ABSTRACT

Drug discovery field has tremendously progressed during last few decades however, an effective radioprotective agent for safe administration to the victims of radiation exposure is still unavailable. A number of herbal, natural and synthetic agents have been evaluated to meet the need, but, associated toxicity with useful doses has precluded their use from bench to bed. This multi-model study is aimed at elucidating the mechanistic aspects of a novel podophyllotoxin and rutin combination (henceforth referred as G-003M) in hematopoietic radioprotection and its involvement in the DNA damage and repair signaling pathways. Using in silico study, we identified the binding sites and structural components of G-003M. The In silico study showed the binding of podophyllotoxin to β-tubulin and presence of a functional hydroxyl group in the rutin, suggested their involvement in G2/M arrest and the free radical scavenging, respectively. This study was further validated through in vitro method. The radioprotective work of this formulation was carried out in mice and rabbit animal models. Study with rabbit model was focused on efficacy evaluation, generation and persistence of γH2AX foci, variability in foci size after acute and fractionated radiation exposure. G-003M provides significant radioprotective efficacy > 85% in the rabbits exposed to whole body irradiation. This study also revealed that γH2AX focus assessment could be used to confirm radiation exposure, and its type (acute or fractionated). G-003M attenuates the level of γH2AX foci in blood cells of animals exposed to both acute and irradiated radiation. Studies conducted in mice model was focused on endpoints related to the DNA repair and cell death pathways. Mice were pre-administered with G-003M, irradiated and subsequently euthanized to collect blood and bone marrow cells. Measurement of DNA double strand break biomarkers and non-homologous end joining repair pathway proteins and various pro and anti-apoptotic pathways proteins were carried out at various time points. We noticed a decrease in the levels of γH2AX, 53BP1, and ATM kinase and an increase in the levels of DNA-PK, Ku80, Ligase IV, Mre11, Rad50 and NBS1 in the blood and bone marrow cells of the G-003M pre-administered and irradiated mice at different time intervals. In-vivo DNA damage and repair studies in mice confirmed that G-003M pre-administration attenuated DNA damage and enhanced repair after whole
body exposure. In cell death studies, we observed a decrease in pro-apoptotic proteins (Bax, P53, Caspase 3, and Bax) and increase in expression of anti-apoptotic protein (Bcl 2) in mice hematopoietic system. The cell death study was validated through Annexin V-FITC-PI staining and DNA fragmentation assays which confirmed the cell death reducing potential of G-003M. We further noticed an overall increase in the pro-survival factors in the G-003M pre-treated and irradiated groups establishing the radioprotective efficacy of this formulation. The whole study has explicitly demonstrated G-003M as an efficient formulation which can minimize DNA damage in hematopoietic tissues by various mechanisms like G2/M arrest, free radical scavenging, altering the DNA double strand breaks signaling and reduction in cell death. Present investigation plays a major role in deciphering the mode of action of G-003M. The lead obtained from this study will certainly help in developing this formulation as a safe and effective radioprotector which could be used for human against any planned or emergency exposure of radiation.