ABSTRACT

Human beings get exposed to ionizing radiation during planned radiation exposures such as radiotherapy, diagnostic scanning, as well as unplanned radiation exposures such as accidents in nuclear industry, nuclear terrorism and natural background radiation. Radiation exposure generates free radicals within cells, resulting in nonselective alterations in various cell targets and macromolecules, leading to acute and chronic deleterious biological effects. Thus, protection of humans and animals from the influence of ionizing radiation is a major challenge in radiation biology. Radioprotector is an agent or a chemical that reduces the damaging effects of radiation, when administered to living organisms.

Drugs, both synthetic and natural in origin have demonstrated radioprotective capabilities in last few decades, but their application was limited due to their toxicity at optimum protective doses. An ideal radioprotective agent should fulfill several criteria. It should be chemically stable, devoid of any toxicity or adverse effects and must confer a general protective effect on majority of organs and tissues. Likewise it should be readily available, affordable and suitable for oral administration, with the ability to get readily absorbed and distributed in mammalian systems. Unfortunately, to date, there is no radioprotector that fulfills all of these criteria, necessitating an urgency in identifying novel, nontoxic, effective, and convenient compounds to protect humans from the damaging effects of ionizing radiation.

Protective abilities of Lipoic acid (LA), Silver, Zinc, Tempol (TPL) and POLY-MVA against different oxidative stress conditions have been well substantiated. The present study focuses on evaluating the radioprotecting potential of– (i) nanoparticle complexes of Lipoic acid viz. Silver nanoparticle complex of LA (SNLA) and Zinc oxide nanoparticle complex of LA (ZNLA), (ii) Tempol and (iii) POLY-MVA. Their usefulness as adjuvants in cancer therapy is also explored.

Silver nanoparticles (SN) or Zinc oxide nanoparticles (ZN) were dispersed and ultrasonicated with Lipoic acid (LA) to obtain nanoparticle complexes of LA viz. SNLA and ZNLA, respectively. The complexes were characterized by Scanning
Electron Microscopic (SEM) analysis. SNLA and ZNLA treatment caused an inhibition in DPPH free radicals indicating their antioxidant potential.

The sub-plantar injection of carrageenan/ dextran/ formalin in mice produced local inflammatory responses. Administration of SNLA, ZNLA or TPL resulted in significant anti-inflammatory effects in both acute and chronic paw edema models.

Administration of SNLA, ZNLA, TPL or POLY-MVA helped to prevent radiation induced membrane lipid peroxidation and lowering of endogenous antioxidants in different tissues of mice. They also had a protective effect on hematopoietic system against deleterious effects of ionizing radiation. Irradiated mice exhibited gastrointestinal damages and administration of mice with the test agents prior to irradiation protected the intestinal epithelial cells from radiation-induced structural alterations.

Radiation exposure to mice resulted in significant depletion of different hematological parameters such as RBC count, platelet count, hematocrit % and hemoglobin concentration which were reversed upon treatment with SNLA, ZNLA or TPL as compared to irradiated control group.

Formation of endogenous spleen colonies is an index of hematopoietic stem cell proliferation. Administration of SNLA, ZNLA, TPL or POLY-MVA significantly enhanced the spleen colony formation and prevented radiation induced loss of spleen weight in animals exposed to a sub- lethal dose of whole body gamma radiation.

Upon exposure to a lethal dose of whole body gamma radiation, mortality rate was higher and survivors exhibited a profound loss in the body weight. Administration of SNLA, ZNLA, TPL or POLY-MVA delayed the onset of radiation induced loss of survival and body weight alterations to a greater extent than radiation exposed control groups.

