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1. INTRODUCTION

Accidental exposure to radiation in a terrorist attack/military operations or industrial accidents is a current and continuing threat. Low LET ionizing radiation are highly penetrating and deposit their energy deep inside the tissues. The damage to the bio-molecules is caused by direct deposition of energy as well as by generating free radicals. Whole body exposure to ionizing radiation leads to multi-factorial events which can damage multiple tissues by multiple pathways. At the time of nuclear/radiological emergency, both civilians and rescue workers need to be protected against radiation injuries. After nuclear/radiological emergency first responders need to enter the contaminated area to re-establish critical life care system and to rescue the affected people from the contaminated area. For such scenarios, a suitable medical intervention to protect the responders/rescue workers against injuries caused by ionizing radiation, is essential and is an optimal choice. Development of protective agents against radiation injuries is a global challenge and till date no radioprotective drug has been approved for human use. To develop effective and suitable radiation countermeasures against radiation injuries, evidence-based biological explanation on the mechanism(s) of action of radiation pathology needs to be adequately understood.

Low LET ionizing radiation damage biological system by directly interacting with biomolecules or indirectly via production of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS). Free radicals are highly reactive and are capable of altering all biological molecules including lipids, DNA and proteins. Radiolysis of water results in production of reactive species which includes, hydroxyl radical (OH), hydrogen peroxide (H₂O₂), peroxyl radical (ROO⁻), superoxide radical (O₂⁻⁻), singlet oxygen (¹O₂) etc. The highly reactive species formed in biological systems during exposure to ionizing radiation are O₂⁻⁻ and OH. DNA is one of the most critical targets for radical damage, contributing towards cell death after exposure to ionizing radiation. Inflammation, disruption of immunity, oxidative stress, depletion of stem cells, organ system dysfunction and death are some of the consequences after exposure to high doses of ionizing radiation. Haemolymphoid system with high proliferative capacity is one of the most sensitive
organ system to radiation doses exceeding 1 Gy. Blood, spleen, liver, thymus and lymph nodes are important haemolymphoid tissues. Blood maintains homeostasis in tissues by circulating essential nutrients and growth factors to the organs; regulating body temperature and oxygen supply and by maintaining the functioning of immune system. Whole body exposure to ionizing radiation has been reported to alter the levels of circulating anti-inflammatory and pro-inflammatory cytokines, which disturbs the homeostasis between inflammation and the counter-balancing anti-inflammatory response (Schaue and McBride, 2010). Depending upon the radiation dose whole body exposure to ionizing radiation results in multiple organ failure. Inflammation, immune dysregulation, stem cell depletion, disruption of vascular integrity and oxidative stress are some of the important pathways playing role in radiation induced multiple organ dysfunction (Williams and Mcbride, 2011). Therefore, protection of haemolymphoid tissues from radiation injuries is important after whole body exposure to ionizing radiations.

A clinically effective radioprotective agent is expected to reduce the damaging effects of radiation, when administered to living organisms prior to radiation exposure. Some of the important mechanisms by which a radioprotective drug is expected to act are (i) scavenging of free radicals, (ii) activation of cellular antioxidant defence mechanism, (iii) enhancing DNA repair by triggering one or more cellular DNA repair pathways, (iv) activation/protection of stem cell compartment.

Upto now several chemicals, vitamins, amino acids, cytokines have been screened for their radioprotective efficacy. But, either due to low efficacy at lethal doses or due to toxicity near radioprotective doses, none of them could be treated as an ideal radioprotective agent. The high toxicity of various synthetic compounds necessitated the search for alternative agents, which could be less toxic but at the same time more effective at non-toxic doses. Whole body exposure to ionizing radiation since, leads to multi-factorial events which can damage tissues by multiple pathways, it was proposed that a radioprotective drug should ideally have multiple bioactive constituents. Plants, due to presence of large number of bioactive molecules, can be potential candidate for developing a radioprotective drug. The bioactive constituents of plants could act as antioxidants, immunostimulants, haemopoietic

