradiation significantly increased the serum and liver lipid peroxide (LP) level and simultaneous BR administration significantly decreased the level. Alkaline phosphatase (ALP) level was also significantly increased in serum and liver of irradiated controlled mice and it was decreased after BR treatment in normal mice and BR treated irradiated mice indicating that BR can protect the liver from radiation induced injury. These experimental findings clearly reveals the possibility of reducing the risk associated with exposure to ionizing radiation by BR, which an excellent antioxidant *in vitro* and *in vivo*.