Protective Effect of *Nardostachys jatamansi* Against Radiation-induced Damage at Biochemical and Chromosomal Levels in Swiss Albino Mice

L. N. MADHU, N. SUCHETHA KUMARI*, P. NAVEEN AND G. SANJEEV

Central Research Laboratory, Nitte University, Deralakatte, Mangalore–575 018, ¹Department of Biochemistry, K. S. Hegde Medical Academy, Nitte University, Medical Sciences Complex, P.O. Nityanandanagar, Deralakatte, Mangalore–575 018, ²Department of Physics, Mangalore University, Mangalagangotri, Mangalore-574 199, India

*Address for correspondence
E-mail: suchethakumarin@gmail.com

*www.ijpsonline.com*
The effect of 100 mg of ethanol extract of *Nardostachys jatamansi* was studied on the mice exposed to 6 Gy electron beam radiation. Treatment of mice with 100 mg of *Nardostachys jatamansi* extract for 15 days before irradiation reduced the symptoms of radiation sickness when compared with the nondrug treated irradiated groups. The irradiation of animals resulted in an elevation in lipid peroxidation and reduction in glutathione, total antioxidants and antioxidant enzymes such as glutathione peroxidase and catalase activities. Irradiated group had shown micronucleus in the bone marrow cells. Treatment of mice with *Nardostachys jatamansi* extract before irradiation caused a significant depletion in lipid peroxidation followed by significant elevation in reduced glutathione, total antioxidants, glutathione peroxidase and catalase activity. It also showed a reduction in the micronucleus formation in the bone marrow cells. Our results indicate that the radioprotective activity of *Nardostachys jatamansi* extract may be due to free radical scavenging and increased antioxidant level in mice.

Key words: Antioxidants, electron beam, free radicals, oxidative stress, radioprotectors

Over the past 50 years, radiation research has focused on screening a plethora of chemical as well as biological radioprotectors[1-3]. The discovery of radioprotectors begins with the study, protection of mice and rats against radiation-induced sickness and mortality by cysteine[6]. The traditional Indian system of medicine the *Ayurveda*, gives a detailed account of several disease and their treatments. The majority of the drug and drug formulations used in *Ayurveda* are principally derived from herbs and plants.

The interest has been developed in search for potential drugs of herbal origins which are capable of modifying immune and radiation responses without their side effects. Several studies concerning radioprotection have been conducted on vitamins[5-7], ginseng[8], garlic[9], *ocimum*[10], *mentha*[11], ginger[12]. Studies carried out in the past decade and half have shown that herbal preparations like Liv.52, protected mice against radiation-induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in peripheral blood cell counts, prenatal development, lipid peroxidation and radiation-induced chromosomal damage[13-16]. The herbal formations such as *Abana* and *Mentat* have shown protection against radiation-induced damage[17,18].

Various sesquiterpenes (such as Jatamansic acid and Jatamansone), lignans, alkaloids, coumarins and neolignans have been reported to be present in the roots of the plant[23,24]. In addition, volatile oils like jatamansic acid and other chemical substances have been isolated from various fractions of roots and rhizomes of the herb[25]. These components provide protection against reactive oxygen species (ROS) induced damage in cells. With this background our aim of the study is to find the protective effect of *Nardostachys jatamansi* root extract against electron beam radiation (EBR) induced cellular damage.

The plant material i.e., rhizome powder of *Nardostachys jatamansi* was collected from Genuine chemical Co., Mumbai, India. This powder was extracted with 95% ethanol at room temperature, concentrated in reduced temperature and pressure on rotary evaporator and stored at 4℃.

Animal care and handling was carried out according to the guidelines set by WHO (World Health Organization; Geneva, Switzerland). The Institutional Animal Ethical Committee (IAEC) has approved this study. Swiss albino mice aged 6-8 weeks and weighing 25±5 g, taken from an inbred colony, was used for this study. The mice were maintained under controlled conditions of temperature and light (light: 10 h; dark: 14 h). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed and water ad libitum.

