Chapter 9

Summary and Conclusion
Oxidative stress is an important initiator of cellular injury in many pathophysiological conditions such as manifestations of xenobiotic toxicity, radiation toxicity, reperfusion injury, tumour promotion, aging and cell death. An evolutionary change in the understanding of nutritional value of dietary components is that inhibition of oxidative damage and antioxidant nutrients are centrally important. The epidemiology and the laboratory data are pointing to the idea that the antioxidant defenses that come through a high intake of antioxidant nutrient can influence the oxidative disease risk.

The modern paradigm of nutrition focuses on the ability of diet and its nutrient and non-nutrient components to reduce the rate of loss of physiological functions leading to chronic diseases. The present investigation continues to add support that generous dietary or supplemental intake of certain non-nutrients with antioxidant property may reduce the disease risk associated with oxidative stress.

The antioxidants used in this study were (a) α-Tocopherol, have widest distribution and greatest biological activity of all tocopherols; (b) Curcumin, (diferuloylmethane) the yellow pigment in turmeric used as a spice.
food colouring agent and as a preservative; (c) Ellagic acid, (a polyphenol) phenolic constituent of many plants; (d) Bixin, a carotenoid carboxylic acid present in the seeds of Bixa orellana L; which used as food colouring agent and (e) Probucol (bisphenol), synthetic hypolipidemic drug with antioxidant property.

A comparative study of curcumin, bixin & ellagic acid in vitro, showed that the activities of these compounds are comparable with that of α-tocopherol. All these compounds were equally effective in scavenging hydroxyl radical (OH'), a powerful initiator of lipid peroxidation. The inhibitory effects of ellagic acid, curcumin and bixin on lipid peroxidation and superoxide generation were found to be more than that of α-tocopherol. The antioxidants were administered orally to normal animals to study their effect on in vivo system. It was found that the key enzymes of antioxidant system, GPx and catalase were reduced and SOD remained without change in α-tocopherol treated group. Both SOD and catalase activities decreased and GPx remained without change in ellagic acid treated rats. While SOD decreased, GPx and catalase remained without change in
curcumin treated animals. The cellular levels of antioxidant - GSH and associated enzymes, GR and GST were also found to be unaltered in α-tocopherol, curcumin and ellagic acid treated animals. This showed the maintenance of antioxidant and detoxification mechanisms in the in vivo defense systems by these compounds.

Gamma Radiation, though a therapeutic modality was found to produce lipid peroxidation and tissue toxicities in dose and time dependent manner. The maximum level of lipid peroxidation was produced at 96h after radiation. Radiation dose above 8Gy could produce a significant increase in lipid peroxidation. It was found that the oral administration of antioxidants to animals with whole body radiation can produce effective reduction in tissue lipid peroxidation. A significantly increased liver and kidney GPT levels were also observed in these animals compared to untreated group. This may show that these compounds would be facilitating the repair processes, thereby depleting the cellular levels of excess aminoacids and other compounds produced due to radiation toxicity. An effective protection against radiation induced morphological changes in small intestine was observed in ellagic acid treated group.
γ-Radiation was also found to produce chromosomal damage. The simultaneous treatment of total four doses of the antioxidants - α-tocopherol, curcumin and ellagic acid to irradiated mice could significantly reduce frequency of micro nucleated poly chromatic erythrocytes (MnPCE). The bone marrow cellularity also was increased by α-tocopherol and curcumin treatment. A significant reduction in radiation induced chromosomal aberration in bone marrow cells were observed in antioxidant treated group. The treatment of antioxidants significantly inhibited the radiation induced DNA strand breaks in rat lymphocytes.

Fractionated dose of γ-radiation, administration of bleomycin and paraquat were found to produce lung fibrosis. The antioxidants, α-tocopherol, ellagic acid, curcumin and bixin were administered subsequent to radiation exposure. From the value of lung hydroxy proline and pathological observations it was found that ellagic acid can protect lung tissue against the occurrence of radiation induced fibrosis. In bleomycin administered animals the treatment of α-tocopherol, curcumin and ellagic acid produced significant protection against the induced lung fibrosis. The
effectiveness was in the order of α-tocopherol < ellagic acid < curcumin at the concentrations used. Both curcumin and α-tocopherol were found to inhibit the occurrence of paraquat induced lung fibrosis.

It was observed that the simultaneous treatment of ellagic acid (100 μmole/kg body wt) to rats could also effectively inhibit the development of liver fibrosis with chronic dose of CCl₄. Normal histopathological findings and significant reduction in liver hydroxy proline were seen in ellagic acid treated animals. It has already been reported that curcumin can reduce liver fibrosis induced by chronic dose of CCl₄ (Soni and Kuttan, 1993) and that increased liver content of vitamin E by dietary supplementation can afford a significant degree of protection against CCl₄ induced liver damage and cirrhosis (Maurizio et al.; 1992).

A single dose of cisplatin was sufficient to produce renal toxicity in rats. The compounds α-tocopherol, curcumin, ellagic acid and probucol were administered orally along with cisplatin and subsequently for 14 days. Blood parameters, biochemical evaluation of kidney and liver levels of GSH and associated enzymes and histopathological
observation of lung tissue were done. Probucol and ellagic acid were found to be effective in reducing the nephrotoxicity due to cisplatin. Probucol was found to be most effective of all. Whereas α-tocopherol at the used concentration (200 μmole/Kg body wt) along with cisplatin may increase the renal toxicity.

In conclusion, antioxidants of naturally origin such as α-tocopherol, curcumin, ellagic acid and bixin including a synthetic antioxidant probucol could prevent various toxicities generated by reactive oxygen species (ROS). ROS has been implicated in the pathogenesis of a wide variety of processes that affect our state of health and longevity. Thus encouraging consumption of foods rich in these specific micronutrients or through supplementation of these compounds may alleviate the gross emergence of many disease processes and dose limiting toxicities of certain therapeutic modalities. Further studies are needed to find out the exact mechanism of action of these compounds.