SNLA, ZNLA, TPL and POLY-MVA were found to have protective effects against radiation-induced genotoxicity. Exposure of plasmid to gamma radiation results in production of strand breaks which, in turn, will result in relaxation of plasmid DNA from covalently closed circular (ccc) form to open circular (oc) form or linear form
in a dose dependent manner. Presence of TPL or POLY-MVA inhibited this conversion. Comet assay was employed to assess the effect of SNLA, ZNLA, TPL and POLY-MVA on gamma radiation induced cellular DNA strand breaks under *ex vivo* and *in vivo* conditions. Different comet parameters *viz.* Tail DNA %, Tail length, Tail moment and Olive tail moment were observed to be elevated upon radiation exposure indicating DNA damage and pre-treatment with these test agents aided in reducing the radiation induced cellular DNA damage to various extents. The test agents were also found to be beneficial in reducing the radiation induced micronuclei formation (*in vivo*) and chromosomal aberrations (*ex vivo* as well as *in vivo*). Effect of these agents on cellular DNA repair was determined by monitoring the comet parameters at different post-irradiation time intervals. Post-irradiation treatment with these test agents, resulted in decrease of various comet parameters (Tail DNA %, Tail length, Tail moment and Olive tail moment) at a faster rate under both *ex vivo* as well as *in vivo* conditions. ‘Cellular DNA Repair Index’ (CRI), a relation based on these comet parameters implied that the test agents significantly increased cellular DNA repair efficiency.

Chemotherapy and radiation therapy are the most common routes for treatment of cancer. In order to obtain a better tumor control with a higher dose, the normal tissues should be protected against radiation or chemotherapy induced injuries. SNLA, ZNLA and TPL were analyzed for their adjuvant role in tumor therapy. DLA solid tumor was found substantially suppressed in the group of animals administered with either of these test agents and then exposed to gamma radiation showing their synergistic effect on antitumor efficacy of radiation. Co-treatment of these test agents and with either of the chemotherapeutic drugs (DOX or CDDP) protected tumor bearing mice from chemotherapy induced bone marrow suppression and micronuclei formation. Levels of major endogenous antioxidants were significantly decreased in the heart tissues of mice injected with DOX. Similar scenario was seen in renal tissues of CDDP treated groups. Administration of these agents under study protected the endogenous antioxidants from chemotherapy induced oxidative damages as evidenced from their increased levels in the respective tissues analyzed. The concentration of MDA was elevated in different tissues of
mice upon DOX or CDDP treatment indicating the formation of lipid peroxides. Administration of agents under study inhibited the peroxidation of membrane lipids in heart and kidney tissues of DOX and CDDP administered groups respectively. Meanwhile, both the chemotherapeutic drugs resulted in an increase in lipid peroxidation levels and decrease in different endogenous antioxidant levels in tumor tissues of mice. Administration of the agents under study did not interfere with the antitumor efficacy of these drugs. Single i.p injection of DOX significantly elevated serum marker levels (LDH, CK-MB, SGOT and SGPT) indicative of cardiotoxicity. Treatment with either of the test agents ameliorated these enzyme activities to different extents. CDDP administration to mice induced marked renal failure, characterized by significant increase in serum urea and creatinine levels indicative of nephrotoxicity and administration of mice with the test agents maintained these parameters to near normal levels. Histopathological analysis of heart tissues of DOX treated and renal tissues of CDDP treated mice exhibited extensive structural alterations and administration of animals with the agents under study helped to decrease the extent of tissue degeneration.

The results thereby demonstrate the applicability of SNLA, ZNLA, TPL and POLY-MVA as radioprotectors in various planned or un-planned radiation exposure scenarios. Moreover, SNLA, ZNLA and TPL effectively ameliorated the side effects of radiotherapy as well as of commonly used cancer chemotherapeutics (DOX and CDDP) in tumor bearing animals. Differential protection to normal cells as compared to tumor cells implies their use as adjuvants in radiotherapy or chemotherapy for cancer.

**Key Words:** Lipoic acid, Silver, Zinc, Nanoparticle, Tempol, POLY-MVA, Radiation, Radioprotection, DNA damage, Comet assay, Antioxidant, Anti-inflammation, Hematopoietic system, Gastrointestinal mucosa, DNA repair, Micronuclei, Chromosomal aberrations, Tumor, Radiotherapy, Chemotherapy, Nephrotoxicity, Cardiotoxicity.