The irradiation work was carried out at Microtron centre, Mangalore University, Mangalore, Karnataka, India. The animals were restrained in well-ventilated
perspex boxes and exposed to whole-body electron beam at a distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

After obtaining an ethical clearance from IAEC, 36 male Swiss albino mice were used for the Survival assay. These animals were divided into six groups. Each group containing six animals each. These animals were irradiated to 4, 6, 8, 10, 12 and 14 Gy radiation dosages. The number of mice surviving 15 days after exposure against each dose will be used to construct survival dose response curve.

The following groups of animals were used. The mice were divided into four groups (n=6 in each group). Group I served as control. Group II animals were received on N. jatamansi crude extract (100 mg/kg body weight-sublethal dosage) orally for 15 days. Group IV animals were also treated with same dosage (100 mg/kg body weight) for 15 days. One hour after the final administration, the group III (radiation control) and Group IV animals were exposed to 6Gy (sublethal dose) EBR.

The animals 15 days after exposure were euthanized on day 16 and the blood was collected by cardiac puncture. Animal blood was collected in Ethylenediaminetetraacetic acid (EDTA) containing tubes for red blood cells (RBC) and in plain tubes for serum. It was then used for the biochemical estimations. The bone marrow cells were flushed into 5% Bovine serum albumin (BSA) to carry out micronucleus assay.

Lipid peroxidase (LPx) was measured by the method of Beuenge and Aust[26]. Briefly, serum was mixed with TCA-TBA-HCl reagent and was heated for 15 min in a boiling water bath. After centrifugation the absorbance was recorded at 535 nm using a UV/Vis double beam spectrophotometer. The LPx has been expressed as melondialdehyde (MDA) in µM per liter.

Total antioxidant capacity of serum was determined by the phosphomolybdenum method as described by Prieto et al.[27]. The serum was precipitated with 5% TCA, it was then reacted with total antioxidant capacity reagent containing phosphomolybdenum at 95° for 90 min. The absorbance was read at 695 nm.

Glutathione peroxidase (GPx) activity was measured as described by Rotruck, et al.[28]. The RBC were mixed with 4 mM reduced glutathione. In the presence of GPx the reduced glutathione converts into oxidized glutathione at 37°. The leftover reduced glutathione reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). The coloured compound formed absorbs maximally at 412 nm. The glutathione content in blood was measured spectrophotometrically using DTNB as a colouring reagent to form a compound, which absorbs maximally at 412 nm. The assay was done according to the method of Beutler et al.[29].

Catalase activity in RBC was measured spectrophotometrically as previously described[30]. The method is based on the fact that catalase causes breakdown of H₂O₂ (30 mM). The H₂O₂ was mixed in 3 ml of phosphate buffer (pH 7.0) and then 50 µl of 1:20 diluted erythrocyte was added and the changes in absorbance at 240 nm were recorded up to 2 min at the interval of 15 s. The enzyme activity was expressed as Units mg/Hb.

The mouse bone marrow micronucleus test was carried out according to the method described by Schmidt[31] by evaluation of chromosomal damage in experimental animals. The bone marrow from femur was flushed in the form of a suspension into a centrifuge tube containing 5% BSA. The cells were dispersed by gentle pipetting and collected by centrifuge at 2000 rpm for 5 min at 4°. The cell pellet was resuspended in a drop of BSA and bone marrow smear were prepared. After air drying the smear were stained with May-Grünwald/Giemsa. Micronucleated polychromatic erythrocytes (MnPCEs) and micronucleated nonchromatic erythrocytes (MnNCEs) were observed under microscope. The percentage of MnPCEs, MnNCEs and ratio of PCE to (PCE+NCE) was calculated.

All values were expressed as Mean±SD. Comparison between the control and treated groups were performed by analysis of variance (ANOVA) with Bonferroni. In all these test criterion for statistical significance was P<0.05.

The radiation dose was determined by exposing the mice with various doses (4, 6, 8, 10, 12 and 14 Gy) of EBR. It was found to be nontoxic up to a dose of 6 Gy, where no radiation-induced mortality was observed. A further increase in the electron beam dose to 8 Gy resulted in 33% mortality. An increase in radiation dose to 10 Gy caused a 50% reduction in
the survival of mice. 100% of the mice died when the electron beam dose was increased to 12 and 14 Gy. The LD$_{50}$ of electron beam for acute radiation-induced mortality was 10 Gy (fig. 1).

