Chapter - 1

Introduction and Review of Literature
1.1 Introduction

Radiotherapy and surgery are the two most successful forms of treatment of cancer. If the tumour is accessible and no vital structures are involved, surgery is the treatment of choice. Both surgery and radiotherapy are treatment of localized disease. Systemic chemotherapy is the main method of treatment for widely disseminated cancer but wholebody or regional irradiation is sometimes used prophylactically. In the therapy of tumours with radiation and other cytotoxic agents, a primary objective is the differential killing of tumour cells relative to normal cells. All localized tumours are potentially curable with radiation, but the dose necessary to achieve that cure may leave such a high level of normal tissue damage. It can be fatal or it makes the quality of life unacceptable. The lethal effects of ionizing radiation on bacterial and mammalian cells have been shown to be the consequence of DNA strand breakage and other damage, caused on chromosome (Kaplan, 1968 a). Although enzymatic repair of certain types of strand breakage has been documented, breaks induced in both strands of the DNA macromolecule appear to be non repairable (Kaplan, 1966).

DNA lesion production and radiation induced cellular lethality are the results of both direct and indirect effects of radiation. Indirect effects are the results of the reactions of free radicals, created by energy absorption primarily in the intracellular water, with critical target molecules. The damaging free radicals can be either the primary water radicals or secondary radicals formed by the reaction of the primary radicals with non-critical cellular components. The primary water radicals are the hydroxyl radical, OH; the hydrated electron, e⁻aq; and the hydrogen atom, H. The hydroxyl radical has been found to damage a wide variety of biological systems. DNA (Achey and Duryea, 1974), enzymes (Adams et al., 1972), bacteriophage (Powers and Gample – Jobbagy, 1972), bacterial spores (Power, 1972), bacterial (Johansen and Howard – Flanders, 1965) and mammalian cells (Chapman et al., 1973). Roots and Okada (1972) have shown OH radicals to play a major role in radiation induced single strand break production in mammalian cells. Bonura and Smith (1976) have shown that a
significant number of DNA double strand breaks in irradiated E.Coli can be suppressed if cells are in the presence of glycerol; these data probably indicate that OH radicals normally contribute to double strand breakage. There is evidence that secondary radicals can also interact with DNA in a lethal fashion in bacterio phage (Dejong et al, 1972)

The effect of radiation on living cells is usually enhanced by the presence of oxygen. The radiation dose required to produce a given effect is about three times greater for hypoxic than for oxic or aerbic cells. This enhancing factor is called the "oxygen enhancement ratio" (OER), is usually independent of level of survival for a given type of radiation. Total body irradiation is now being used together with intensive chemotherapy in the treatment of certain leukemic patients. Because of the success of this treatment in controlling the leukemia proliferation, these patients are surviving long enough to develop late normal tissue injury, particularly to the CNS. Van der kogel et al, 1976 have recently evaluated the risk of severe CNS damage in prophylactic irradiation of the brain is often combined with intrathecal methotrexate.

Ionizing radiation has been called a "Universal carcinogen" in that it will induce cancer in most species at all ages, including the fetus. It is, however, a relatively weak carcinogen and mutagen when compared to certain chemical agents. The cancers induced by radiation are of the same histologic types as occur naturally but the distribution of types may differ. There is a distinct latent period between exposure to radiation and the clinical appearance of a tumour. The earliest step in the overall process of carcinogenesis is the transformation of one or more normal cells in a tissue (in vivo) (Little, 1989). Transformation induced by radiation or chemical agents indicate that it is a progressive, multistep process by which normal cells acquire the various phenotypic characteristics of cancer cells. There are three major independent stages in the malignant transformation of cells in vitro. The development of morphologic changes, cellular mortality and tumorigenicity (Cox and Little, 1992). Morphologic changes are many and varied and include the development of abnormalities in cytology, growth pattern, and the control of cell proliferation.
Three major advances in cancer chemotherapy are (1) the development of new drugs, (2) the concept of combined chemotherapy, and (3) the use of drug therapy in conjunction with radiation and surgery for early cancer patients with a poor prognosis. At the present time, there are about eighty active anti-tumour drugs available, and the rate of new drug development is increasing steadily. Thus chemotherapy is becoming common place and standard practice (Susan Golder, 1976).

Most of the drugs used in the chemotherapy of malignancy have been shown to affect the immunologic system. The mechanisms of action are different for different classes of drugs, but the end result is the same — interference with the proliferative capacity of the cells involved in the immune response. The immunosuppressive effect of cytotoxic agents have been known for over half a century, since Hektoen and Coper (1921) demonstrated that mustard gas impaired the ability of rabbits to form antibodies to sheep erythrocytes. In addition to immuno suppressive potential, several anti cancer drugs may be directly oncogenic. In animals, all the drugs act by alklylation or binding to DNA have been shown to be carcinogenic, in addition to this antimetabolities such as methotrexate may act as “cocarcinogens” in animal system by enhancing the carcinogenic effect of certain chemicals (Harris 1976). Cyclophosphamide (CTX) an alkylating agent used extensively for both malignant and non-malignant conditions, has also been associated with malignancies of the bladder (Worth 1971; Wall and Clausen, 1975). In breast cancer, women with four or more positive axillary nodes are being treated with either L-phenylalanine mustard (Fisher et al., 1973) or a regimen containing cyclophosphamide (Bonadonna, 1976) — both alkylating agents with known carcinogenic potential inhibits DNA synthesis, arrests cells in M-phase and inhibits the transition into S-phase (Lemplain et al, 1971).

Under normal conditions the antioxidant defence system of the body protects against the metabolic free radicals and oxidative stress. (Wilson, 1983., Chance et al, 1979). The natural antioxidant system of the body consisting of glutathione (GSH) and the related enzymes as well as superoxide dismutase are believed to be the major cellular constituents involved in the defense against
oxidative stress. Almost every organ or system in the body is affected by oxidative stress. Oxidative stress is a disturbance in the pro-oxidant to antioxidant balance and produce reactive oxygen species (ROS). ROS damage the molecules such as DNA, proteins, carbohydrates and lipids and affect the enzyme processes and genetic machinery. Antiagents such as radioprotectors and anticarcinogens can in principle interfere with particular free radical steps and either prevent or minimize the biological consequences. They are also found to protect from radiation induced mutagenesis (Wattenberg, 1978).

Traditional use of herbal medicine is usually an integral part of culture, which was developed within an ethnic group before the development and spread of modern science. The term "Herbal medicine" means that a plant derived material or preparation with therapeutic or other human health benefits from one or more plants. These potential herbal medicine that are capable of modifying immune responses with comparatively low side effects (Srima, 1997). Several natural and synthetic compounds were developed during the past several years, clinical values of most of the immunotherapeutic regimens tested so far as not yet been proved unequivocally (Mihich, 1982). There are two important hindrances in standardizing and assuring quality of herbal formulations:

1. There are ecotype and genotype variations of medicinal plants with regard to their chemical compositions, efficiency and toxicity. This is an area where urgent scientific studies remain to be done. The pharmacological and phytochemical variations with regard to ecotype variations of medicinal plants are not systematically studied.

2. In most of the herbal formulations the active principles or the compounds producing synergistic effects are not known or clear. Therefore meaningful chemical standardization becomes extremely difficult. Pharmacokinetic studies cannot be done in a scientific manner. However pharmacological and toxicological studies can be used along with chemical profiles for determining the quality of herbal drugs (Kamala Purkar et al, 2000).
Recently there is an increasing interest in the search of potential drugs that are capable of modifying the immune responses with less side effect. Herbal with "Rasayanas" property are prescribed for this purpose. According to Ayurvedic texts, the Rasayana therapy arrests ageing, increases intelligences, vigour, enables to prevent diseases and nourishes blood, lymph, bone marrow, bone flesh, adipose tissue, semen etc. (Singh, 1990). However there are only scanty scientific evidences on the activity of Rasayanas drugs.

