SUMMARY AND CONCLUSION

Mankind has always been exposed to ionizing radiation. An exposure to radiations are wide spread and of varied nature. Radiation cause damage and death of the cell. It is a matter of concern that their levels in environment are continuously increased. However, radiations are useful and beneficial. Radiation therapy is one of the most important and popular tool. The use of radioisotopes in research, medicine, agriculture and industry has not only unfolded the hidden facts of science but also significantly contributed to overall progress of man.

An ionizing radiation is detrimental to life, in absence of proper assessment some practices of today involving exposures of man could lead to serious health problems. Indeed, not only from the biological but also the radiotherapy point of view, any study which reveals new facts of radiation effects is of paramount interest. Moreover, to exploit the radiations for peaceful uses, we must ascertain the maximum risk involved so that the risk vs benefit balance can be tilted in favour of the later. Apart from DNA, cellular membranes are critical targets for radiation action. Lipid peroxidation is an important effect of radiation on membranes. It is highly destructive process and induces a plethora of alterations in cellular membranes. Iron plays an important role in lipid peroxidation.

It seems unlikely that free iron exist in biological systems. Organisms take great care in handling of iron, using transport protein such as transferrin and storage protein such as ferritin and minimizing the size of the intracellular iron pool. Though proteins and enzymes are very well designed, none is

perfectly designed i.e. none is able to withstand all of the insult inflected by nature and man. All of the iron used in life sustaining processes is potentially harmful when released from its natural environment. It will be interesting to know whether free iron could be available for the catalysis of deleterious reactions induced by radiation.

In the present work, therefore, an attempt has been made to understand radiation induced lipid peroxidation and to evaluate and assess the potentiality of iron-containing proteins like transferrin, ferritin and lactoferrin to release the iron for catalysis of reactions leading to enhancement of radiation induced damage. In addition, we have studied the effect of divalent cations on radiation induced lipid peroxidation. Efforts have also been made to examine the modifying effect of extract of plants that are consumed frequently by Indian people on large scale and are consisted to be anticarcinogenic, to see whether they have the capacity to scavenge the free radicals.

Radiation induced lipid peroxidation in liposomes was found to increase with radiation dose upto 400 Gy and decrease in dose beyond 400 Gy. Our results have confirmed that lipid peroxidation is not a linear function of radiation dose.

Vitamin E, ferrous ions and molecular oxygen, well known modifiers of radiation effect, could not alter the non-linear pattern of lipid peroxidation. These results are important from biological point of view as lipid peroxidation is used as a measure of membrane damage.

Our results have clearly shown that ionizing radiation induced significant release of iron from transferrin, ferritin and lactoferrin. The release was increased with increase in radiation dose. The chemical effects produced on irradiation of dilute aqueous solutions are a consequence of the production of the free radicals (e^-aq, $H^\cdot$ and $HO^\cdot$) and molecular species like H_2O_2. The $HO^\cdot$ radical and H_2O_2 are oxidizing, and $\bar{e}aq$, $H^\cdot$ and $O_2^{\cdot -}$ are reducing species. Therefore radiation-induced release of iron might have occured by more than one mechanisms. A significant release of iron from proteins due to superoxide $HO^\cdot$ and mixture of $\bar{e}aq$, $H^\cdot$ and $HO^\cdot$ is also indicative of more than one mechanisms. The relative ability of free radicals to release the iron was found to be : $O_2^{\cdot -} < HO^\cdot <$ mixture of $\bar{e}aq$, $H^\cdot$ and $HO^\cdot$ in transferrin and lactoferrin ; and $HO^\cdot < O_2^{\cdot -} <$ mixture of eaq, $H^\cdot$ and $HO^\cdot$ in ferritin.

The radiation induced iron release in anaerobic condition is an important observation from radiation therapy point of view. Hypoxia is known to enhance the mobilization of iron. Low pH promoted the release of iron in all these proteins. In tumour cells pH is suggested to be acidic in nature. Therefore, low pH and high content of iron in the hypoxic region of tumour ; and further release of iron due to radiation might be useful for differential sensitization of cancer cells.

