CHAPTER -7-

SUMMARY AND CONCLUSION
Ionizing radiation inflicts a spectrum of deleterious effects to living cells through the generation of reactive oxygen species that react with almost all the biological cellular components to induce oxidative damage. ROS attack lipids, proteins and DNA, induce oxidation and membrane damage, enzyme inactivation and DNA damage and finally can induce cell damage, dysfunction and death. Therefore protection of biological systems from ionizing radiation is of paramount importance during accidental and unavoidable exposures to radiation, and development of novel and effective approaches to combat radiation damages using non-toxic radioprotectors are of considerable interest for defense, nuclear industries, radiation accidents, space travels, etc, besides the protection of normal tissues during radiotherapy of tumours.

The results from present study with phyto phenolic compounds SM, GA and their silver nanoparticle complexes SNSM, SNGA showed their potential free radical scavenging activities indicating their antioxidant capacity. The results of the toxicity studies showed that, neither the compounds, nanoparticles nor their complexes caused significant changes in the parameters studied, confirming their non-toxic nature to proceed with in vivo studies. The sub plantar injection of carrageenan/ dextran/ formalin in mice produced local inflammatory responses. Administration of the study compounds and complexes resulted in significant anti-inflammatory effects in both acute and chronic models.

Free radicals generated by radiation, attack the fatty acid component of membrane lipids and produce lipid peroxidation products such as peroxyl radicals and malonaldehyde, which have effects at other targets away from the site of generation, cause interphase cell death. Irradiation of liver homogenate led to peroxidation of membrane lipids and was found to increase with increasing doses of radiation. The results showed that the presence of phenolics or their silver nanoparticle complexes during irradiation protected the cellular membranes from radiation-induced lipid peroxidation. The administration of SM, SNSM, GA or SNGA helped to prevent radiation-induced membrane lipid peroxidation and lowering of endogenous antioxidants in different tissues of whole body gamma irradiated mice.

Mortality of animals following radiation results from several factors like damages to the hematopoietic system and gastrointestinal system which may ultimately lead to immune suppression. The administration of SM, SNSM, GA or SNGA had a protective effect on hematopoietic system against deleterious effects of ionizing radiation as evident from data on bone marrow cellularity and blood GSH content. A close microscopic examination of the stained sections of the intestine of radiation exposed animals revealed the altered structures of mucosa.
and sub-mucosa layers. The irradiated mice exhibited the gastrointestinal damage with crypt epithelial cell necrosis, blunting of the villi and diffused lymphatic and plasmacellular infiltration. Administration of mice with study compounds and their silver nanoparticle complexes prior to irradiation protected the intestinal tissues from radiation-induced damages.

Radiation exposure resulted in significant depletion of different hematological parameters in mice. The administration of SM, SNSM, GA or SNGA significantly increased the total erythrocyte and leukocyte counts, and hemoglobin levels when compared to irradiated control animals. Formation of endogenous spleen colonies is an index of hematopoietic stem cell proliferation. Administration of SM, SNSM, GA or SNGA 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. Survival studies conducted suggest that the oral administration of phenolics and their silver nanoparticle complexes bestowed survival advantage to the animals following exposure to the lethal dose of gamma radiation and also delayed the onset of radiation induced body weight alterations.

SM, SNSM, GA or SNGA was found to have protective effects against radiation-induced genotoxicity. Exposure of plasmid to gamma radiation results in the 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 SM, SNSM, GA or SNGA inhibited the radiation induced conversion of ccc form to oc form. Gamma irradiation-induced strand breaks in cellular DNA \textit{ex vivo} and \textit{in vivo} was assessed by Comet assay. Different comet parameters \textit{viz}. Tail DNA \%, Tail length, Tail moment and Olive tail moment were observed to be elevated upon radiation exposure. SM, SNSM, GA or SNGA treatments aided in reducing this radiation induced cellular damage to various extents.

Since the studies suggested the prophylactic radioprotecting potential of SM, SNSM, GA and SNGA, their therapeutic radioprotective activity was ascertained by post-irradiation administration of these agents in mice and analyzing radiation induced DNA damage and repair in terms of strand breaks and micronucleus assay.

The post-irradiation treatment with these compounds or their silver nanoparticle complexes, resulted in decrease of comet parameters at a faster rate compared to radiation control group. This would suggest the potential of SM, GA and their silver nanoparticle complexes SNSM and SNGA on enhancement in repair rate of radiation-damaged cellular DNA. To quantify the efficiency of the cells to repair and rejoin strand breaks in DNA, a relation based on the comet
parameters of the cellular DNA named ‘Cellular DNA Repair Index’ or CRI was arrived at. From the CRI data, it was inferred that SM, SNSM, GA or SNGA significantly increased cellular DNA repair efficiency. A similar trend in enhancement of DNA repair was also observed under \textit{in vivo} conditions. Post-irradiation administration of these compounds and complexes resulted in faster repair inferred from decrease of various comet parameters when compared to radiation exposed control group.

SM, SNSM, GA or SNGA administration significantly decreased the radiation induced micronuclei frequency in peripheral blood lymphocytes of mice at both time points in comparison with the respective irradiated control groups. Radiation exposure also produced a significant increase in the percent aberrant cells under \textit{ex vivo} and \textit{in vivo} conditions. Pre-treatment with SM, SNSM, GA or SNGA resulted in significant decrease in the percent aberrant cells. There was a decrease in all types of aberrations, as well as polyploidy and cells with pulverization when compared to the irradiated control groups.