The present study is devoted to assess the usefulness of Brahma Rasayana (BR) to ameliorate the radiation and chemotherapy. BR is a non-toxic polyherbal drug preparation (approximately contain 35 plant extracts) which are used to improve the general health in normal and sick people by stimulation of the immune system. (Singh, 1990). Most of the plant extracts in BR may have immunopotentiating activity. One of the main ingredient in BR is Emblica officinalis (20%) has high antioxidant potency (Jose and Kuttan, 1995), anticarcinogenic and antimutagenic (Jose et al, 1997) and also immunomodulatory activity. Extracts of Terminalia chebula (6.67%), Boerrhavia diffusa (0.4%), Asparagus racemosus (0.4%), Curcuma longa (0.16%), Glycyrrhiza glabra (0.16%) etc. are present in BR preparation. Many of these plants have been found to immunostimulating activity as well as shown to the anticarcinogenic. Cost of production of BR is very low, locally available, lack of adverse effects with high nutritive value are very effective to reduce myelosuppression when given orally to cancer patients receiving chemotherapy and radiation therapy (Joseph et al, 1999)

The present investigation highlights the role of BR as an antioxidant, enhancer of endogenous antioxidant defence mechanism systems in mice during radiation and chemotherapy treatment, amelioration of radiation induced damage on immune system and as anticlastogenic activity during radiation treatment in experimental animals. Based on these aspects the thesis has been divided into following chapters.
Chapter I - Introduction and Review of Literature.
Chapter II - Antioxidant Activity of Brahma Rasayana, In Vitro and In Vivo
Chapter III - Role of Brahma Rasayana on Antioxidant Systems in Normal Mice and Mice Treated with Radiation.
Chapter IV - Effect of Brahma Rasayana in Amelioration of Radiation Induced Damage in Mice.
Chapter V - Effect of Brahma Rasayana on Antioxidant Systems and Cytokine Level in Mice Treated with Cyclophosphamide.
Chapter VI - Role of Brahma Rasayana in Immune Responses of normal and Irradiated Mice.
Chapter VII - Anticlastogenic Activity of Brahma Rasayana.
Chapter VIII - Summary and Conclusion
Appendix
Bibliography
List of Papers Published
1.2 Review of Literature

Cancer is defined as the disorder in which some cell type in the organism begins to grow in an apparently unchecked fashion. It is a multistep process; during this proliferation of cells irrespective of the tissue of its origin tends to lose or change some of its normal genetic characteristics lead to formation of neoplasia. The cause of cancer are numerous. Substances can initiate neoplasia are called carcinogens. Substances that enhance the abnormalities in the gene expression are called promoters. The stable and heritable genetic alternations are carried in the form of chromosomal translocations, deletions, amplifications, point mutations or certain oncongenic viral infections and integration of its DNA into the genome.

1.2.1 Causes of cancer

This section is intended to provide a brief overview of cancer risk factors with special emphasis on ionizing radiation based mainly on evidence from analytical epidemiology, including recent observations relevant to the practicing oncologist.

a. Tobacco.

Tobacco smoking is the most important carcinogenic hazards identified in Western countries as well as in developing countries. Smoking has been firmly linked to cancers not only of the lung but also of the larynx, mouth, pharynx, esophagus, bladder and pancreas (Brinton et al, 1986). Oral cancer is common in people who use tobacco quids often mixed with betel, lime and other agents (International agency for research on cancer, 1985).

b. Alcohol

Consumption of alcoholic beverages has been shown to potentiate the effects of tobacco smoking on cancers of the mouth, pharynx, esophagus, and larynx and has been estimated to account for about 3% of all cancer deaths (Tuynx J, 1982; Rothman, 1980). Combined exposures were found to account for about three fourths of all oral and pharyngeal cancers. The risks were not
uniform for all forms of alcohol, being higher with hard liquor or beer than with wine. Alcohol is an important cause of hepatic cirrhosis, which is sometimes complicated by hepatocellular carcinoma.

**c. Occupational hazards**

Occupational exposures may account for about 5% of all cancer deaths, while the proportion is higher in certain areas for particular cancers, such as those of bladder and lung. Most carcinogenic exposures in the workplace were first detected by clinicians, while others were noted initially by epidemiologists as in the case of asbestos (lung cancer), inorganic arsenic (lung cancer) and the leather industry (nasal cancer) or by experimentalists as in the case of 4-amino biphenyl (Doll, 1975). Many of the occupational cancers are characterized by high relative risks and specificity of cell type.

**d. Environmental pollution**

Pollutants in the urban air have long been suspected in the etiology of lung cancer. Polycyclic aromatic hydrocarbons are one of the major ingredient of fossil fuel combustion product. Nevertheless, there is suggestive evidence that atmospheric pollution plays a limited role in the causation of lung cancer (Doll and Peto, 1981). There are many case reports suggesting that mesotheliomas may result in neighborhood exposed to asbestos industries (Tagnon et al., 1980). Naturally occurring zeolite fiber is one of the environmental carcinogen cause of pleural mesothelioma (Artvinll and Baris, 1979) and another one is air-borne arsenic (lung cancer) (Brown et al, 1984). Prolonged use of chlorinated surface water increases the risk of bladder cancer (Cantor et al, 1987).

**e. Ultra violet (UV) radiation**

Ultra violet (UV) radiation from sunlight is the major risk factor for skin cancer, both squamous and basal cell carcinomas and melanoma (Scotto et al, 1982). The evidence includes the tendency of tumours to arise on sun-exposed sites, the high incidence is associated with outdoor activities and the predisposition of fair-complexioned people who sunburn easily. Exceptionally high risks of skin cancer occur among persons with genetic diseases exposed by sunlight (xeroderma pigmentosum and albinism). In experimental animals
repeated doses of UV radiation (UV-B spectral range 290-320 nm), can induce skin cancer, in addition to about one half of the melanomas appear to arise from dysplastic nevi (Greene et al, 1985). The steady rise in the incidence and mortality rates for melanoma may be related to short term intense sun exposures that have accompanied changes in leisure–time activities and clothing habits. There is no evidence so far that ground-level measures of UV-B have increased (Scotto et al, 1988). But recent reports of stratospheric ozone depletion have promoted concerns about future trends in skin cancer that would presumably result from increases of UV-B reaching the earth surface. International efforts are under way to lower the production of chlorofluorocarbons (used in aerosol propellants, air conditioners etc.) that may reduce the protective ozone layer.

f. Medications

From studies of patients exposed to medicinal agents that may account for as much as 2% of all cancers. Synthetic estrogens given during pregnancy-produced adenocarcinomas of the vagina and cervix. This was the first demonstration of Trans- placental carcinogenesis in humans. Endometrial cancer can result from conjugated estrogens taken for menopausal symptoms and excess of breast cancer in long term users (Brinton et al, 1986). An excess risk of acute non-lymphocytic leukemia has been noted among patients receiving alkylating agents, especially melphalan, cyclophosphamide and chloramucil (Greene et al, 1986). Semustine (Methyl-CCNU) was evaluated as adjuvant therapy for gastrointestinal cancer, the risks of leukemia and preleikemia were found to be elevated with dose - response relationship (Boice et al, 1983; Boice et al, 1986). Immunosuppressive agents, particulary azathioprine, have been assessed mainly by studies of renal transplant recipients. The risk of non-Hodgkin’s lymphoma is very high within a few months of transplantation and remain at about the same level (Hoover and Fraumeni 1973; Kinlen 1982). A predominance of lymphomas has been seen also with primary immunodeficiency disorders such as ataxia-telangiectasia, Wiskott-Aldrich syndrome, and the X-linked lymphoproliferative syndrome (Filopovich et al, 1980). For lymphomas in the latter group as well as in the transplant patients, there is evidence of causation by the Epstein-Barr Virus
EBV) (List et al., 1987). This finding is consistent with animal experiments, indicating that immunosurveillance primarily operate against viral-induced neoplasms.

g. Viruses

Epstein-Barr virus (EBV) is widely considered to be the cause of endemic Burkitt's lymphoma and nasopharyngeal cancer (Levine et al., 1987). Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infection are important causes of hepatocellular carcinoma, the oncogenic effects of hepatitis B are enhanced by early-life infection and dietary exposures to aflatoxin. Another human retrovirus, called the human immunodeficiency virus (HIV) has been shown to cause the acquired immunodeficiency syndrome (AIDS) (Goedert and Blattner, 1988) and Kapossi sarcoma.

