

Effect of brahma rasayana on antioxidant system after radiation

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Oral administration of brahma rasayana (BR; 50 mg/animal for 10 and 30 days) significantly increased the liver antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and tissue and serum levels of reduced glutathione (GSH). Whole body irradiation suppressed the levels of SOD, CAT and GSH. Reduced activity of SOD, CAT and GSH was significantly elevated by treatment with BR after radiation treatment. Similarly radiation exposure induced increase in serum and liver lipid peroxides was significantly reduced by further treatment with BR. The results indicate that BR could ameliorate the oxidative damage produced in the body by radiation and may be useful as an adjuvant during radiation therapy.

Rasayanas are a group of non-toxic polyherbal preparation commonly used in indigenous medical practice (Ayurveda) in India to improve the health and longevity. Rasayana has been shown to stimulate both humoral¹ and cell-mediated immunity in mice². It has antioxidant property *in vitro*³ and reduced the radiation induced clastogenicity in experimental animals⁴. Brahma rasayana (BR) has been found to protect the haematological system in patients undergoing radiation and chemotherapy⁵. One of the main ingredient in BR is *Embolica officinalis* which has very high antioxidant potential *in vitro*⁶. In the present study the effect of BR has been evaluated on the endogenous antioxidant enzymes in normal mice and mice treated with radiation.

Brahma rasayana (BR) was purchased from Vaidyaratnam Oushadhasala, Ollur. Majority of chemicals used in this study were of analytical grade and procured from standard chemical laboratories. Glutathione peroxidase (GPX) kit was obtained from Randox Laboratories Ltd., Ardmore, U.K.

Inbred strains of Swiss albino mice (4-5 weeks old, 20-25g), reared in Centre's animal house were housed in ventilated cages in air controlled rooms and fed with normal mouse chow (Lipton, India) and water *ad libitum*. One dose whole body radiation (600 rads) was given using Cobalt 60 teletherapy unit (Theratron 780, Canada) at the rate of 100 rads/min.

The animals were divided into following groups of 6 animals each as given below.

Group	Treatment
I	Normal control
II	BR-50mg/animal (day 1- day 10, po).
III	BR-50mg/animal (day 1- day 30, po).
IV & V	Radiation control (600 rads/animal).
VI	Radiation (day 1) followed by BR-50mg/animal (day 1 - day 10, po).
VII	Radiation (day 1) followed by BR-50mg/animal (day 1 - day 30, 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 cytosol were used for the biochemical analysis.

The biochemical parameters estimated were protein⁷, superoxide dismutase⁸, catalase⁹, glutathione¹⁰, glutathione peroxidase¹¹, glutathione-S-transferase¹², glutathione reductase¹³ and lipid peroxidation¹⁴.

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

Table 1 represents the effect of BR on the biochemical parameters in normal and irradiated animals. 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 enzymes¹⁵. Superoxide dismutase (SOD) catalyses

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Table 1—Effect of brahma rasayana (BR) on various biochemical parameters

[Values are mean $\pm$ SD of 6 observations, except in blood glutathione peroxidase, where 4 observations are from 4 animal]

