

Studies on Brahma rasayana in male swiss albino mice: Chromosomal aberrations and sperm abnormalities

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ABSTRACT

Ayurveda, the Indian holistic healthcare system encompasses traditional medicines with a principle of creating harmony and maintaining balance within the natural rhythms of the body. Rasayana is one of the branches of Ayurveda frequently used as rejuvenating therapy to overcome many discomforts and prevent diseases. It has been reported that rasayanas have immunomodulatory, antioxidant and antitumor functions. However, the genotoxic potential of many rasayanas remains to be evaluated. The present study was undertaken to assess the role of Brahma rasayana (BR) on genotoxicity *in vivo* in a mouse test system. The older mice (9 months) were orally fed with rasayana for 8 weeks. The treated groups showed no signs of dose-dependent toxicity at the dosage levels tested. The body weight loss/gain and feed consumption were unaffected at tested doses. Furthermore, sperm abnormalities and chromosomal aberrations were insignificant in the treatment group when compared to controls. However, there was a marginal increase in sperm count in the BR treated animals. These findings clearly indicate that there are no observed adverse genotoxic effects elicited by BR in experimental animals such as mice.

Key words: Aging, Brahma rasayana, chromosomal aberrations, genotoxicity, sperm abnormalities.

INTRODUCTION

Ayurveda, Yoga, Unani, Siddha, and other forms of traditional medical practice are still the mainstays of human health maintenance and disease prevention despite the era of modern medicine. Ayurveda is considered holistic, since it takes into consideration body, mind and spirit as a whole.^[1, 2] It also includes concepts of rejuvenation. Of Ayurveda's eight branches, 'Rasayana' is considered the primary method for maintaining health and vigor. Rejuvenation therapy can help the elderly to regain vigor and youthfulness,^[3] overcome drowsiness and negate fatigue. It helps preserve harmony in the three psychobiological dimensions known as *doshas*,^[1] biological rhythms which regulate the entire functioning of the physiology.^[4]

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Rasayanas constitute a group of single or polyherbal preparations made from plant extracts, commonly used to improve health and longevity. They have been reported to improve memory, intelligence, youthfulness, lustre, complexion and efficiency.^[4,5] Examples include Brahma rasayana (BR), Narasimha rasayana, Ashwagandha rasayana, Amruth Prash, Chyavana Prash, and Student rasayana, and many others. In contrast to single chemical medicines, complex formulations such as these may have multiple benefits.

BR is a case in point. It is recommended for management of mental conditions, including anxiety, poor cognition and lack of concentration. In this regard, it has been shown to improve learning and memory in mice^[4], but, in addition, antioxidant^[12,13] and immunostimulatory^[14,15] activity have been reported. Furthermore, it has been found to decrease palpable tumor incidence, and to reduce the tumor weight in MAT-LyLu cell inoculated Copenhagen rats.^[10] In addition, treated animals showed significant reductions in Factor VIII, pro-angiogenic factors like VEGF, MMP-9 and MMP-2, suggesting that the rasayana may also have anti-angiogenic properties.^[10,11]

Not all phyto-preparations are free from toxicity, however.^[6] In general, comprehensive quality evaluation^[7] for possible

side effects^[8] is needed. In the case of Rasayanas, most research has focused on mechanisms of known actions, and relatively work has been done in relation to genotoxicity. In the case of BR, for example, no information regarding possible genotoxicity is available. In view of its many other effects, potential genotoxicity should be assessed. The present investigations were therefore undertaken to study potential genotoxic effects of Brahma rasayana in mice by analyzing chromosomal aberrations, sperm abnormalities, mitotic index and the body weight. This article presents results so obtained.

MATERIALS AND METHODS

Animals

Male swiss albino mice aged 9 months and weighing 35-40 g were employed. All were reared in the Manipal University animal house, and maintained under standard conditions. To eliminate influence of weight variation on induction of chromosomal aberrations, animals of identical weight (36g) were employed in one set of experiments. They were provided with standard diet and water ad libitum.

