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Radiation Induced DNA Damage and Its Modification by Herbal Preparation of Hippophae rhamnoides L. (SBL-1): An in vitro and in vivo Study in Mice

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Abstract : Ionising radiation exposure induces generation of free radicals and the oxidative DNA damage. SBL-1, a radioprotective leaf extract prepared from leaves Hippophae rhamnoides L. (Common name; Seabuckthorn), showed > 90% survival in mice population that was treated with lethal dose (10 Gy) of ^{60}Co gamma irradiation. In this study, early effects of pre-treatment with or without SBL-1 in blood peripheral blood lymphocytes (PBMCs) were investigated by cell viability assays (trypan blue and MTT). The quantitative in vitro study of Hoescht/PI staining was performed to check the apoptosis/necrosis in PBMCs irradiated at 2 Gy with or without pretreatment of SBL-1 (at different concentrations) up to 24 and 48h. Comet assay was performed in vivo, to detect the DNA strands breaks and its repair mechanism on peripheral blood lymphocytes at lethal dose (10 Gy). For this study, male mice (wt. 28 ± 2g) were administered radioprotective dose (30mg/kg body weight) of SBL-1, 30 min prior to irradiation. Animals were sacrificed at 24h and 48h. Blood was drawn through cardiac puncture, and blood lymphocytes were separated using histopaque column. Both neutral and alkaline comet assay were performed using standardized technique. In irradiated animals, alkaline comet assay revealed single strand breaks (SSBs) that showed significant ($p < 0.05$) increase in percent DNA in tail and Olive tail moment (OTM) at 24 h while at 48h the percent DNA in tail further increased significantly ($p < 0.02$). The double strands breaks (DSBs) increased significantly ($p < 0.01$) at 48 h in neutral assay, in comparison to untreated control. The animals pre-treated with SBL-1 before irradiation showed significantly ($p < 0.05$) less DSBs at 48 h treatment in comparison to irradiated group of animals. The SBL-1 alone treated group itself showed no toxicity. The antioxidant potential of SBL-1 were also investigated by in vitro biochemical assays such as DPPH ($p < 0.05$), ABTS, reducing ability ($p < 0.09$), hydroxyl radical scavenging ($p < 0.05$), ferric reducing antioxidant power (FRAP), superoxide radical scavenging activity ($p < 0.05$), hydrogen peroxide scavenging activity ($p < 0.05$) etc. SBL-1 showed strong free radical scavenging power that plays important role in the studies of radiation-induced injuries. The SBL-1 treated PBMCs showed significant ($p < 0.02$) viability in trypan blue assay at 24-hour incubation.

Keywords : radiation, SBL-1, SSBs, DSBs, FRAP, PBMCs
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