h. Diet and nutrition

Fat is more calorogenic than other nutrients, calories may influence the risk of breast and other reproductive cancers by increasing body weight or size, it is an established risk factor for certain cancers in women, especially cancer of the endometrium (Weiss, 1983). The risk factor elevates the risk of endometrial and breast cancers by increasing the serum levels of circulating estrogens through a conversion from androst-enedione in adipose tissue and also by a lowering of the sex-hormone binding globulin. (Hederson et al., 1988; Pike, 1985). Vitamin C may protect against gastric and certain other cancers and also by blocking the endogenous formation of nitrosamines. Indole compounds in cruciferous vegetables that may decrease the risk of colon cancer (Graham et al., 1978) and allyl sulphide in garlic and onions that may lower the risk of gastric cancer (You et al., 1988). Cooking practices may generate hydrocarbons or other carcinogens in the food at high temperatures, but no relevant epidemiologic data are available.

i. Genetic susceptibility

Genetic susceptibility is most evident for skin cancer, with geographic and ethnic variations corresponding to the degree of protective skin pigmentation. Although only a small fraction of cancer is inherited in a mendelian fashion, over 200 single-gene disorders have been linked to neoplasia (Mulvihill, 1989). This
does not include several constitutional cytogenetic disorders that predispose to cancer, such as Down's syndrome with leukemia, Klinefelter's syndrome with mediastinal teratoma, gonadal dysgenesis with gonadoblastoma and aniridia with Wilm's tumour (Miller, 1986). Genetically determined neoplasms tend to occur earlier in life than other cancers of the same anatomic type and often have a multifocal origin.

**j. Ionizing radiation**

Ionizing radiation is also known as one of the human carcinogen (Boice and Fraument, 1984). Approximately 3% of all cancer deaths may be attributed to radiation (Jablon and Bailar, 1980). Nearly all sites of the body appear vulnerable to the carcinogenic effects of radiation, with the most radiosensitive tissues being the bone-marrow, breast and thyroid (Boice and Land, 1982). Radiogenic leukemia shows a distinctive wave-like pattern with the excess risk starting 2 to 4 years after exposure, peaking at 6 to 8 years and decline to normal within 25 years. In contrast, radiogenic carcinomas have a minimal latent period of 5 to 10 years. Despite a reasonably linear dose-response curve for breast cancer, the radiation effect is most pronounced among young women and is not evident among those who were exposed after age 40.

**Mechanism of radiation induced damage**

A schematic representation of the interaction of ionizing radiation with biologic tissues and the subsequent development of radiation injury are shown in figure 1.
Figure 1
Development of Radiation Injury

Gamma and X-ray

Ionizing particles
(alpha, beta, protons etc)

Ionization and excitation

Heat

Chemical change
(free radical formation)

Chemical “repair”
(energy dissipation)

Oxygen effect
chemical protection

Biologic change
(DNA damage)

Enzymatic DNA
(repair processes)

Malignant transformation of cells

Mutations

Inhibition of cell division (cell death)

Promotion and suppression

Cancer

Genetic effects

Acute somatic effects, teratogenic and developmental effects
Direct and indirect damage by ionizing radiation

Radiation has both direct and indirect effects at a molecular level. The direct action is ionization of atoms in the nucleus. Indirect action on the cell nucleus of radiation induced free radicals. When X-ray interact with water, free radical ions are produced (H\(_2\)O\(^+\) and a free electron e\(^-\)). The H\(_2\)O\(^+\) is chemically unstable and quickly forms a hydrogen ion (H\(^+\)) and a hydroxyl ion (OH\(^-\)). These free radicals are highly reactive and result in breaks in the chromosomes. The lethal damage caused to the cell may be due to the delivery of a large amount of energy to particularly sensitive molecules within the nucleus.

Radiation tolerance of normal tissues

The purpose of radiotherapy planning is to confine the radiation to the tumor and minimize the dose to normal tissues. The maximum dose of radiation that a tissue will tolerate is referred to as tolerance dose. Radiosensitivity means the relative vulnerability of cells to damage by ionizing radiation. Both normal and malignant tissues have different sensitivities, determined by their different growth rates.

The survival of a single cell, whether normal or malignant, which has been irradiated is measured by assessing its ability to form a colony of daughter cells in tissue culture. The number of colonies formed is a measure of the capacity of surviving cells to proliferate. The percentage of cells which develop into colonies is called the plating efficiency (PE). If the number of colonies of a given size is counted it can be plotted graphically against radiation dose. The cell survival curve represent the relationship between the number of cells surviving following irradiation and the dose of radiation delivered. A logarithmic scale is used to express the surviving fraction of cells.

Surviving fraction of cells = \( \frac{\text{Number of colonies counted}}{\text{Cell seeded} \times \text{PE/100}} \)
A given dose will kill a certain percentage of cells. The remaining cells, after temporary inhibition of mitosis, will recover. If the same dose is applied again, the same percentage of cells will again be killed.

1.2.2. Biological factors influencing radiosensitivity

a. Intrinsic radiosensitivity

Tumor cells are most radiosensitive as normal tissue. They do vary in their intrinsic radiosensitivity. These differences are expressed in $\alpha/\beta$ ratio for early responding tissues. A relatively high $\alpha/\beta$ ratio occurs in very sensitive acutely responding tissues such as skin and low values in relatively radioresistant tissues such as connective tissue.

b. Repair of cellular damage

Damage to DNA following irradiation is generally repaired over a period of few hours. However, the degree of repair will vary from tissue to tissue. There is a distinction is made between sublethal and potentially lethal damage. Sublethal damage (SLD) refers to irradiated proliferating cells where the damage caused is insufficient to kill the cell. The amount of recovery from SLD varies with the amount of oxygenation within normal and tumour cells during and after irradiation.

Potentially lethal damage (PLD) may occur in non-proliferating cell population and dose-dependent. The damage is "potentially lethal" because the degree of recovery can be modified by changing the environment of the cells following irradiation.

c. Repopulation by tumour cells.

The occurrence of repopulation in response to cellular injury is a major reason for fractionating radiotherapy rather than giving limited numbers of fraction. In tumours with a high growth fraction, repopulation of tumour cells between daily fractions may outstrip the tumoricidal effects of irradiation and lead to persistent disease after a course of radiotherapy is completed.
d. Reoxygenation during the course of irradiation

As tumours grow, their increased demand for nutrients often cannot be met by their vascular supply. Poorly vascularised tumours are therefore prone to hypoxia and necrosis. Hypoxic tumour are known to be 2-3 times more radioresistant than well oxygenated cells. After each dose the hypoxic cell population has the opportunity of reoxygenation and being removed by next dose of radiation.

e. Redistribution of cells in the cell cycle.

Most sensitive phase of the cell cycle to radiation is the mitotic (M) phase. Once the radiosensitive population has been killed and removed, the residual cell population is virtually synchronous in radioresistant phases of the cell cycle.

1.2.3. Effects of radiation on normal tissues.

When ionizing radiation passes through tissue resulting the damage of DNA, is mainly through the effects of free radicals. The pathological effects of ionising radiation on cells are due to chemical changes of DNA molecule. Damage to DNA may result in immediate cell death or at the next mitosis be fully repaired or result in a permanent change in genotype which is transmitted to future generations of cells. Low dose irradiation is most likely to cause a change in the genotype since it may be below the threshold for cell death. The abnormal genotype may therefore survive in subsequent cell divisions.

The most marked acute effects of radiation on normal cells will be on those with the highest mitotic activity (eg. gut and bone marrow). The renewal of the cell population from a pool of less differentiated cells is stopped either permanently or temporarily, while the process of cell loss continues. There is also damage to the vascular lining (endothelium), which results in protein and fluid leakage.

The pathogenesis of the chronic cellular changes of irradiation are less well understood than the acute ones. The lining of collagen is exposed when the vascular endothelium is damaged. Exposed collagen may act as a focus for platelets to gather and thrombosis to be initiated. Vascular endothelial cell loss will result in exposure of the underlying collagen. This will prompt platelet
adherence and the formation of thrombus, which is incorporated into the vessel wall. The initial lining of vessel wall proliferates. This process is known as endarteritis obliterans. Chronic vascular insufficiency may lead to atrophy and fibrosis of the tissues supplied.