Phyto-medicine

Brahma rasayana (Batch Nos. 8647 and 50323) was obtained from Arya Vaidya Sala, Kottakal, India. Its stated contents include extracts of the following plants: *Terminalia chebula* (Pathya), *Phyllanthus emblica* (Dhatri), *Cinnamomum zeylanicum* (Twak), *Elettaria cardamomum* (Ela), *Cyperus rotundus* (Musta), *Curcuma longa* (Rajani), *Piper longum* (Pippali), *Aquilaria agallocha* (Agaru), *Santalum album* (Chandana), *Centella asiatica* (Mandukaparni), *Atropa belladonna* (Kanaka), *Convolvulus pluricaulis* (Shankhpushpi), *Acorus calamus* (Vacha), *Cyperus scariosus* (Plava), *Glycyrrhiza glabra* (Yashtyahwa), *Embelia ribes* (Vidanga), Ghee (Sarp), Sesamum oil (Taila), Milk (Kshaudra), Sugar candy (Sitopala), *Desmodium gangeticum* (Shalaparni), *Uraria picta* (Prishnaparni), *Solanum indicum* (Brihati), *Solanum xanthocarpum* (Kantakari), *Tribulus terrestris* (Gokshura), *Aegle bruhata* (Bilva), *Clerodendron premnoses* (Agnimantha), *Oroxylum indicum* (Shyonaka), *Gmelina arborea* (Gambhari), *Pterospermum suaveolens* (Patala), *Sida cordifolia* (Bala), *Boerhavia diffusa* (Punarnava), *Ricinus communis* (Eradamula), *Phaseolus trilobus* (Mudgaparni), *Teramnus labialis* (Mashaparni), *Asparagus racemosus* (Shatavari), *Tinospora cordifolia* (Geevaka), *Leptademia reticulata* (Jivanthi), *Withania somnifera* (Medha), *Bambusa arundanacia* (Rishabhaka), *Imperata cylindrica* (Darba), *Saccharum spontaneum* (Kshara), *Oryza sativum* (Kshalimula) and *Saccharum officinalis* (Ikshumula). The feeding method adopted was mixing the normal feed powder with rasayana in equal proportions. This was then pressed into pellets, which become hard and ideal for chewing by mice.

Slide preparation and chromosome analysis

The animals were sacrificed after eight weeks of Rasayana treatment. Colchicine (0.05%) at a dose of 0.5 mL was injected into each animal 90 min prior to sacrifice. Bone marrow was processed and slides prepared by routine air-drying technique.^[16] In brief, the femur bones were dissected and bone marrow was flushed into hypotonic solution [0.56% potassium chloride (KCl)]. The resulting cell suspension was mixed thoroughly and incubated at 37°C for 30 min. After centrifugation, the cell pellets were resuspended in chilled methanol/acetic acid (3:1 v/v) as fixative and this procedure was repeated thrice. Finally the pellet was resuspended in fresh fixative, and the cell suspension dropped on clean, non-greasy, pre-chilled microslides using a fine-tipped Pasteur pipette. Air-dried slides were coded and stained with giemsa. They were screened microscopically for the presence of chromosomal aberrations such as chromatid breaks, chromatid exchanges, intrachromatid deletions, triradials, chromosome breaks, dicentric, rings and minutes. In each treatment group, a minimum of 1000 well-spread metaphase plates were scored. Statistical analysis of results was carried out using one way ANOVA.

Analysis of mitotic index (MI)

Mitotic Index was employed to analyze potential toxicity of BR in terms of cell proliferation. To analyze mitosis rates in different treatment groups, the number of mitotic cells in a population of at least 5,000 cells (1000 cells/animal) was scored for each treatment schedule and controls. Mitotic index was calculated using % MI = (No. of cells in mitosis/ Total No. of cells scored) (100).

Sperm count and sperm morphology assay

Sperm counts were carried out according to the method described by Searle and Beechey^[17] Paired capita were macerated in 1-2 ml 1% (w/v) trisodium citrate, and the resulting suspension was further diluted with 2 or 4 ml of trisodium citrate, depending on caput weight. After thorough mixing, the number of spermatozoa was counted on an improved Neubauer haemocytometer. Statistical significance was calculated using one way ANOVA for observed differences between treatment groups and controls.

For analysis of Sperm shape abnormalities,^[18] each suspension was mixed with 1% Eosin Y in a 10:1 ratio. The mixture was allowed to stand for 30 minutes and slides were then made by smearing on staining solution and allowing them to air dry overnight. Coded slides were then examined by microscopy for the presence of different sperm shape abnormalities – pin head, sword head, triangle, hookless, amorphous, double headed, double tailed and other conspicuous abnormalities. A minimum of 2000 sperms were counted per animal, and results subjected to one way ANOVA.

