

Title: The combination of Podophyllotoxin, podophyllotoxin-β-D-glucosides and rutin enhance the non-homologous end joining (NHEJ) signaling to repair radiation induced DNA double strand breaks

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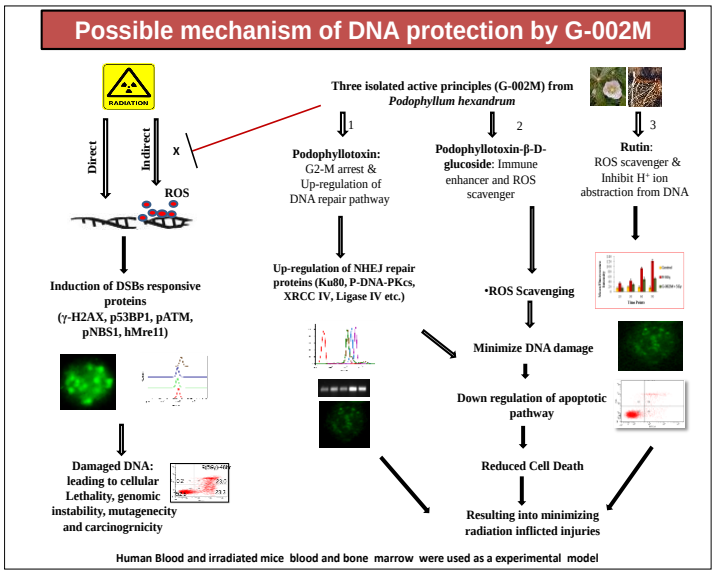
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A combination of three active principles (G-002M) podophyllotoxin, podophyllotoxin-β-D-glucosides and rutin has been revealed for its DNA radioprotective ability. The present study has been conceptualized to elucidate its mechanism in reducing radiation mediated DNA double strand breaks (DSBs) and regulation of DNA repair pathway. The levels of γ-H2AX and P53BP1, DSBs detection signalling molecules in mammalian cells, were found decreased in G-002M pre-treated irradiated human blood and PBMCs as compared to cells irradiated without G-002M. The flowcytometry and foci formation studies in these samples also showed that G-002M pre-treatment attenuates the phosphorylation of ATM, DNA-PKcs, SMC1, NBS1 and hMre11. However, Ku80 and XRCCIV and ligase IV protein levels in the same group samples had enhanced. Effect of the formulation also modulated the expression of various genes such as *ATM and 53BP1* were found down regulated and *DNA-PKcs, Ku80* got upregulated. Mechanistically, the protective efficacy of the formulation can be attributed to its free radical scavenging potential termed as indirect mode of action. Same was experimentally confirmed using DHE and DCFH2-DA fluorescence dye, analysed in the form of significant decline in radiation mediated ROS generation. Additionally, this formulation also demonstrated significant reduction in radiomimetic drug (Bleomycin sulphate) induced foci and MFI of γ-H2AX. Since bleomycin sulphate induced DSBs are known to be independent of ROS, results clearly suggest that G-002M may have a direct mode of action in regulation of DSBs signaling and its repair molecules. The flowcytometry data of annexinV, a cell viability marker-based study, demonstrated efficacy of G-002M by indicating reduced number of dead cells. The regulation of apoptotic proteins such as BCl_2, Bax and Caspase 3 was also efficiently modulated by pretreatment of this formulation.

The *in vitro* study (human blood and PBMCs) observations have been further strengthened with *in vivo* study in mice. Mice pretreated (-1h) with G-002M and exposed to moderate doses (5and 7Gy) of ionizing radiation revealed significant decrease in γ-H2AX foci formation and DNA fragmentation in blood and bone marrow cells as compared to the respective radiation alone groups.

The present study clearly emphasizes protective ability of G-002M against radiation induced damage to DNA, a major cellular biomolecule. This task has also conveyed a clear understanding in explaining the possible mechanisms involved in overall radioprotection delivered by this formulation. Thus, this effort has been of immense importance in identifying a

potential lead, necessary for the development of a new generation, safe and effective radioprotective agent for human applications against planned or emergency exposure of radiation.



References:

1. Pannunzio, Nicholas R., Go Watanabe, and Michael R. Lieber. "Nonhomologous DNA End Joining for Repair of DNA Double-Strand Breaks." *Journal of Biological Chemistry* (2017): jbc-TM117
2. Yashavarddhan, M. H., et al. "Targeting DNA Repair through Podophyllotoxin and Rutin Formulation in Hematopoietic Radioprotection: An in Silico, in Vitro, and in Vivo Study." *Frontiers in pharmacology* 8 (2017): 750.
3. Guleria, Ayushi, and Sudhir Chandna. "ATM kinase: Much more than a DNA damage responsive protein." *DNA repair* 39 (2016): 1-20.
4. Dutta, A., M. L. Gupta, and B. Kalita. "The combination of the active principles of Podophyllum hexandrum supports early recovery of the gastrointestinal system via activation of Nrf2-HO-1 signaling and the hematopoietic system, leading to effective whole-body survival in lethally irradiated mice." *Free radical research* 49.3 (2015): 317-330.
5. Mladenov, Emil, and George Iliakis. "Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 711.1 (2011): 61-72.

Biography

Dr. Nityanand has completed his Ph.D from Institute of Nuclear Medicine and Allied sciences, Defense Research Development Organization, India and currently pursuing postdoctoral studies from July, 2016 in Department of Pharmacology, Center for lung and Vascular Pharmacology, University of Illinois, Chicago. He has also published his work in different scientific journals and few more research manuscripts are under communication. He has been considered for New Investigator Paper award in 2015. He has recipient of many awards like DRDO Laboratory Technology Group Award-2013, Best Paper award, Travel grant award. He has also qualified Radiation Safety, Level I (RSO Level) exam in 2013, conducted jointly by Bhabha Atomic Research Centre (BARC) and Atomic Energy Regulatory Board (AERB), INDIA. He has also active member of Indian Society for Radiation Biology, SIT Member of Radiation Research society and Indian Science Congress.

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