The irradiation of mice to 6 Gy of EBR induces lipid peroxidation. The irradiated group (Group III) had showed significant increased MDA level and decrease in the reduced glutathione (GSH) level. Presupplementation of Nardostachys jatamansi extract (NJE) before irradiation, Group IV, had showed the decreased level of lipid peroxidation and increased level of GSH when compared to Group III. This proves that the jatamansi extract helps in lowering the oxidative stress in irradiated mice.

The activity of various enzymes such as GPx and catalase in all the four groups which is given in Table 1. Significant lowering of enzyme activity was observed in electron beam exposed group in comparison with that of control (Group I) and drug control group II ($P<0.05$). The result of NJE pre supplemented group demonstrated its protective effect for enzyme activity.

A significant alteration in the total antioxidant level was observed in group II. But there was no much difference was observed in antioxidant level between control and irradiated group. There was no significant difference observed between the control and treated groups ($P=0.0504$).

The effect of EBR with and without NJE on the induction of micronucleus in bone marrow cells is shown in Table 2. The frequency of micronuclei was increased in group III. But the NJE treatment (Group IV) has helped in lowering the micronuclei formation (Table 2).

The recent radiation biology research is mainly focused on the identification and development of nontoxic and effective radioprotective compounds that can reduce the effect of radiation. Such compounds could potentially protect the biological system against the genetic damage, mutation, alteration in the immune system which acts through the generation of free radicals.

A single whole-body exposure of mammals to ionizing radiation results in a complex set of syndromes whose onset, nature and severity are a

![Fig. 1: Effect of electron beam dosage on the acute toxicity in mice. $P<0.05$ is statistically significant. Values are express as Mean±standard deviation.](image-url)

**Table 1.** Significant lowering of enzyme activity was observed in electron beam exposed group in comparison with that of control (Group I) and drug control group II ($P<0.05$). The result of NJE pre supplemented group demonstrated its protective effect for enzyme activity.

| Parameters                  | Group I       | Group II      | Group III     | Group IV      |
|-----------------------------|---------------|---------------|---------------|---------------|
| MDA (µM)                    | 0.26±0.13     | 0.90±0.30     | 5.19±1.38     | 3.68±0.99*    |
| TAC (µg/ml)                 | 343.60±9.03   | 535.6±11.7    | 335.40±64.75  | 396.00±5.29   |
| GSH (µg/ml)                 | 810.00±46.5   | 567.00±23.4   | 355±27.90     | 563.00±66.58* |
| GPx (GSH consumed/min/mgHb) | 0.28±0.005    | 0.19±0.05     | 0.08±0.016    | 0.13±0.03*    |
| Catalase (Units/mgHb)       | 19.89±0.91    | 17.50±3.90    | 10.71±1.55    | 14.90±1.80*   |

*P<0.05. MDA=Melondialdehyde, TAC=Total antioxidant capacity, GSH=Reduced glutathione, GPx=Glutathione peroxidase. Group I=Control, Group II=Drug control, Group III=Radiation control, Group IV=Treatment Group

**Table 2.** Effect of treatment with Nardostachys Jatamansi extract on protection against micronucleus formation induced by electron beam radiation.

| Number of cells counted per 100 cells | Group I       | Group II      | Group III     | Group IV      |
|--------------------------------------|---------------|---------------|---------------|---------------|
| PCE                                 | 48.72±2.22    | 46.8±1.23     | 27.1±0.90     | 34.21±1.95    |
| MnPCE                                | 0.00          | 0.14±0.01     | 6.00±1.09     | 1.07±0.05     |
| NCE                                 | 51.27±1.78    | 53.15±2.11    | 72.90±3.21    | 65.78±1.22    |
| MnNCE                                | 0.00          | 0.28±0.03     | 12.00±1.25    | 1.46±0.60     |

PCE=Polychromatic erythrocytes, NCE=Nonchromatic erythrocytes, MnPCE=Micronucleated polychromatic erythrocytes, MnNCE=Micronucleated normochromatic erythrocytes
function of both total radiation dose and radiation quality. At the cellular level, ionizing radiation can induce damage in biologically important macromolecules such as DNA, proteins, lipids and carbohydrates in various organs[32,33].