Recovery from cellular effects of radical therapeutic irradiation is very limited. The doses given are often close to the tissue tolerance of the particular organ. For this reason radical irradiation is not generally repeated, for fear of precipitating tissue breakdown (radionecrosis).

1.2.4. Effect of radiation on haemopoietic tissues

The effect of radiation on haemopoiesis depend on the radiation dose, age of the individual and the volume of the marrow and lymphoid tissues irradiated. Localized irradiation, used in the treatment of breast cancer, head and neck tumours does not usually produce appreciable changes in the blood counts except for lymphopenia. Significant reduction in blood counts occurs when large radiation portals are used such as that used for the management of lymphomas and cerebrospinal irradiation for medulloblastoma (Blomgren et al, 1981). Lymphopenia usually results itself within 1-3 days of fractionated wide field therapy and reaches its nadir during the second week of the treatment. The rapid fall in the lymphocyte level is the result of intermitotic cell killing (Plowman, 1983). In addition to these changes, there is a long-term impairment of T-cell mediated immune functions (Anderson and Warren, 1976). Neutrophil and platelet counts begin to decrease one week after treatment, reaching a nadir in the second and third week, and generally returned to near normal values 1 - 2 months after completion of therapy (Blomgren et al, 1981). There is usually little change in the hemoglobin level. Reduction in monocyte counts and relative eosinophilia are sometimes observed during wide-field radiation. These haematological effects of radiation are largely the consequence of the elimination of uncommitted marrow stem cell within the portals (Orchard et al, 1989).
1.2.5. Effects of ionizing radiation on DNA

Radiation may produce single or double strand breaks within DNA. Double strand breaks are more difficult to repair and can irreversibly damage the cell. Radiation damage may result in changes (mutations) in the structure of DNA. The direct or indirect effects of irradiation may also result in breaks appearing in the chromosomes. Broken ends of different chromosomes may rejoin. This is called as chromosomal rearrangement. The incidence of chromosomal abnormalities increases with the dose of ionizing radiation.

The quality of radiation is also important to chromosomal damages. As ionizing radiation passes through tissue it gives up energy along its track by setting electrons in motion. The amount given up per micron of tissue (1 microns = 1mm) is called the Linear Energy Transfer (LET). Radiation with a high LET (e.g. alpha particles, fast neutrons and soft X-rays) and high density of ionization increases the probability of damaging chromosomes along the track of the particle. Low LET irradiation (e.g. gamma rays) the density of ionization per unit of distance along the track is lower and chromosomal damage is reduced.

1.2.6. Radiation and the cell cycle.

The growth of both normal and malignant tumours are influenced by the different proportions of cells in the cell cycle. The cell cycle for proliferating cells is composed of a 4 phases, S (DNA synthesis), M (mitosis) and the gaps before and after S phase, G1 and G2 respectively. Proliferating cells in this cycle called as growth fraction (i.e. the proliferating portion of the total cell population). The proportion of tumour cells in the growth fraction may vary were widely from under 1% to nearly 100%. Cells that are not proliferating (i.e. they are resting) or incapable of division (a sterile phase) is called as Go.Cells from the proliferating phase or the sterile phase may undergo cell death called as cell loss factor. The proportions of a cell population in proliferating, resting or sterile phases can affect the radiosensitivity of a cell population.
1.2.7. Radiation induced mutagenesis

The mutagenic effects of ionizing radiation were first described by Herman Muller in 1927 in his classic experiments with the fruit fly Drosophila. The dose-response relationship for such mutations to be a linear function of exposure over a wide range of radiation doses from as low as 10 to 1,000 cGy. DNA structural analyses have shown that the majority of radiation induced mutations in human cells can result from large-scale genetic events involving loss of entire active gene and extending to other loci on the same chromosome (Li, C-Y et al, 1992). When all of the experimental data for the various genetic end points are considered the genetic doubling dose (radiation dose necessary to double the spontaneous mutation rate) for low dose-rate exposure appears to be in the range of 100 cGy. 100 cGy represents approximately the lower 95% confidence limit for the human doubling dose (National Research Council, 1990).

Radiation can induce two types of chromosomal aberrations. The first have been termed “aberrations”, they are lethal to dividing cells. They include such changes as dicentrics, ring chromosomes, large deletions and fragments. These type of aberration do not allow the equal contribution of genetic materials into daughter cells. The frequency of such aberrations correlates with the cytotoxic effects of radiation. The second type has been termed stable aberrations, include changes such as small deletions, reciprocal translocations and aneuploidy. Radiation induced reciprocal translations have occurred in the cell may be passed through many generations of cell replication and emerge in the clonal cell populations (Kano and Little, 1984; 1985). Deletions and translocations can result in gene mutations, they may play a more fundamental role in the process of carcinogenesis.

1.2.8. Radiation induced carcinogenesis

Whole body radiation may induce cancer by a number of different mechanism, since the radiation induced damage causes a cascade of events. Some of them involving complicated restoration process as well as disturbances in homeostasis in a number of system. There have been extensive studies of the
effect of exposure to radiation and the incidence of radiation induced cancer (NCRP, 1980).

The energy from ionizing radiations is delivered to biological material in discrete energy deposition on events, the intensity and frequency of which depend on the dose and type of radiation (Lea, 1947). The basic biophysical mechanisms and dose response relationship of radiations pertain to cell reproductive death, chromosomal aberrations, mutation and transformation (Barendsen, 1979; Committee on the Biological effects of ionizing radiation, 1980). The origins of the chromosomal changes may be obscured by a series of molecular and cellular events (Evans, 1977) and the consequences of such changes are much more obvious. Modifications of chromosome changes take place during the course of disease, altered chromosomes disappear in remission and reappear in relapse. These anomalies, usually translocations or deletions may not though be primary events leading to disease. Most human cancers result from genetic transpositions (Klein, 1981) rather than point mutations.

Ionising radiation obviously causes cancer, will either directly or indirectly enhance carcinogenesis. Mutagens (Xrays, urethane etc.) exposure of parents led to transmitted heritable changes that substantially increased tumour incidence in offspring, in a dose-dependent manner. Hence induced “cancer proneness” enters the list of heritable genetic changes and serves to emphasis the protection of germ cell line genetic material. Energy released in cells breaks chemical bonds producing ions and free radicals which, being inherently unstable, have a high propensity of interaction with the atoms and molecules around them. Free radicals also implicated in chemical carcinogenesis (Slaga, 1980). A few features could be indicative of free radical participation (Miller and Miller, 1976).

(a) Carinogens or their intermediate metabolic form are electrophile - the higher electrophilicity , the higher probability of free radical generation.
(b) Some carcinogens, e.g. organic peroxides (ROOR) and hydroperoxides (ROOH), easily yield alkoxy radical (RO). These radicals, provided they do not degrade unimolecularly, could be extremely damaging reactants.
1.2.9. Role of oncogenes on radiation induced cancer

The role of specific oncogene activation in radiation induced cancer is less clear (Bowden et al., 1990; Cox and Little, 1992). Myc and ras oncogenes play a significant role in radiation carcinogenesis, activation or amplification of these genes should be found in vitro as well as in vivo. In in vitro the dominant transforming activity has not been associated with a number of known oncogenes (Krolewski and Little, 1994, Leuthauser et al., 1992). Whether oncogene activation arose as a consequence of a direct interaction of radiation with cellular DNA or from a complex series of events initially triggered by DNA damage. The activation of ras oncogenes by chemical carcinogens may be either an early or a late event. Ras activation usually occurs by point mutations, it may not be unexpected that activation of the ras proto-oncogene is not an important initiating event in radiation induced transformation and carcinogenesis. The pattern of oncogene activation differs significantly for transformation and carcinogenesis induced by radiation as compared with chemical carcinogens.

1.2.10. Role of tumor suppressor genes in radiation induced carcinogenesis.

p53 gene appears to play an important role in cell cycle control, radiosensitivity, the development of genetic instability leading to cell transformation, and perhaps in the response of human tumors to radiation or chemotherapy. p53 mutations have been found in a wide spectrum of human cancers (Greenblatt, et al., 1994) and in mouse skin tumors induced by ionizing radiation (Ootsuyama et al., 1994). When normal human diploid fibroblasts are exposed to radiation, a significant fraction of the population remains irreversibly blocked in the G1 phase of the cell cycle (Little and Nagasawa, 1985; Nagasawa and Little, 1983).