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stimulators, cell proliferation stimulators, anti-inflammatory and antimicrobial agents. Some of which may act in isolation as well as in combination with other constituents from the same plant. The traditional systems of medicine like Ayurveda, Chinese medicine or the European pharmacotherapy generally assume that a synergy of various ingredients of the plants is responsible for the maximum therapeutic efficacy (Ullrich-Merzenich et al., 2010). It was therefore, thought that products/compounds isolated from plants could be of substantial use as non-toxic radioprotectors. A number of medicinal plants, so far evaluated for their radioprotective efficacy are Ocimum sanctum, Tinospora cardifolia, Mangiferin, Mentha piperitita, Amaranthus paniculatus, Hawthorn fruit extract. (Uma devi et al., 2000; Goel et al., 2004; Jagetia et al., 2005; Smarth and kumar, 2003; Krishna and Kumar, 2003; Hossienmehr et al., 2006). Most of these plants showed radioprotective efficacy at sub-lethal radiation doses, but none of them could be regarded as ideal radioprotector as they did not protect the organisms exposed to lethal doses of ionizing radiation. Therefore, globally, the search for an effective yet non-toxic radioprotector is still in progress.

Plant growing under extreme climatic conditions e.g. at high altitudes, are constantly exposed to extreme environmental perturbation such as drought, intense light, extreme temperature, wounding, infection and radiation. To counter the adversaries of nature a highly efficient defence system is expected to have been evolved in plants growing in adverse climatic conditions. Therefore, these high altitude plants in the process of adaptation to adverse conditions tend to develop more bioactive molecules. Hippophae rhamnoides is one such plant growing at high altitude. Hippophae commonly known as Seabuckthorn or Chharma is a deciduous spiny species widely distributed throughout the temperate zones of Asia, Europe and all over the subtropical zones of high altitude. Hippophae is highly rich in lipids, fatty acids, vitamins, flavonoids, tannins, phenols, progestin, amino acid, minerals, coumarin, triterpene, steroids and organic acids. H. rhamnoides has been used in Indian, Chinese and Tibetan medicinal system for treatment of various disorders. H. rhamnoides berry juice, seed oil and leaf tea have been used as dietary products. We were the first to report the radioprotective properties of different extracts prepared from leaves of H. rhamnoides, which were coded as SBL-1 (Bala et al., 2009) and
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SBL-2 (Bala, 2008). One time treatment with SBL-1 or SBL-2 before irradiation with lethal doses of $^{60}$Co-gamma rays (10 Gy) could protect more than 90 % mice population while all the non-drug treated irradiated (10 Gy) mice died within 12 days after irradiation.

This study was targeted at comparing the radioprotective efficacy of SBL-1 and SBL-2 based on their potential to counter the radiation induced oxidative stress \textit{in vitro}, \textit{ex vivo} and \textit{in vivo}. It was also proposed to investigate the radioprotective effects of the better herbal preparation amongst SBL-1 and SBL-2, on the haemolymphoid system and understand the underlying mechanisms of radioprotection.

Accordingly the objectives of the present study were:-

1. To study the radioprotective efficacy of composite herbal preparations of \textit{Hippophae rhamnoids} L. (Seabuckthorn) both \textit{in vivo} and \textit{in vitro}.
   - The \textit{in vitro} studies were performed in terms of free radical scavenging activity, reduction potential and presence of antioxidants in drug.
   - The \textit{in vivo} studies were performed in murine experimental model system. Studies undertaken to assess protection from oxidative stress, by measuring changes in antioxidant enzyme activities viz. glutathione reductase, superoxide dismutase and catalase etc.

2. To investigate radioprotective effect of herbal preparation in haemolymphoid system as below:
   - Immunomodulation investigated by identifying key immunological entities viz. GM-CSF, interleukins and antibody titer etc.
   - Protection at molecular level studied in proliferative cells of the system by recording expression of proliferation associated genes.