*Nardostachys jatamansi* root extract has shown both *in vitro* and *in vivo* antioxidant property[32]. It attenuates stress induced elevation of biochemical changes such as membrane lipid peroxidation, elevated NO production in brain as well as stomach, levels of antioxidant enzymes like catalase, which are consistent with its antistress properties. The similar mechanism might be the reason for the protection of mice against EBR induced lipid peroxidation followed by oxidative stress.

Depletion of intracellular GSH level has been implicated as one of the causes of radiation-induced damage, while increased levels of this are responsible for the radioprotective action. NJE presupplementation helped to restore the GSH when compared to the concurrent irradiation control group (Group III). This inhibits the radiation-induced lipid peroxidation, thereby protecting against radiation-induced damage. Ionizing radiation induces lipid peroxidation, which causes DNA damage and cell death[34,35]. Some of the plants *Embelia ribes*[36], *Piper longum*, *Zinger officinalis*, *Santalum album*[37], *Ocimum sanctum* have been reported to increase GSH. While the other plants like *Asparagus racemosus*, *Glycyrrhiza glabra*, *Phyllanthus emblica*, *Boerhavia diffusa*, *Ocimum sanctum*, *Eclipta alba* have been found to possess *in vitro* antioxidant properties[38].

The survival assay results revealed that the death of animals in 12 and 14Gy irradiated mice were observed after 10th day of irradiation. Death between 11th and 30th day postirradiation is due to haemopoietic damage inflicted by radiation[39]. The result obtained by micronucleus assay justifies the haemopoietic damage. NJE treatment helps in lowering the micronucleus formation after radiation exposure.

In conclusion, NJE pretreatment reduced radiation-induced stress by protecting against the radiation-induced biochemical and chromosomal level damage. Free radical scavenging, elevation in antioxidant status and GSH levels and reduction in lipid peroxidation appear to be important in providing radioprotection.

ACKNOWLEDGEMENTS

The authors are greatly thankful to Board of Research in Nuclear Science, Government of India for the financial support (2009/34/18/BRNS) and also we would like to thank all the staff members of Microtron centre, Mangalore University, Mangalore, India for providing the radiation source.

REFERENCES

1. Sweeney TR. A Survey of Compounds from the Antiradiation Drug Development Program of the US Army Medical Research and Development Command. Washington, DC: Government Printing Office; 1979. p. 308-18.

2. Maisin JR. Chemical radioprotection: Past, present, and future prospects. Int J Radiat Biol 1998;73:443-50.

3. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology 2003;189:1-20.

4. Patt HM, Tyree EB, Straube RL, Smith DE. Cysteine Protection against X Irradiation. Science 1949;110:213-4.

5. Sarma L, Kesavan PC. Protective effects of vitamins C and E against gamma-ray-induced chromosomal damage in mouse. Int J Radiat Biol 1993;63:759-64.

6. Felemovicius I, Bonsack ME, Baptista ML, Delaney JP. Intestinal radioprotection by vitamin E (alpha-tocopherol). Ann Surg 1995;222:504-8.

7. Konopacka M, Rzeszowska-Wolny J. Antioxidant vitamins C, E and beta-carotene reduce DNA damage before as well as after gamma-ray irradiation of human lymphocytes *in vitro*. Mutat Res 2001;491:1-7.

8. Pande S, Kumar M, Kumar A. Evaluation of radio modifying effect of root extract of *Panax ginseng*. Phytother Res 1998;12:13-7.

9. Gupta NK. Hypolipidemic action of garlic unsaturated oils in irradiated mice. Nat Acad Sci Lett 1986;11:401-3.

10. Uma Devi P, Ganasoundari A, Rao BS, Srinivasan KK. *In vivo* radioprotection by *Ocimum flavonoids*: Survival of mice. Radiat Res 1999;151:74-8.

11. Samarth RM, Goyal PK, Kumar A. Modulatory effect of *Mentha piperita* (Linn.) on serum phosphatases activity in Swiss albino mice against gamma irradiation. Indian J Exp Biol 2001;39:479-82.

12. Jagetia GC, Baliga MS, Venkatesh P, Ulloor JN. Influence of ginger rhizome (*Zingiber officinale Rosc*) on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. Radiat Res 2005;160:584-92.