SOD, catalase and peroxidase inhibited iron release in transferrin and they enhanced in lactoferrin. In transferrin catalase inhibited, peroxidase promoted and SOD did not show any effect on the iron release. In presence of EDTA the effect of

iron release was quite low in all three proteins. With EGTA the release was seen to be decreased in transferrin but in lactoferrin it promoted. Ferrozine enhanced the release in ferritin and lactoferrin, but reduced in transferrin.

We have observed that the radiation induced release of iron from proteins was inversely proportional to the dose rate. Similar inverse relationship was also seen in O_2,N_2O and N_2 saturated solutions of proteins. The importance of the inverse dose rate effect as well as its mechanism has now been well understood.

To investigate the ability of iron released from proteins to promote radiation damage, liposomes prepared from L - α - lecithin were irradiated with various doses of radiation in presence of iron-containing proteins. The presence of iron-containing proteins resulted into enhancement of lipid peroxidation. It indicated that iron released from proteins might be responsible for the enhancement of lipid peroxidation.

The mechanism by which iron stimulates lipid peroxidation is not clear. Recently it has been suggested that ratio of Fe^{2+} to Fe^{3+} is a critical determinant of peroxidative activity of iron. The rate of peroxidation was found maximum when the ratio of Fe^{2+} to Fe^{3+} ions is 1:1. Iron ions released as Fe^{2+} from proteins are likely to undergo oxidation by radiolytically formed species like $HO^{\cdot}$ and H_2O_2. This gives the possibility of presence of both Fe^{2+} and Fe^{3+}. Liposomes which irradiated at various doses of radiation in presence of ferritin probably created ratio of Fe^{2+} : Fe^{3+} so as to increase lipid peroxidation linearly with dose

resulting into elimination of the non-linear pattern of lipid peroxidation. But the same might have not occured in the presence of transferrin and lactoferrin. Transferrin and lactoferrin probably could not change the non-linear patteren of lipid peroxidation due to lack of suitable Fe^{2+} : Fe^{3+} ratios.

Addition of SOD, catalase, peroxidase, EDTA, EGTA and ferrozine resulted into no appreciable change in transferrin dependent lipid peroxidation, but they inhibited lactoferrin dependent lipid peroxidation. In case of ferritin dependent lipid peroxidation SOD and catalase have shown stimulatory and peroxidase has shown inhibitory effect. The chelators have enhanced the ferritin dependent lipid peroxidation. These observations have been attributed to their effect on the redox state of iron released from proteins.

The modifying effects of divalent cations ie. Co^{2+}, Cu^{2+}, Mn^{2+} and Ni^{2+} on lipid peroxidation have also been studied in the present work. Cu^{2+} and Co^{2+} inhibited whereas Mn^{2+} and Ni^{2+} enhanced the lipid peroxidation. The pro-and antioxidant effects could be due to altered reactivity of these divalent cations towards molecular oxygen, O_2, H_2O_2 and peroxidases.

Extract of brassica, papaya, bittergourd and mint have inhibited lipid peroxidation significantly. These results are indicative of the presence of antioxidants in the extract which have the ability to scavenge the free radicals.

Results of the present study have clearly shown that radiation-induced lipid peroxidation is not a linear function of radiation dose. The non-linear pattern of lipid peroxidation was

not changed even in the presence of vitamin E, Fe^{2+} ions or molecular oxygen. These results are important from biological point of view as lipid peroxidation is used as a measure of membrane damage. We have also found that ionizing radiation could also induce significant release of iron from transferrin, ferritin and lactoferrin. These proteins were found to stimulate lipid peroxidation. It suggests that iron containing proteins like transferrin, ferritin and lactoferrin may function in vivo as a source of iron and catalyze various deleterious free radicals leading to enhancement of radiation damage. The mobilization of iron due to radiation action may occur by more than one mechanisms as different free radicals and molecular products are formed on irradiation of aqueous systems. Under anaerobic condition the release of iron from proteins was more compared to aerobic condition. Moreover, low pH also promoted the release of iron. Low pH and higher concentrations of iron in tumours suggests the possibility to device therapeutic strategies for the differential killing of cancer cells based on appropriate manipulation of Fe^{2+} to Fe^{3+} ratios.