RESULTS AND DISCUSSION

Most Ayurvedic drugs and drug formulations are principally derived from herbs and plants. In general, Rasayanas are nutrition promoters, but some are specific to certain organs or tissues; for example, Medhya Rasayana for brain, Hridya Rasayana for heart, Twachya Rasayana for skin, Chaksusya Rasayana for eyes and others. Similarly, Rasayanas promoting nutrition relevant to bio-losses occurring in different phases of the life span are age-specific.^[19]

BR is an example of such a Medhya Rasayana comprising more than 35 different plant extracts. Its main ingredients, listed in toto above, are extracts of *Terminalia chebula* and *Emblica officinalis*. It is reported to retard brain aging, and help in regeneration of neural tissues, besides producing antistress, adaptogenic and memory enhancing effects.^[19] However, whether it damages DNA, or possesses other genotoxic effects is not known. The present study was undertaken to address these questions in *in vivo* mouse systems.

In this context, the Table 1 data show no evidence for BR-induced chromosomal aberrations. The great proportion of observed abnormalities are chromatid breaks the frequencies of which are equal to those of controls at the 2g and 4g levels, where most effects would be expected. Even the slightly elevated 1g level (0.024 ± 0.011) is neither significantly different from controls (0.016 ± 0.008), nor of any absolute significance. The only possible note of caution is the appearance of a tiny proportion of chromosomal breaks B'' at the 4g level, a result again not suggestive of problems, which might even be spurious, and should be checked.

In short, Table 1 data suggest that BR does not elicit genotoxicity in *in vivo* mouse test systems. This may not be surprising, for Sharma *et al.*^[20] have demonstrated that *Emblica officinalis* plant extract, one of Brahma Rasayana's chief ingredients, has an

inhibitory effect on mutagen-induced chromosomal anomalies in Swiss albino mice. Oral administration of plant extract (250 and 500 mg/kg body weight) for 7 days together with single doses of the mutagens, Benzo(a)pyrene (125 mg/kg body weight) or cyclophosphamide (40 mg/kg body weight intraperitoneally), significantly reduced observed frequencies of chromosomal aberrations and micronuclei in Swiss albino mice compared to treatment with mutagens alone. They also observed increased levels of antioxidant and detoxification enzymes such as glutathione peroxidase, glutathione reductase and glutathione-S transferase.^[20]

In our experiments, dosages (1g to 4g) were between 2.5% and 13% of body weight (30-40g) i.e. 25 g to 130g/kg body weight, so that the ingredient Embilica officinalis was being consumed at a comparable rate, and might be expected to have conferred a similar degree of protection on the experimental animals.

In toxicology testing, the US Federal agencies, Environmental Protection Agency (EPA) and Food and Drug Administration (FDA), and international bodies like Organization for Economic Cooperation and Development (OECD), have suggested protocols and guidelines for identifying adverse reproductive effects, for industry to use when testing pesticides, industrial chemicals, pharmaceuticals and food additives for potential reproductive toxicity in laboratory animals^[21]. In the male reproductive system, reduced sperm count and increased sperm abnormalities are among standard criteria used to characterize toxic agents that may cause fertility problems in treated subjects^[8]. Results of sperm abnormality analysis [Table 2] are very interesting. Rasayana treatment apparently gives total protection against sword shaped, banana shaped and double tailed deformations, and excellent protection against triangle and hookless deformations, although differences in total sperm abnormalities between rasayana-dosed animals and controls did not appear significant, possibly because of increases in pin-head abnormality. The two remaining deformations, double headed and amorphous produced mixed results. Overall, the data therefore indicates that BR confers protection against certain kinds of sperm abnormality. Moreover, sperm count data in rasayana-treated animals consistently showed slight but not significant increases in sperm count compared to controls [Table 3]. Similarly, Mitotic analysis showed consistent increases in the number of mitotic cells in all three treatment groups [Table 4] of which none reached significance.

