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Thesis Title –

'Studies on the effect of a herbal preparation on pro-inflammatory and hemopoeitic cytokine expression in spleens of gamma-irradiated mice'.

Abstract

Radiation-induced damage to biological system depends on cell type - proliferating cells like bone marrow being more radio-sensitive than slowly dividing cells like neurons. Radio-sensitivity of mature lymphocytes makes the immune system extremely vulnerable. Cytokines expression is also altered after radiation exposure leading to altered homoestasis and damage.

Herbal radioprotection has emerged as a promising alternative to chemical radioprotection, owing to less toxicity of herbs. In the current study the effect of an aqua-alcoholic extract of *Tinospora cordifolia* (RTc) on cytokine expression in mouse spleens and peritoneal macrophage models was analysed. Phyto-chemical analysis of RTc revealed its anti-oxidant potential *in vitro*. At the *in vivo* level RTc inhibited fall in radiation-induced hemoglobin and total leukocyte counts and conversely decreased lipid peroxidation (LPO) whereas expression of anti-oxidant enzymes like SOD, catalase and GSH was increased in the sera of pre-treated mice. RTc also protected bone-marrow progenitor cells as revealed by CFU assay. It also countered radiation-induced effects like decreased splenocyte survival, increased DNA ploidy, phosphatidyl serine externalization and DNA fragmentation. The extract also exhibited mitogenic properties in splenocytes, which was higher than concanavalin A and lower than lipopolysaccharide. RTc

also inhibited the fall in expression of IL-1 beta and TNF-alpha at early post-irradiation intervals and conversely decreased their expression beyond day 5. It also significantly increased GM-CSF levels in serum and spleen.

In macrophages, RTc decreased PMA-induced superoxide and hydrogen peroxide release. It also inhibited LPS-induced death of irradiated macrophages (ex vivo) by decreasing DNA fragmentation, NO and TNF-alpha production.

RTc, therefore, protected mice from radiation damage by various mechanisms. It inhibited radiation-induced damage in two ways, viz., a) by itself functioning as a strong anti-oxidant and, b) by increasing endogenous anti-oxidant enzyme levels. It protected progenitor cells from radiation damage by increasing circulatory and spleen GM-CSF levels. This condition increased blood levels of leukocytes and splenocyte counts. RTc also protected mature splenocytes by decreasing apoptotic death. It showed the ability to activate macrophages as revealed by MTT analysis. It also increased adherence, spreading and phagocytosis in these cells and decreased radiation-induced respiratory burst response. *In vivo* analysis of cytokine expression in spleens of mice showed that both IL-1 beta and TNF-alpha were expressed in significantly large amounts after day 5 in irradiated animals, correlating with the onset of radiation-induced immunosupression that ultimately led to the death of the exposed animals. Irradiation alongwith bacterial infection (LPS) leads to increased production of TNF-alpha and nitric oxide that causes macrophage death implicating that rise in infection further causes immunosuppression in exposed animals. In an *ex vivo* study on irradiated and LPS treated macrophages pre-irradiation administration of RTc inhibited the elevated levels of DNA fragmentation, nitric oxide release and TNF-alpha expression by peritoneal macrophages. Thus, RTc also had the ability to protect the irradiated animals from invading pathogens.

Owing to its ability to affect multiple systems this extract may be exploited for clinical applications for the development of a safe radioprotector.