		Normal	BR (50mg)	Radiation alone	Radiation + BR(50mg)
SOD ^a	A	4.5 $\pm$ 1.1	5.4 $\pm$ 0.4 ^{##}	3.9 $\pm$ 0.8	4.8 $\pm$ 1.0
	B	—	5.9 $\pm$ 0.6 [#]	1.0 $\pm$ 0.8	2.3 $\pm$ 0.5*
CAT ^a	A	53.1 $\pm$ 4.5	64.0 $\pm$ 3.1*	37.6 $\pm$ 6.1	48.3 $\pm$ 5.6**
	B	—	80.8 $\pm$ 6.7*	62.2 $\pm$ 5.8	77.6 $\pm$ 5.8**
Serum	A	77.5 $\pm$ 10	158 $\pm$ 21*	30.0 $\pm$ 7.0	115 $\pm$ 12.6*
GSH ^b	B	—	146.8 $\pm$ 12*	25.6 $\pm$ 3.1	85 $\pm$ 23*
Liver	A	2.3 $\pm$ 0.4	6.6 $\pm$ 0.9*	1.6 $\pm$ 0.2	4.6 $\pm$ 0.6*
GSH ^c	B	—	4.2 $\pm$ 1.0*	0.9 $\pm$ 0.4	2.2 $\pm$ 0.8**
Blood	A	2160 $\pm$ 151	1467 $\pm$ 50*	1490 $\pm$ 169*	1756 $\pm$ 95 [#]
GPX ^d	B	—	2427 $\pm$ 301	2233 $\pm$ 67.6	2429 $\pm$ 133 [#]
Liver	A	30.5 $\pm$ 6.9	31.9 $\pm$ 4.0*	34.6 $\pm$ 6.6	40.6 $\pm$ 6.1 ^{##}
GPX ^a	B	—	29.6 $\pm$ 5.3*	28.0 $\pm$ 6.9	29.6 $\pm$ 3.7
Liver	A	638 $\pm$ 248	1580 $\pm$ 377*	1807 $\pm$ 321*	3140 $\pm$ 331*
GST ^e	B	—	780 $\pm$ 216	464 $\pm$ 341	553 $\pm$ 331
Liver	A	124 $\pm$ 19	233 $\pm$ 62*	204 $\pm$ 69 [#]	308 $\pm$ 107 ^{##}
GR ^f	B	—	139 $\pm$ 25	93 $\pm$ 26	176 $\pm$ 63***
Serum lipid	A	1.8 $\pm$ 0.3	1.8 $\pm$ 0.2	5.5 $\pm$ 0.5*	3.0 $\pm$ 0.3*
peroxidation ^h	B	—	1.8 $\pm$ 0.5	4.4 $\pm$ 1.4*	2.0 $\pm$ 0.3*
Liver lipid	A	2.2 $\pm$ 0.4	0.2 $\pm$ 0.1*	2.8 $\pm$ 0.4 [#]	0.5 $\pm$ 0.1*
Peroxidation ^c	B	—	2.0 $\pm$ 0.2	4.6 $\pm$ 0.6*	2.0 $\pm$ 0.4*

A— day 11; B— day 31

P values * <0.001 , ** <0.005 , *** <0.01 , # <0.02 , ## <0.05

Units:

^a: U/mg protein^d: U/L of haemolysate^b: n mol/ml^e: n moles CDNB-GSH conjugate^c: n mol/mg protein formed/min/mg protein^f: n moles NADPH consumed/min/mg protein

conversion of superoxides to hydrogen peroxide, which in turn either detoxified by catalase (CAT) or reduced by glutathione (GSH) dependent reactions. In the present study animals exposed to radiation (600 rads) had suppressed endogenous antioxidant enzymes such as SOD, CAT and serum and tissue GSH. 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*³ and significant increase in the levels of antioxidant enzymes, modulation of the levels of GSH is perhaps most important in reducing the oxidative stress produced by BR. GSH level in tissue and serum were significantly reduced by radiation which was increased by BR administration. The decreased level of GSH may be due to effect of electrophilic burden and oxidative stress induced by radiation. BR also produced an increase in glutathione reductase (GR) which reduces the oxidised glutathione (GSSG) and reduces

the activity of blood glutathione peroxidase (GPX) conserving the glutathione (GSH) levels. In fact radiation alone also increased GR in tissue and reduced GPX in blood thus reducing the depletion of GSH in the cell similarly levels of GST were also enhanced after irradiation and BR treatment, summarizing

BR treatment enhanced the level of SOD, CAT, GSH, GST and GR and reduced GPX activity in normal and irradiated animals 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 (LPO) level and simultaneous BR administration significantly decreased the level.

Similar results were also seen using normal (non-irradiated) animals. Administration of BR was found to increase the levels of SOD and CAT (Table1), indicating that BR increases the oxidative defense mechanism in normal animals. More significantly, there

was a two fold increase in GSH level in serum and in liver. GSH forms one of the major reducing agent in the body and is a protector of several enzymes. Increase in GSH was reflected in the increased efficiency of the body to control not only the body defense against oxidative insult but also its efficiency to reduce xenobiotic injury.

BR is made with plant extract available indigenously. A detailed composition of BR has been reported earlier³. *Embllica officinalis* was one of the major component which have very high concentration of polyphenolic compounds which can undergo spontaneous and enzymatic oxidation generating reducing potential in the body. Moreover increased reducing potential can generate increased NADPH levels thus increasing ATP production and may be one of the reason for the adaptogenic properties of Rasayana.

This data also suggest that many indigenous products such as BR can actively reduce the radiation induced damage. Radiation has been reported to produce several biological effects after its exposure including myelosuppression, immunosuppression, genetic damage etc.. The finding as reported in this paper that BR could reduce radiation damage is highly significant as many other antioxidants needed to be given before the radiation exposure. Preparation is highly cost effective and could be given as a adjuvant during radiation treatment as well as in condition such as accidental exposure to radiation.

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