CONCLUSIONS

These results suggest that use of BR as an adjuvant in cancer radiotherapy may produce beneficial effects. Together with our results, they suggest that BR does not possess genotoxic

Table 1: Frequencies of chromosomal aberrations observed after treatment of Brahma rasayana in mouse bone marrow cells

Treatments	Types of chromosomal aberrations (in %)		Total no. of breaks	Total breaks/cell
	B'	B''		
Control	1.8 ± 0.83	0	1.8 ± 0.83	0.018 ± 0.005
BR-1 g	2.4 ± 1.14	0	2.4 ± 1.1	0.024 ± 0.011
BR-2 g	1.6 ± 0.89	0	1.6 ± 0.89	0.016 ± 0.008
BR-4 g	1.4 ± 0.54	0.2 ± 0.44	1.8 ± 0.83	0.018 ± 0.008

Note: Chromatid break - B'; Chromosome break - B''; Mean ± SD

Table 2: Percentage of sperm abnormalities observed after treatment of mice with BR in mice

Treatments	Types of sperm head shape abnormalities								Total sperm abnormalities	% of sperm abnormalities
	Hookless	Sword shaped	Banana shaped	Double headed	Double tailed	Pin head	Amorphous	Triangle		
Control	11.6 ± 7.7	0.4 ± 0.89	3.6 ± 4.5	29.8 ± 18.8	1.6 ± 2.1	19.2 ± 7.6	17.6 ± 7.02	10.6 ± 5.3	94.4 ± 3.64	1.9 ± 0.072
BR-1 g	8.8 ± 3.56	0	0	16.8 ± 9.93	1.2 ± 2.6	25.2 ± 7.1	27.6 ± 10.7	4.2 ± 3.42	83.8 ± 20.6	1.67 ± 0.412
BR-2 g	6 ± 3.31	0	0	14.4 ± 2.30	0	21.8 ± 8.46	39.6 ± 15.1	1 ± 0.70	82.8 ± 23.3	1.65 ± 0.466
BR-4 g	6.6 ± 6.30	0	0	21.2 ± 10.84	0	24.2 ± 10.5	5.4 ± 14.94	1.6 ± 1.67	77 ± 25.90	1.54 ± 0.52

Mean ± SD

Table 3: Sperm counts in BR treated and control mice

Treatment groups	Mean sperm count/caput ($\times 10^{-6}$)
Control	2.99
Brahma rasayana -1 g	3.1
Brahma rasayana -2 g	3.3
Brahma rasayana -4 g	3.0

Table 4: Mitotic index in mouse bone marrow cells of the control and rasayana treated mice

Treatment groups	Percentage of Mitotic index (Mean ± SD)
Control	5.8 ± 0.09
Brahma rasayana -1 g	6.1 ± 0.15
Brahma rasayana -2 g	7.0 ± 0.06
Brahma rasayana -4 g	6.8 ± 0.19

activity. We noted absence of significant body weight changes in BR-treated animals compared to age-matched controls, nor was there any treatment related mortality, or any other sign of adverse effects at the tested dosage levels [Figure 1].

In conclusion, our own and others' results taken together suggest that BR does not elicit genotoxic effects in the mouse system, rather that it is a moderate enhancer of reproductive and mitotic cellularity, and a protector against certain kinds of sperm abnormality.

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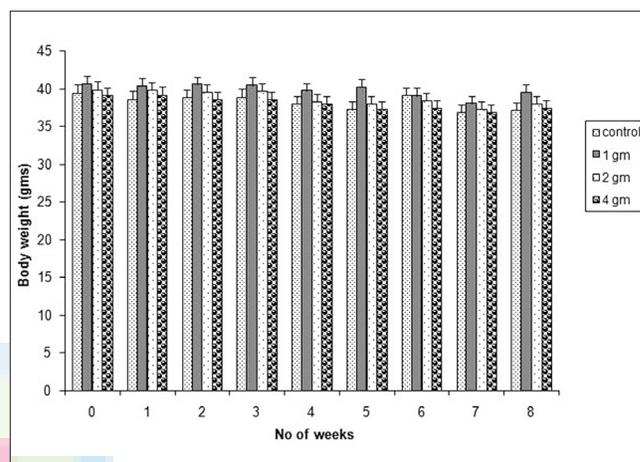


Figure 1: Body weight of BR treated mice compared to control groups. The groups of mice were either treated with BR or with the regular feed at indicated proportions as mentioned in materials and methods. Animals were weighed weekly and recorded. Duration of treatment was for two months.

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