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

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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[4]. 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].

Nardostachys jatamansi (family Valerianaceae), an indigenous medicinal plant induces in organism a state of resistance against stress. It helps to promote physical and mental health augment resistance of the body against disease and has shown potent antioxidant activity. It has also shown marked tranquillizing activity, as well as hypotensive, hypolipidemic, antiischemic, antiarrhythmic, hepatoprotective, anticonvulsant, neuroprotective, antioxidant activities[19-22].

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^{o}.

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 Beuege 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 95º 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 37º. 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 H_2O_2 (30 mM). The H_2O_2 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 4º. 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-Grunwald/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

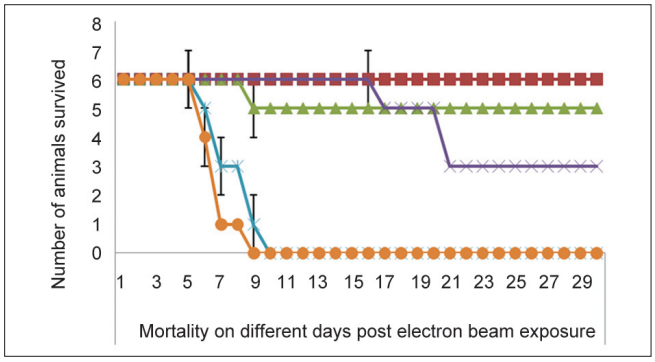

Fig. 1: Effect of electron beam dosage on the acute toxicity in mice. *P*<0.05 is statistically significant. Values are express as Mean±standard deviation. 4 Gy, 6 Gy, 8 Gy, 10Gy, 12Gy, 14Gy

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

TABLE 1: EFFECT OF TREATMENT WITH *NARDOSTACHYS JATAMANSI* EXTRACT IN THE MICE EXPOSED TO ELECTRON BEAM RADIATION

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±0.90	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

	Group I	Group II	Group III	Group IV
Number of cells counted per 100 cells				
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[22]. 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 officinale, Santalum album*[37], *Ocimum sanctum* have been reported to increase GSH. While the other plants like *Asparagus racemosus, Glycyrrhiza glabra, Phyllanthus embelica, Boerrhaavia 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 10^{th} day of irradiation. Death between 11^{th} and 30^{th} 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.

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