13. Saini MR, Kumar S, Jagetia GC, Saini N. Effect of Liv. 52 against radiation sickness and mortality. Indian Pract 1984;37:1133-8.

14. Jagetia GC, Ganapathi NG. Inhibition of clastogenic effect of radiation by Liv. 52 in the bone marrow of mice. Mutat Res 1989;224:507-10.

15. Jagetia GC, Ganapathi NG. Treatment of mice with a herbal preparation (Liv. 52) reduces the frequency of radiation-induced chromosome damage in bone marrow. Mutat Res 1991;253:123-6.

16. Ganapathi NG, Jagetia GC. Liv. 52 Pretreatment Inhibits the Radiation-induced Lipid Peroxidation in Mouse Liver. Curr Sci 1995;68:601-3.

17. Baliga MS, Jagetia GC, Venkatesh P, Reddy R, Ulloor JN. Radioprotective effect of abana, a polyherbal drug following total body irradiation. Br J Radiol 2004;77:1027-35.

18. Jagetia GC, Baliga MS. Treatment of mice with a herbal preparation (menta) protects against radiation-induced mortality. Phytother Res 2005;17:876-81.

19. Rao VS, Rao A, Karanth KS. Anticonvulsant and neurotoxicity profile of *Nardostachys jatamansi* in rats. J Ethnopharmacol 2005;102:351-6.

20. Arora RB, Singh KP, Das PK, Mistry PN. Prolonged hypotensive effect...
of the essential oil of Nardostachys jatamansi. Arch Int Pharmacodyn Ther 1958;113:367-76.
21. Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, et al. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J Ethnopharmacol 2007;109:359-63.
22. Lyle N, Bhattacharyya D, Sur TK, Munshi S, Paul S, Chatterjee S, et al. Stress modulating antioxidant effect of Nardostachys jatamansi. Indian J Biochem Biophys 2009;46:93-8.
23. Chatterji A, Prakshi SC. The Treatise on Indian Medicinal Plants, Vol. 5. New Delhi: National Institute of Science Communication; 1997. p. 99-100.
24. Bagchi A, Oshima Y, Hikino H. Neolignans and Lignans of Nardostachys jatamansi Roots. Planta Med 1991;57:96-7.
25. Rucker G, Panicker Mayor R, Breitamaier E. Revised structure and stereochemistry of jatamansic oil. Phytochemistry 1993;33:141-3.
26. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302-10.
27. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
28. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
29. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
30. Aebi H. Catalase in vitro. In: Colowick SP, Kaplan NO, editors. Methods in Enzymology. Vol. 105. Florida: Acad Press; 1984. p. 114-21.
31. Schmidt W. The Micronucleus test. Mutat Res 1975;31:9-15.
32. Jagetia GC, Baliga MS, Malagi KJ, Sethukumar Kamath M. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to gamma-radiation. Phytomedicine 2002;9:99-108.
33. Jagetia GC, Baliga MS. Cystone, an ayurvedic herbal drug imparts protection to the mice against the lethal effects of gamma-radiation: A preliminary study. Nahrung 2002;46:332-6.
34. Raleigh JM, Shum FY. Radioprotector in model lipid membranes by hydroxyl radical scavengers: Supplementary role of α-tocopherol in scavenging secondary peroxyradicals. In: Nyaard OF, Srim G, editors. Radioprotectors and Anticarcinogens. New York: Academic Press; 1983. p. 87-102.
35. Leyko W, Bartosz G. Membrane effects of ionizing radiation and hyperthermia. Int J Radiat Biol Relat Stud Phys Chem Med 1986;49:743-70.
36. Chitra M, Shyamala Devi CS. Protective action of embelin against lipid peroxidation on tumor bearing mice. Fitoterapia 1994;65:317-21.
37. Banerjee S, Evacuee A, Rao AR. Modulator influence of sandalwood oil on mouse hepatic glutathione s-transferase activity and acid soluble sulphydryl level. Cancer Lett 1993;68:105-9.
38. Joy KL, Kuttan R. Antioxidant activity of selected plant extracts. Amala Res Bull 1995;15:68-71.
39. Bond VP, Fliedner TM, Archambeau JO. Mammalian Radiation Lethality. New York, USA: Academic Press; 1965.