1.2.11. Effect of chemotherapy on normal tissues

Chemotherapeutic agents generally have a higher therapeutic index and also have significant toxicity to normal tissues even at the dose used in routine patient treatment (Fukets et al., 1976). Immunosuppression is a major drawback of antineoplastic agents. Cyclophosphamide (CTX) is considered to have the greatest
immunosuppressive potential, which is an alkylating agent. Normal bone marrow is capable of supplying the peripheral blood with mature cells for 7-10 days after precursor cells have been damaged by cytotoxic agents. Leukopenia and granulocytopenia are major consequence of myelosuppressive effects of antineoplastic agent. Granulocytopenia is often associated with a high mortality rate, secondary to bacterial and fungal infections in the immuno suppressive patient. Cytotoxic agents can produce a significant decrease in phagocytic and bactericidal activity of granulocytes, affect both B-cell and T-cell functions. (Schwartsmann et al, 1988). Intra-cerebral, renal or gastrointestinal hemorrhage is the most serious complication of thrombocytopenia. Mature monocyte macrophage is more resistant to the damaging effects of anticancer agents, helps to increase the phagocytic activity.

1.2.12. Radioprotectors and chemoprotectors

Many chemical compounds have been identified that confer protection against radiation and chemotherapy. These anti-agent can in principle interfere with particular free radical steps and either prevent or minimize the respective biological consequences. Some antioxidants have been shown by Wattenberg (1978) to be capable of reducing the effects of chemical carcinogens, and by others (Ben-Hur et al, 1981) to protect from radiation induced mutagenesis. Thiol compounds were very effective in protecting against the acute effects of low-LET radiation (Thomson, 1962)

The most chemoprotector and radioprotectors are sulfhydryl compounds such as cystamin disulphide, mercaptopropyl amine and its disulphide, aminoethyl isothiournoium, mercaptoethyl guanidine and its disulphide -guanyl ethyl disulphide, mercaptopropyl guanidine and amino alkyl thiophosphates and phosphorothioates include WR-2578, WR-638, WR-2721, WR-2822, WR-2823 and VVR -2824. Phosphorothioates [WR-2721 s-2-(3- aminopropyl amino) ethyl phosphorothiolic acid] and 2 mercapto- propionyl glycine (MPG) (Yuhas 1977) have proven to be the most effective radio- protector in clinical use. Maisin et al (1977) compared the protective effects of combination of agents with that obtained with individual drugs. It was found that combination of AET(2-β-amino
ethyl isothiouronium-Br-HBr), GSH (glutathione), 5-HT (5-hydroxytryptamine creatinine sulfate), MEA (mercaptoethylamine), CYST (cysteine) were very effective in protecting against life shortening in Balb/c mice.

Several antioxidants such as superoxide dismutase, vitamin E (α-tocopherol), ellagic acid, bixin and plant products like curcumin were also reported to have radioprotective effects (Thresiamma et al., 1995: 1996). Moreover glutathione monoethyl ether, cysteine and sodium thiosulphate are other potential chemoprotective agents (Ainsworth, 1988).

1.2.13. Protection of tissue oxidative damage

Reactive Oxygen species (ROS) are produced by cellular metabolic reaction (figure 2) include the superoxide anion radical \( O_2^- \), peroxide ion \( O_2^{2-} \) and its hydrated form hydrogen peroxide \( H_2O_2 \) and hydroxyl radical \( OH \). ROS appear to have broader significance in the production of tissue injury under conditions of “oxidative stress”. Oxidative stress is a state of imbalance between prooxidant and antioxidant in which the former predominate ROS damage the biomolecules such as DNA, proteins, Carbohydrates and lipids and affect the enzyme processes and genetic machinery. Almost every organ or system in the body is affected by oxidant stress (Table 1).
Table 1

Free Radical Mediated Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>Genetic disorders</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Inflammatory disorders</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Muscular dystrophy</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Parkinson’s dementia</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td>Radiation injury</td>
</tr>
<tr>
<td>Cancer</td>
<td>Skin disease (prophyria)</td>
</tr>
<tr>
<td>Cataract</td>
<td>Senile dementia</td>
</tr>
<tr>
<td>Drug induced GIT/liver disorders</td>
<td>Stroke</td>
</tr>
</tbody>
</table>
Figure 2
Mechanisms of formation of reactive oxygen species

Superoxide anion ($O_2^-$) can be produced from a variety of sources. It is either hydrated to form hydrogen peroxide (reaction II), or forms hydroxyl radicals in Haber-Weiss cycle (reaction III). Hydroxyl radicals are also formed by Fenton reaction (reaction IV) which is catalysed by ferrous iron (or another transitional metal) $O^2$ also promotes the release of storage iron (although other reductants such as ascorbate may be more potent releasers) and reductases $Fe^{3+}$ to $Fe^{2+}$. The various endogenous and exogenous sources of oxidative damage is shown in figure 3.
Figure 3

Sources of Oxidative Damage

Oxidative damage

- Endogenous Source
  - * Mitochondrial Electron Transport
  - * Defence – phagocytes
  - * Membrane oxidases
  - * Catecholamines

- Exogenous Source
  - * Alcohol
  - * Chemical carcinogens
  - * Pollutants
  - * Radiation
  - * Smoking (tobacco)
  - * Stress

ROS mediated disease
1.2.14. **Antioxidant Defense System**

In order to survive from oxidative stress, aerobic organisms have developed an antioxidant defense system. Antioxidant defense system consists of enzymatic and non-enzymatic components. Enzymatic system includes superoxide dismutase, catalase etc. Non enzymatic system consists of both nutrient and non nutrient components, nutrients are α-tocopherol, β-carotene etc. and non nutrients are ceruloplasmin, transferrin etc. (Fig. 4).

**Figure 4**

**Antioxidant Defense System**

![Antioxidant Defense System Diagram]

* Superoxide dismutase (SOD)
* Glutathione peroxidase (GPX)
* Catalase (CAT)
* Glutathione-S-transferase (GST)

* Nutrient
  * Alpha-tocopherol
  * β-carotene
  * Ascorbate
  * Glutathione
  * Selenium

* Non-nutrient
  * Ceruloplasmin
  * Transferrin
  * Uric acid
  * Peptides
  * Carnosine
  * Anserine
1.2.14.a. Enzymatic antioxidants (endogenous)

1. Superoxide dismutase (SOD)

SOD are metalloenzymes (Halliwell, 1983) which catalyse the protonation of superoxide anion radical (O$_2^-$) to form hydrogen peroxide (H$_2$O$_2$). SOD is ubiquitous in aerobic cells and has been isolated from bacteria, plants, birds and mammals. In mammalian cells there are two isoenzymes of SOD which contain different metals at their active sites, differ in molecular weight, amino acid sequence and number of subunits. Mammalian cytosolic SOD contains one copper (Cu) and two Zinc (Zn) atoms per molecule. The copper ions appear to function in the dismutation reaction by undergoing alternative oxidation and reduction, ie.

\[
\text{Enzyme} + \text{Cu}^{2+} + \text{O}_2^- \rightarrow \text{Enzyme} - \text{Cu}^+ + \text{O}_2
\]

\[
\text{Enzyme} + \text{Cu}^+ + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{Enzyme} + \text{Cu}^{2+} + \text{H}_2\text{O}_2
\]

Net reaction: \( \text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \)

The Zn $Z^+$ does not function in the catalytic cycle but it appears to stabilize the enzyme. Dismutation of $O_2^-$ by SOD to form $H_2O_2$ which detoxified by glutathione peroxidase (GPX).

