SUMMARY
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Low LET ionizing radiation are highly penetrating and deposit energy deep inside the tissues. Whole body exposure to low LET ionizing radiation leads to pathologies in multiple organs depending upon the dose of radiation. Haemopoietic system is a critical and vulnerable organ system which undergoes extensive damage after whole body exposure to ionizing radiation. Development of effective and non-toxic radioprotector against lethal doses of ionizing radiation is essentially needed, because of increasing risk of accidental exposure to radiation during terrorist attacks/military operations, space programs or industrial accidents. Up to now various chemicals, vitamins, amino acids, phytochemicals have been screened for their radioprotective potential. However, none of them could be treated as ideal radioprotective agent, either due to their low efficacy at lethal doses or due to high toxicity near radioprotective doses. During whole body exposure to ionizing radiation, all organs and organ systems are affected and if the radiation dose is high enough, it can cause multiple organ failure. Whole body radiation exposure affects multiple pathways simultaneously, leading to inflammation, infection, immune deficiency, massive cell death, wounds etc. The complexities of the pathways affected during whole body radiation exposure, suggest that protecting any single pathway or process is unlikely to provide full protection from the radiation damage. Therefore, a combined agent regimen is needed to achieve holistic radiation protection.

Plants contain large number of bioactive molecules and therefore, can be potential source for developing radioprotective agent. However, so far, most of the radioprotective drugs from plants were effective at sub-lethal radiation doses only. The bioactive extracts from the high altitude plants were thought to be better radioprotective agents due to their induced adaptive response to adverse climatic conditions. *Hippophae rhamnoides* L. common name Seabuckthorn (family Elaeagnaceae), is a high altitude plant, known to Tibetan and Chinese medicinal system for treating various disorders. All parts of *Hippophae* contain large number of bioactive compounds such as ascorbic acid, tocopherols, carotenoids, flavonoids, tannins, minerals, lipids, sugars, organic acids and phytosterols. A number of *Hippophae* preparations have been recommended for treatment of various cardiac and
gastric disorders (Zeb, 2004); as well as for nutraceutical purposes. It was reported that treatment of mice with preparation from Seabuckthorn leaves rendered significant survival benefit (> 90 %) to mice population, when administered 30 minutes before irradiation with lethal dose of $^{60}$Co-gamma radiation (10 Gy) at concentration 30 mg/kg b.w. for SBL-1 (Bala et al., 2009) and 20 mg/kg b.w. for SBL-2 (Bala, 2008).

To achieve the objectives defined in this study, first both the herbal preparations SBL-1 and SBL-2 from leaves of *H. rhamnoides* were evaluated for *in vitro* and *in vivo* antioxidant potential. Thereafter, the best herbal preparation was tested for its efficacy to protect haemolymphoid system of animals irradiated with lethal doses of $^{60}$Co-gamma radiation.

- Performance of SBL-1 was better in comparison to SBL-2 in three out of four key free radical scavenging assays performed *in vitro*. These assay were to test the reducing potential, scavenging potential of Fenton reaction generated 'OH and scavenging potential of $O_2^{-}$. Performance of SBL-2 was better than SBL-1 only in scavenging radiation generated 'OH.

- *Ex vivo* studies demonstrated that both SBL-1 and SBL-2 were equally efficient in inhibiting radiation induced RBC haemolysis and lipid peroxidation in liver cells.

- *In vivo* antioxidant potential of SBL-1 and SBL-2 was compared at three radiation doses, 5 Gy, 7.5 Gy and 10 Gy in liver as well as plasma of whole body irradiated mouse. At sub-lethal doses (5 Gy and 7.5 Gy) both SBL-1 and SBL-2 were equally efficient in countering the radiation induced oxidative stress in plasma and liver. At lethal doses (10 Gy), SBL-1 was more efficient than SBL-2 in countering the radiation induced oxidative stress.

- SBL-1 had 39 % more polyphenols and 12.5 % more tannins, but had 46 % less flavonoids in comparison to SBL-2. Further studies revealed that tannins and polyphenols had better correlation with 'OH scavenging and $O_2^{-}$ scavenging activity while, flavonoids had better correlation with scavenging of radiation generated 'OH.
• Although both the herbal preparations SBL-1 and SBL-2, provided more than 90 % survival benefit to animals treated with drug in comparison to non-drug treated and irradiated mice, SBL-1 was more effective in reducing oxidative stress under *in vitro* and under *in vivo* conditions at lethal doses of $^{60}$Co-γ rays in comparison to SBL-2. Therefore, SBL-1 was selected over SBL-2 for investigating its effects on haemolymphoid system of whole body lethally irradiated mice.

• Administration of SBL-1 before irradiation (2 Gy) significantly reduced radiation induced formation of micronuclei and apoptotic bodies in bone marrow as well as peripheral blood in comparison to non-drug treated and irradiated animals.

• SBL-1 treatment before whole body irradiation caused the complete recovery of radiation induced decrease in haematological parameters viz., total leukocyte count, differential leukocyte count, platelets and haemoglobin by day 25.

• Mice treated with SBL-1 before irradiation displayed no observable inflammation viz., hair fall and swelling at head and snout region, swelling and haemorrhage of internal organs, which was generally observed in mice on day 5 after irradiation without any prior drug treatment.

• Treatment with SBL-1, before whole body irradiation delayed the radiation induced early augmentation of HMGB1 in serum. Total body lethal irradiation had significantly increased the levels of TNF-α and TGF-β and decreased the levels of IgG in serum. Treatment with SBL-1, before irradiation normalized the levels of TNF-α, TGF-β and IgG and upregulated the levels of anti-inflammatory cytokine IL-10 (24-48 h), suggesting that SBL-1 treatment before irradiation countered the radiation induced inflammatory proteins and also the immunesuppression.