Treatment of EAC ascites tumour bearing mice with SM, SNSM, GA or SNGA helped to inhibit the ascites growth and also increased the survival period than the control tumour animals. Likewise, DLA solid tumour was found substantially suppressed in the group of animals administered with SM, SNSM, GA or SNGA and exposed to gamma radiation showing their synergistic effect on anti-tumour efficacy of radiation. The \textit{in vitro} and \textit{in vivo} studies also showed that these phenolic compounds and their silver nanoparticle complexes significantly induce apoptosis in these cancer cells.

The combined therapy with SM, SNSM, GA or SNGA and chemotherapeutic agents (DOX or CDDP) significantly protected chemotherapeutics-induced bone marrow suppression and micronuclei formation. Activities of major endogenous antioxidant defence systems 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 SM, SNSM, GA or SNGA could protect these endogenous antioxidant defense systems from DOX or CDDP induced oxidative stress which was evidenced from their increased levels in the respective tissues analyzed. The concentration of MDA was found to be elevated in mice upon DOX or CDDP treatment indicating the formation of lipid peroxides in different tissues. The co-treatment of SM, SNSM, GA or SNGA with DOX or CDDP inhibited the peroxidation of membrane lipids in heart and kidney tissues of DOX and CDDP treated tumour animals respectively.
Meanwhile the administration of the phenolics and their silver nanoparticle complexes under study did not interfere with the anti-tumour efficacy of these chemotherapeutic agents. It was seen that a single i.p dose of DOX significantly elevated serum LDH, CK-MB, SGOT and SGPT levels indicate cardiotoxicity. The co-treatment with SM, SNSM, GA or SNGA ameliorated the toxic effects and enzyme activities to near normal. CDDP administration to mice induced marked renal failure, characterized by significant increase in serum urea and creatinine levels. Administration of mice with SM, SNSM, GA or SNGA restored the urea and creatinine levels to their normal. Histopathological analysis of heart tissues of DOX treated mice, showed myocardial degeneration. In mice pre-treated with SM, SNSM, GA or SNGA and then given DOXO, there were marked reductions in the severity of myocardial degeneration. Likewise, CDDP treatment resulted in decreased cellularity of the glomeruli, oedema of the lining of epithelial cells in the renal tubules and interstitial tissue also showed oedema as can be evident from histopathological sections analyzed. The renal tissues of CDDP treated mice administered with SM, SNSM, GA or SNGA showed almost normal glomerular, renal tubules and interstitial tissue structure.

Hence from all these data we could substantiate that the compounds and complexes demonstrated excellent free radical scavenging and anti-inflammatory activities as well as protected mice from radiation-induced endogenous antioxidant depletion, hematopoietic and gastrointestinal system dysfunctions, and genotoxicity. The results thereby open the door for their applicability in various planned or un-planned radiation exposure scenarios. Moreover, the phenolics and the complexes effectively ameliorated the side effects of radiotherapy as well as of commonly used cancer chemotherapeutics (DOX and CDDP) in tumour bearing animals, and their differential protection to normal cells as compared to tumour cells implies their use as adjuvants in radiotherapy or chemotherapy for cancer. Even though the potential usefulness of phytophenols and their silver nanoparticle complexes in radiation exposure or oxidative stress scenarios and their adjuvant action during radio and chemotherapy is evident from the present study, further detailed investigations are needed to be done for understanding their actual molecular mechanism of action and their possibility in clinical applications.

Application of nanoparticles and a hypoxic cell sensitizer for targeting and enhancing the efficacy of chemotherapeutics to tumour cells for effective tumour therapy was explored by complexing the anthracycline anticancer drug, DOX with silver nanoparticles and AK. The complex, SN-AK-DOX exhibited more toxicity towards DLA cells and induced a higher rate of apoptosis in these cells under in vitro as well as in vivo conditions than the drug or its single
combinations alone treated groups. The complex also showed effective induction of DNA damages in the tumour cells under *in vitro* and *in vivo* conditions, the extent of DNA damages was analysed using comet assay. The oral administration SN-AK-DOX efficiently reduced the tumour growth in DLA solid tumour bearing animals when compared to control untreated tumour animals. So the present work also projects the feasibility of controlling tumour growth effectively by complexing and targeting anticancer drug DOX with silver nanoparticles and hypoxic cell sensitizer AK.

As silver nanoparticles might exhibit toxicity to mammalian system, their clinical acceptance is limited. The present study with silver nanoparticle complexes demonstrated their potential application in tumour therapy, both by use of phytophenolics and targeting antineoplastic drug directed by sanazole.

The preclinical investigations on hydro-alcoholic extract of medicinal mushroom *Ganoderma lucidum* (GLE) as an adjuvant in tumour- radiotherapy, showed that GLE significantly ameliorated the radiation-induced damages to cellular DNA in normal tissues, at the same time showed a sensitizing effect by promoting the DNA damage in tumour cells, indicating preferential protection to normal tissues. The tumour regression study carried out with DLA solid tumour-bearing animals revealed that the oral administration of GLE to the animals significantly inhibited the fast growth of the tumour and this tumour growth delay was more prominent in groups administered with GLE and subjected to 4 Gy gamma-radiation treatment. Thus the present work in addition suggest the possibility of using medicinal mushroom *Ganoderma lucidum* extract (GLE) as adjuvant in cancer radiotherapy to protect normal tissues from radiation damages and also to enhance the anti-tumour activity by sensitizing the tumour to radiation and resulted in effective radiotherapy of cancer.

The medicinal macrofungi *Ganoderma* is used for treatment of several human ailments and also in cosmetics. The present work on the use of GLE as an adjuvant in radiotherapy of tumour in preclinical situations was significant and this could be translated in to human situations, because of lack of toxicity and neutraceutical human use of *Ganoderma lucidum*. It is therefore worth undertaking clinical trials on the use of GLE as an adjuvant in radiotherapy of human cancers.