Illumination of riboflavin solution in the presence of either EDTA or of the amino acid methionine causes a reduction of the flavin. It then reoxidizes and simultaneously reduces oxygen to $O_2^-$, which is allowed to react with a detector molecule such as nitrobluetetrazolium (NBT) which is reduced by $O_2^-$ to form a deep blue coloured formazan. The enzyme SOD inhibited the formazan production.

\[
\text{NBT} + \text{O}_2^- \rightarrow \text{NBT radical} + \text{O}_2
\]

SOD may be more important at sites of inflammation than GPX and catalase, both of which are rapidly inactivated by hypochlorous acid.
Superoxide generating system
(eg. Xanthine plus, Xanthine oxidase) → Superoxide → radical
Reacts with detector molecule to give an observative change which is inhibited by SOD
[eg. Cyt-c (Fe³⁺) cytc (Fe²⁺)]

2. Glutathione peroxidase (GPX)

GPX, a selenium containing enzyme found in cytosol and mitochondria of animal tissues, helps to dispose of hydrogen peroxide (H₂O₂) by catalyzing the reaction.

\[ 2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{GSSG} \]

GPX is specific for GSH as a substrate but will act in vitro on a range of peroxides in addition to hydrogen peroxide. In the liver, both GPX and catalase (CAT) can destroy H₂O₂. The Km of GPX for H₂O₂ is much lower than that of catalase. Two enzymes are likely to be complementary in protecting against oxidative reactions caused by H₂O₂. GPX may act on relatively low levels of H₂O₂.

GPX is a preventive antioxidant.

GPX plays a critical role in the protection of membrane lipid against oxidation (in vivo). GPX, by catalyzing the reduction of H₂O₂ to water interrupts the propagation of peroxidation through membrane lipids. The subsequent rejuvenation of GSH via glutathione reductase - mediated reduction of GSSG is dependent on protons and NADPH. GPX is the more efficient system for removal of H₂O₂.

3. Catalase (CAT)

Most aerobic cells contain catalase activity. It is a preventive antioxidant. In animals catalase is present in all major body organs, being especially concentrated in liver and erythrocytes. Catalase have been shown to consist of four protein subunit, each of which contains a haem(Fe(III) - protoporphyrin) group bound to its active site. Dissociation of this molecule occurs during storage, freeze drying or exposure of the enzyme to alkali or acid. Catalase may act on relatively high
concentrations of $H_2O_2$. The $K_m$ of catalase for $H_2O_2$ is much higher than of GPX.

The catalase reaction mechanism:

$$\text{Catalase} - \text{Fe (III)} + H_2O_2 \xrightarrow{K_1} \text{Compound I}$$

$$\text{Compound I} + H_2O_2 \xrightarrow{K_2} \text{Catalase- Fe(III)} + 2H_2O_2 + O_2$$

For rat liver catalase, the two second order rate constants $K_1$ and $K_2$ have values of $1.7 \times 10^{-7} \text{ M}^{-1} \text{s}^{-1}$ and $2.6 \times 10^{-7} \text{ M}^{-1} \text{s}^{-1}$ respectively. Catalase activity of animal and plant tissues is largely located in subcellular organelles bounded by a single membrane and known as peroxisomes (Halliwell and John Gutteridge, 1985)

4. **Glutathione reductase (GR)**

GR catalyses the reaction of oxidised glutathione (GSSG) to reduced glutathione (GSH) by converting NADPH to NADP. By this mechanism GSH level is maintained in cells.

$$\text{GR}$$

$$\text{GSSG} \xrightarrow{\text{NADPH}} \text{GSH} \xleftarrow{\text{NADP}}$$

5. **Glutathione – S – transferase (GST)**

GST is now widely recognised as important and versatile enzyme involved in the detoxification of an extensive variety of compounds. GST catalyzes the first step in the formation of mercapturic acids (N-acetyl cysteine derivatives). In 1879 (Baumann and Preusse, 1879; Jaffe, 1879) mercapturic acids were first characterised as metabolites of several compounds. The purpose of mercapturate pathway appears to be aimed at inactivating the potentially toxic electrophilic center of the substrate molecule and at the same time forming a more hydrophilic conjugate for excretion.

1. Glutathione (GSH)

GSH is an intracellular reductant (tripeptide-L-gamma glutamyl-L-
cysteinyl-Glycine). GSH is widely distributed in most cell types at concentrations
ranging from 1 - 12mM (Kosower and Kosower, 1978). Glutathione was first
isolated from yeast in 1888 and its structure was established by chemical synthesis.
GSH is capable of preventing or limiting both ionic alkylating events and free
radical mediated damage to cellular macromolecules (Reed and Beatty, 1980). Its
metabolic and protective functions also involve reactions in which both
endogenously produced compounds and exogenous compounds react with the
sulfhydryl group of glutathione to form glutathione conjugates in reactions
catalyzed by glutathione-S-transferases (Meredith and Reed, 1982). Rapid
depletion of intracellular GSH can occur during GSH protection processes which
can necessitate replenishment of both GSH and a much smaller pool of cysteine.
Figure 5 represents the antioxidant roles of the glutathione system.
Antioxidant Roles of the Glutathione System (Meister, 1981)

SOD- superoxide dismutase, GR - glutathione reductase, GSH- reduced glutathione, GGT - gamma glutamyl transpeptidase, GSSG- glutathione disulphide, GPX - glutathione peroxidase. X is a substrate for one of the glutathione S- transferases (these substrates include endogenous compounds like oestrogens and leukotrienes, xenobiotics and epoxides).

1.2.14.c. Non-enzymatic nutrient antioxidants (exogenous)
a. Natural antioxidants

Regular consumption of some antioxidants present in the diet appears to be very effective in the protection against oxidant insults. Although vitamins such as E and C, selenium and carotenoids have been extensively studied for their
beneficial effects, it is only recently that the antioxidant defense offered by these nutrients has received recognition (Krinsky and Sies, 1995).

1. **Vitamin E (α-tocopherol)**

   The active vitamin was isolated from wheat germ oil in 1936 and named as tocopherol (tokos = child birth, pheros = to bear; ol= alcohol). Vitamin E is a powerful chain breaking antioxidant, inhibits lipid peroxidation (Packer, 1990). So it act as a cell membrane stabiliser. Vitamin E quenches singlet oxygen, reacts with free radicals. Vitamin E is accepted as the most potent biological antioxidant which protects the tissues from the harmful effect of free radicals. Vitamin E has been reported to specifically modify signal transduction at several steps, including PKC and arachidonic acid metabolism (Balasubraminan et al, 1994; Traber, 1995).

2. **Selenium**

   A naturally occurring element. Selenium is present in glutathione peroxidase (GPX). Selenium has been found to decrease the requirement of Vitamin E and vice versa. They act synergistically with each other in minimising lipid peroxidation. Selenium has a sparing effect on vitamin E. (Balasubramanian et al, 1994).

3. **Vitamin C (ascorbic acid)**

   In the absence of transition metal ions, Vitamin C is an outstanding antioxidant in the aqueous phase (Frei et al, 1989). The outstanding property of this is the capacity for reversible oxidation reduction between ascorbic acid and dehydroascorbic acid. Most of the physiological properties of Vitamin C are due to its redox system. In the presence of excess of Fe$^{3+}$ or Cu$^{2+}$(1mM), vitamin C may act as a strong pro-oxidant and may actually induce lipid peroxidation and oxidative modification of genomic structures. Under such conditions, Vitamin C may reduce Fe$^{3+}$ to Fe$^{2+}$ which in turn facilitates the generation of hydroxyl radical.