• Whole body irradiation caused increase in levels of CD14 and MPO in spleen and increase in levels of TNF-α and MPO in liver. Administration of SBL-1 before irradiation resulted in normalization of radiation induced increase in the
levels of CD14 and MPO by day 2 in spleen. Treatment with SBL-1 before irradiation also normalized the levels of TNF-α and MPO in liver by day 10. This suggested the anti-inflammatory potential of SBL-1 at tissue level.

- Whole body irradiation decreased the FRAP and total thiols in liver from day 5 onwards till the death. The SBL-1 treatment before lethal irradiation increased the FRAP (8 h-48 h) in plasma and total thiols (16 h-72 h) in liver at earlier time points which were normalized at later time points (day 10). SBL-1 treatment alone to mice had also increased the FRAP in plasma and thiols in liver at early time point till day 5, suggesting the in vivo antioxidant potential of SBL-1.

- The SBL-1 treatment before lethal irradiation normalized the radiation induced decreased levels of catalase and SOD in spleen by day 10. SBL-1 treatment before irradiation also normalized the radiation induced decreased levels of catalase, SOD, GR and GST enzymes in liver (day 7- day 10). This indicated the potential of SBL-1 in inducing endogenous antioxidant enzymes.

- Radiation induced increase in lipid peroxidation in spleen and liver was normalized in the animals treated with SBL-1 before irradiation. This further supported the antioxidant potential of SBL-1.

- Histological studies with spleen demonstrated that administration of SBL-1 before whole body irradiation normalized the white pulp area, PALS and the follicle numbers by day 10. Consistent decrease in the white pulp area, PALS and the follicle numbers; with increase in apoptotic bodies, necrotic cells and fibrosis was observed in animals irradiated with lethal doses of radiation without treatment with SBL-1. The animals treated with SBL-1 before irradiation did not show visible appearance of apoptotic bodies, necrosis and fibrosis in spleen by day 10. These results demonstrated the radioprotective potential of SBL-1 at tissue level.

- In irradiated animals, levels of VEGFR-3 in spleen decreased significantly while levels of sVEGFR-3 in serum increased significantly in a time dependent manner from day 2 till day 10, indicating radiation damage to vasculature. In
animals treated with SBL-1 before irradiation the levels of VEGFR-3 in serum as well as spleen were normalized by day 10, indicating the role of SBL-1 in protecting the tissue vasculature. This was also supported by the observations such as normalization of the radiation induced changes in levels of (i) GM-CSF in spleen as well as in serum by day 10; (ii) levels of CD4 and CD8 in spleen by day 7; in the animals treated with SBL-1 before irradiation. Therefore, demonstrating the radioprotective role of SBL-1 at molecular and biochemical level.

- DNA is one of the most critical targets responsible for cell death following whole body irradiation. Administration of SBL-1 before irradiation caused decrease in radiation induced DNA damage in comparison to the irradiated animals as observed in genomic DNA from spleen of mouse using pulsed field gel electrophoresis technique.

- SBL-1 treatment before whole body irradiation up-regulated the levels of anti-apoptotic protein bcl-2 and normalized the levels of pro-apoptotic protein bax by day 2. On the other hand increased levels of bax and decreased levels of bcl-2 protein were observed in spleen of animals irradiated with lethal doses of radiation without SBL-1 treatment. This indicated the anti-apoptotic effect of SBL-1.

- Addition of SBL-1 to the splenocyte cultures showed enhancement in the proliferation of cells isolated from whole body irradiated mice.

- Histological studies with liver demonstrated that whole body irradiation caused derangement of hepatic chords, decreased sinusoidal space, increased hyaline bodies and pyknotic cells till the death of animals (day 12). Administration of SBL-1 before whole body irradiation normalized the (i) arrangement of hepatic chord and sinusoidal space with no visible hyaline bodies and pyknotic cells in liver by day 10. This suggested protection to liver at tissue level by SBL-1.

- Treatment with SBL-1 before irradiation normalized the mRNA levels of TWEAK and FN14 by day 10 in liver; whereas consistently elevated levels of
TWEAK (day 2-day 10) and delayed elevation in levels of FN14 (day 10) were observed in animals irradiated with lethal doses of radiation without SBL-1 treatment. This suggested potential of SBL-1 in regulating cell proliferation associated genes.

- Radiation induced damage is reported to adversely affect the liver function. In our study elevated ratios of liver function enzymes (AST/ALT) as well as increased levels of ALP in plasma were observed in animals irradiated with lethal doses of radiation without SBL-1 treatment. SBL-1 treatment before irradiation normalized the ratio of liver function enzymes (AST/ALT) and levels of ALP in plasma. This suggested that treatment with SBL-1 before irradiation normalized the functioning of liver.

This study clearly demonstrated that herbal preparations SBL-1 and SBL-2 developed from the leaves of Hippophae rhamnoides had significant radioprotective properties. Amongst the two herbal preparations, SBL-1, had demonstrated better radioprotective efficacy at lethal doses (10 Gy) of 60Co-gamma radiation in comparison to SBL-2. The extensive experiments performed at \textit{in vitro} and \textit{in vivo} levels corroborated these findings and also fulfilled the first committed objective of this study. The administration of SBL-1 to experimental mice prior to whole body irradiation with lethal dose (10 Gy) of 60Co-gamma radiation offered protection to major tissues of haemolymphoid system, which were investigated as per commitment in the second objective of this study. The major underlying mechanisms of radioprotection by SBL-1 were by countering radiation-induced inflammation, immunesuppression, DNA damage and apoptosis. Further, SBL-1 treatment prior to irradiation supported the normal regulation of biochemical and cellular markers associated with cell proliferation.