4. **Vitamin A and β-carotene**

   They act as potent antioxidants (Halliwell and Gutteridge, 1985). β-carotene plays an important role in reducing the incidence of cancer (Peto et al
β-carotene has the ability to trap certain organic free radicals, and to deactivate the excited oxygen molecules. The most consistent evidence of protective effect of high intake of carotene rich foods comes from studies of lung cancer and cancer of oesophagus. β-carotene act as antioxidants under normal physiologic conditions (low oxygen tension), can also act as prooxidants at high concentrations and more oxidizing conditions (Ostrowski et al, 1987; Hennekens et al, 1986)

5. Polyphenols

Among the most investigated non-nutritive antioxidants are plant phenols, flavanoids, coumarins, benzyl-isothiocynates etc. Phenolic acid like curcumin, caffeic acid, ferulic acid, gallic acid and ellagic acid have been investigated in great detail for antioxidant activity (Rice-Evan et al, 1996). These compounds in general influence the quality, acceptability and stability of foods by acting as flavorants, colourants and antioxidants. Flavanoids are present in a wide variety of fruits, vegetables, nuts, whole seeds, spices, tea and wine. Quercetin is the major flavanoid in vegetables, fruits and wine. Flavanoids are a group of polyphenolic compounds which have cancer blocking property. Flavanoid have a common skeleton of diphenyl pyrons, two benzene rings linked through a heterocyclic pyran or pyrone ring. The basic ring structure allows a multitude of substitution patterns giving rise to flavanoids, flavones, catechins, anthocyanadines and isoflavanoids. They are low molecular weight compounds having three phenolic rings based on flavon nucleus. The conjugated ring structure and the heterogenous group allow the phenois to actively scavenge and stabilize free radicals. The carboxylic group inhibits lipoxidation by metal chelation (Rice-Evans et al, 1996)

b. Synthetic antioxidants

1. Butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT)

BHA and BHT are widely used as food additives to prevent the oxygen induced peroxidation of lipids (Halliwell and Gutteridge, 1985). BHA was shown
to inhibit the carcinogenesis induced by benzo(a)pyrene, DMBA, diethylnitrosamine, uracil mustard, urethane, and methyl azoxy methanol acetate (Das et al, 1985). The protective effects of these phenolic compounds on tumor promotions have been attributed to their antioxidant functions. Moreover these compounds have also been reported to produce substantial alterations in the activities of hepatic biotransformation enzymes in experimental animals (Benson et al, 1979; Cha and Bueding, 1979).

2. Mercaptoalkylamines

General formula of these compounds RN(CH₂)nSResponsibility. They are -‘S’ containing compounds derived from cysteinamine (MEA). The main ones among them are :- cystamin (MEA disulphide), mercaptopropyl amine and its disulphide, aminoethyl isothiouranium, mercaptoethyl guanidine and its disulphide, mercaptopropyl guanidine and amino alkyl thiophosphates (Yarmonenka, 1988). This phosphorothioates include WR – 2573, WR – 638, WR-2721, WR-2822, WR-2823, WR-2824.

The protective action of these aminothiols is assumed to be associated with their ability to scavenge free radicals. WR-2721 has been proven to be least toxic and therefore most effective protector (Yuhas, 1977). Studies of differential protection of WR-2721 against alkylating agent injury in tumours and normal tissues is that the protection is greater in normal tissues than in tumours (Yuhas et al, 1980).

1.2.14.d. Non-nutrient antioxidants

Several chronic diseases such as coronary heart disease, cancer, cataract etc. have been positively correlated with low vegetable and fruit consumption (American Institute for Cancer Research, 1997). Although vitamins such as E and C, selenium (Se) and carotenoids have been extensively studied for their beneficial effect such as antioxidant mechanism (Krinsky and Sies, 1995). Further, the phytochemicals, which primarily serve in plant protection are now considered as vitamins of 21st century (Messina et al, 1994). Non-nutrients antioxidants is shown in table 2.
Table 2

Non-Nutrient Antioxidants

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple phenols</td>
<td>Vanilin</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Catchin, Epicatechin gallate, Quercetin, Chrysin, Cyanidin</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>Ferulic acid, Caffeic acid, p-Coumaric acid</td>
</tr>
<tr>
<td>Coumarins</td>
<td>mono, di and tri terpinoids</td>
</tr>
<tr>
<td>Lignans</td>
<td>Carnosine, Anserine</td>
</tr>
<tr>
<td>Essential Oils</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td></td>
</tr>
<tr>
<td>Glycoudes</td>
<td></td>
</tr>
<tr>
<td>Peptides</td>
<td></td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td></td>
</tr>
<tr>
<td>Inositol hexaphosphate</td>
<td></td>
</tr>
<tr>
<td>Sterols</td>
<td></td>
</tr>
<tr>
<td>Isothiocyanates, Thiocyanates</td>
<td></td>
</tr>
<tr>
<td>Conjugated linolic acid</td>
<td></td>
</tr>
<tr>
<td>Phytoesterogens</td>
<td></td>
</tr>
</tbody>
</table>
1.2.15. Biological response modifiers (BRMs)

BRMs are those agents that modify the relationship between tumor and host by modifying the host biological responses to tumor cells with resultant therapeutic effects. It can employ the body’s own natural defences to destroy the tumor cells either indirectly (except few cytokines) or as adjuncts to chemotherapy and radiation by facilitating regeneration of normal bone-marrow elements (Hersh, 1982; Tennebaum, 1995). The two most common symptom complexes experienced by the patients undergoing BRM therapy are flu like syndrome and fatigue. BRMs are classified into two groups, immunomodulating agents and tumoricidal cytokines. Immunomodulating agents alter host immunological responses. They were divided into three groups. Immunooaugmenting agents, immunoregulating agent and immunorestorative agents.

Immunooaugmenting agents are those agents augment the immune response by stimulating cells of reticuloendothelial system to initiate action against foreign invaders. Examples are Bacillus Calmette – Guerin (BCG), methanol extracted residue of BCG (MER), Corynebacterium parvum and interferones etc. Immunoregulating agents effect the developmental or functional balance inherent in the immune response. Enhance or inhibit T-cell and B-cell production, function and stimulate secretion of a variety of cytokines. Examples are interleukins, interferons, colony stimulating factors, tumour necrosis factor etc. Immunorestorative agents possess no direct cytotoxic effects. Restore or stimulate depressed antigen response and cellular immune function. Examples are levamisole, thymosine etc.

Tumoricidal cytokines exert a direct anti tumor effect which is either cytotoxic or cytostatic. Examples are tumor necrosis factor (TNF), lymphotoxin etc. They may have some immunomodulating properties that are not yet well defined. (Table 3)(Blackwill et al, 1995)
### Table 3

**Major Cytokines and Their Antitumour Properties**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Cytokines activity</th>
<th>Antitumour effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferons (IFNs)</td>
<td>Yes</td>
<td>Direct and indirect</td>
</tr>
<tr>
<td>Tumour necrosis factor (TNF)</td>
<td>Yes</td>
<td>Direct and indirect</td>
</tr>
<tr>
<td>Colony stimulating factors</td>
<td>Yes</td>
<td>Macrophage mediated cytotoxicity</td>
</tr>
<tr>
<td>(G-CSF, M-CSF, GM-CSF, IL-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukins (IL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>Yes</td>
<td>Direct and indirect</td>
</tr>
<tr>
<td>IL-2</td>
<td>Yes</td>
<td>Lymphokine-activated killer cells (LAK-cells)</td>
</tr>
<tr>
<td>IL-4</td>
<td>Yes</td>
<td>Direct, LAK-cells and cytotoxic cells</td>
</tr>
<tr>
<td>IL-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Yes</td>
<td>Direct, cytotoxic-T-lymphocytes (CTLs)</td>
</tr>
<tr>
<td>IL-7</td>
<td>Yes</td>
<td>T-cell dependent tumor rejection</td>
</tr>
<tr>
<td>IL-8-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8, MCP, MIP1, RANTES, GRO/MGSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncostatin M</td>
<td>Yes</td>
<td>Direct effect</td>
</tr>
<tr>
<td>Leukoregulin</td>
<td>Yes</td>
<td>Direct effect</td>
</tr>
</tbody>
</table>


1.2.16. Immunomodulators from plant sources

Recently there have been a renewed interest in the search of potential drug especially of plant origin for diseases for which the modern medicine seldom offers a cure. Immunomodulators not only have immunorestorating function as in the case of cancer, AIDS etc but also can be used to produce an immunocompetent state so as to ward off many diseases. Therefore great attention has been given for the isolation and characterization of plant materials that can stimulate immune system. Some of the immunomodulatory agents and their use in cancer are given in table 4. They include mono and polysaccharides, terpenes, steroids, phenolic compounds, furanocoumarins, cannabinoids, aminoacids, peptides, proteins, alkaloids and other nitrogen containing compounds and several immunomodulatory plant extracts with unidentified structures. These plant products are comparatively non-toxic to that of synthetic drugs and can be administered for longer duration (Landquist and Teuscher, 1985). Some of these plant products also have anticancer properties and may have great significance in cancer therapy. It has been shown that they can be used as adjuvants with the cytoreductive therapies used in cancer treatment.
Table 4
Plant Immunomodulators with Anticancer Activity

<table>
<thead>
<tr>
<th>Plants</th>
<th>Plant material</th>
<th>Immunomodulatory activities</th>
<th>Antitumor effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panax ginseng</td>
<td>Polysaccharides, Saponin</td>
<td>Phagocytosis, antibody production, increase in serum complement content, IgG level, B/T cells ratio (Im et al, 1990)</td>
<td>Yes</td>
</tr>
<tr>
<td>Trichosanthes rhizome</td>
<td>Polysaccharides</td>
<td>Increase leukocyte counts, peritoneal exudate cells, antibody forming activity (Chung et al, 1990)</td>
<td>Yes</td>
</tr>
<tr>
<td>Traxcum platycarpum</td>
<td>Polysaccharides</td>
<td>Increase peritoneal exudates cells (Jeong et al, 1991)</td>
<td>Yes</td>
</tr>
<tr>
<td>Astragalus Membraneaceous</td>
<td>Crude</td>
<td>Immunotherapy along with IL-2</td>
<td>No</td>
</tr>
<tr>
<td>Paris formosana</td>
<td>Formosanin-C(saponin)</td>
<td>Enhancement of PHA stimulated peripheral whole blood proliferation, proliferation of mouse lymphocytes to ConA, proliferation of GM-colony, NK-cell activity, IFN induction (Wa et al, 1990)</td>
<td>Yes</td>
</tr>
<tr>
<td>Pinecone extract</td>
<td>Lignin related structures complexed with sugars or polysaccharides</td>
<td>Peripheral plaque forming cells, induction of cytokine production(TNF), macrophages PMNs and spleenocytes were stimulated (Sakagami et al, 1991)</td>
<td>Yes</td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Therapeutic Compound</th>
<th>Therapeutic Activity</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Viscum album</em></td>
<td>Peptide</td>
<td>Peripheral plaque forming cells, macrophage activation, antibody production, NK cell and ADCC activity (Kuttan et al, 1990)</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Alsophila Spinulosa</em></td>
<td>Partially purified</td>
<td>Lymphocyte proliferation, antibody production, CTL activation (Kao et al, 1994)</td>
<td></td>
</tr>
<tr>
<td><em>Picrorhiza Kurroa</em></td>
<td>Crude</td>
<td>Enhance phagocytosis, lymphocyte proliferation, complement and neutrophil activation (Simmons, 1990)</td>
<td></td>
</tr>
<tr>
<td><em>Asparagus racemosus</em></td>
<td></td>
<td>(Dhanukar and Thatte, 1986; 1988)</td>
<td></td>
</tr>
<tr>
<td><em>Tinospora Cordofolia</em></td>
<td></td>
<td>(Dhanukar and Thatte, 1986; 1988)</td>
<td></td>
</tr>
<tr>
<td><em>Glycyrrhiza glabra</em></td>
<td></td>
<td>Lymphocyte proliferation, interferon production (Hikino, 1985; Shinada et al, 1986)</td>
<td></td>
</tr>
</tbody>
</table>

The traditional Indian system of medicine (Ayurveda) has given great emphasis on the promotion of health in normal and sick people. Rasayanas are a group of non-toxic polyherbal preparations which are used to improve the general health by stimulation of the immune system (Singh, 1990). According to Ayurveda, Rasayana therapy nourishes blood, lymph, bonemarrow, flesh and semen, which in turn arrests aging, increases intelligence, strength and enable to prevent diseases.

Recently our laboratory has shown that Rasayana treatment can to enhance the production of stem cells and its maturation after their depletion so that the animals could recover from the myelosuppressive effect of...
cyclophosphamide (Praveen Kumar, et al, 1994). BR treatment was found to protect mice from lethal effects of radiation and promotes the recovery of bone marrow cells and immunological functions such as natural killer (NK) cell activity and antibody dependent cellular cytotoxicity (ADCC) activity (Praveen Kumar et al, 1996). Rasayanas Treatment was found to enhance the immunopotentiating activity in normal mice (Praveen Kumar et al 1999) and also found to increase the cell mediated immune responses in tumour bearing mice (Praveen Kumar et al 1999). Brahma Rasayana (BR) has been shown that leukopenia, neutropenia and lymphopenia produced in cancer patients by the simultaneous administration of chemotherapy along with radiation therapy was significantly reduced by the administration of BR. Doses of BR given in these patients were 50 g/day in three divided doses (Joseph et al, 1999). BR was found to inhibit the chemically induced sarcoma developed by the administration of 20-methylcholanthrene and increased life span of animals (Menon et al, 1996). BR was also found to inhibit the lung tumour metastasis produced by B16F10 melanoma cells in C57 BL/6 mice (Menon et al, 1997).

The scope of present study is to find out the use of BR in ameliorating radiation and chemotherapy induced toxicity. Initially we investigated the antioxidant activity of BR in vitro and in vivo as studied by inhibition of lipid peroxidation, superoxide radical formation and nitric oxide radical formation. Superoxide and nitric oxide radicals generated in mice peritoneal macrophages were found to be scavenged by BR. BR was also found to enhance the endogenous antioxidant systems such as non enzymatic glutathione (GSH), enzymatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione – S – transferase (GST) activity in mice during radiation treatment (600 rads/mouse) and cyclophosphamide treatment (25mg/kg.b.wt.). BR treatment enhanced the total leukocyte count, polymorphonuclear cells (PMN), bone marrow cellularity and α - esterase activity in normal and irradiated mice. BR also enhanced the stem cell proliferation of bone marrow, spleen and thymus (in vitro and in vivo) in normal mice. Cytokine levels was found to increase in BR treated mice after radiation or
cyclophosphamide treatment. BR was also found to have anticlastogenic effect as seen from the inhibition of the production of micronuclei and chromosomal aberrations in irradiated mice.

BR is a polyherbal preparation made up of approximately 35 plant extracts. The composition and mode of preparation of BR are given in Table 5. The immunological action of BR may be due to the synergistic activity of several of the immunostimulatory plants present in the preparation.

Table 5
Composition of Brahma Rasayana (BR) and mode of preparation (Vaidya Jadavaji Trikanji Acharya, 1981)

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Emblica officinalis</td>
<td>(20%)</td>
</tr>
<tr>
<td>2. Terminalia chebula</td>
<td>(6.67%)</td>
</tr>
<tr>
<td>3. Uvaria pitca</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>4. Desmodium gangeticum</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>5. Gmelina arborea</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>6. Solanum nigrum</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>7. Tribulus terrestris</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>8. Aegle marmelos</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>9. Premna tomentosa</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>10. Stereospermum suvaceolens</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>11. Sida rhombifolia</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>12. Boerhaavia diffusa</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>13. Ricinus communis</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>14. Vigna vexilata</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>15. Phaseolus adenanthus</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>16. Asperagus racemosus</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>17. Holostemma annulare</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>18. Leptadenia reticulata</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>19. Desmostachya bipinnata</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>20. Saccharum officinarum</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>21. Oryza malampuzhensis</td>
<td>(0.4%)</td>
</tr>
<tr>
<td>22. Cinnamomum iners</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>23. Elettaria cardamomum</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>24. Cyperus rotundus</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>25. Curcuma longa</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>26. Piper longum</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>27. Aquilaria agallocha</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>28. Santalum album</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>29. Centella asiatica</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>30. Mesua ferrea</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>31. Clitoria ternata</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>32. Acorus calamus</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>33. Scirpus eossus</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>34. Glycyrrhiza glabra</td>
<td>(0.16%)</td>
</tr>
<tr>
<td>35. Embelia ribes</td>
<td>(0.16%)</td>
</tr>
</tbody>
</table>
Items 1 – 21 were made into small pieces and washed it well. 16 part of water was added to the total quantity of drugs and allowed to boil to get one fourth of original volume. The seeds from fruit of *Emblica officinalis* and *Terminalia Chebula* were removed and the pulp was roasted well by adding sufficient quantity of ghee and sesame oil. Then sufficient quantity of sugar was added to the above decoction to get a paste like formation. The plants 22-35 were cleaned and dried well and was made into fine powder and this powder was mixed with above paste and stirred well. When the preparation comes to normal temperature, sufficient quantity of honey was added, mixed well and stored at room temperature.