Chapter 1

INTRODUCTION

Breast Cancer: An Overview

Among various diseases attributed to mortality in humans all over the world, cancer is a leading cause. **Cancer** is one of the most dreaded diseases of 20th century and spreading further with continuance in 21st century. The estimated number of World new cancer cases annually is expected to rise from 10 million in 2000 to 15 million by 2020. With increased environmental awareness and health consciousness, it is clear that environmental mutagens and carcinogens are responsible for such genetic disorders including cancer. **Breast cancer** is one of the most commonly occurring cancerous diseases. More than 10.5 lakhs new breast cancer cases occur worldwide annually while nearly 5.8 lakhs in developed countries remainder in developing countries. The lifetime risk of any particular woman getting breast cancer ranges from 2-5 % in the world. In general, breast cancer rates have risen about 30 % in the past 25 years in western countries. Breast cancer now ranks first among the causes affecting women throughout the world. It can also occur in men but rarely. As developing nations such as India, breast cancer is the leading cancer site in its female residents. The population based cancer registry data from various parts of the country, has revealed breast cancer as the commonest cancer among women in Delhi, Mumbai, Ahmadabad, Kolkata and Trivandrum. Several factors found to be responsible for initiation and promotion of breast cancer are genetic factors including genes like breast cancer antigens BRCA 1 and 2 having 85 % and 65 % risk of mammary carcinoma development respectively and non genetic factors like use of hormone replacement therapies, high fat diet, intake of heterocyclic amines, late pregnancy, use of oral contraceptives, later age of menarche, earlier age of menopause etc. The present trend in the management of the cancer development involves either reduction of the exposure of an individual to known carcinogen to the extent possible and or seeking advantage of the inhibitors of carcinogenesis for their eventual applications as anticancer agents. Since exposure to the environmental carcinogens is often unavoidable, the later field has been widely explored. Unfortunately, an alarming situation of drug resistance along with severe side effects is experienced and found environmentally harmful just because of the widespread and continued use of synthetics. A dose of chemotherapeutic drugs sufficient to kill tumor cells is often toxic to the normal tissue and lead to many side effects which in turn limit their treatment efficacy.

Reactive Oxygen Species and Cancer: Interdependence

**Reactive oxygen species** generated by normal physiological processes or exogenous factor, are known to induce oxidative damage to biological macromolecules such as membrane lipids, proteins and DNA. Such oxidative macromolecular and cellular damage lead to the development of various diseases including cancer, neurodegenerative diseases, aging and inflammation. Cancer is considered to be a complex disease caused by oxidative damage which is again severe problem now a day. In recent years, considerable efforts have been directed towards naturally occurring substances that can protect against oxidative stress. Antioxidants have been used to inhibit apoptosis because it is initially thought to be mediated by oxidative stress. Many antioxidant substances have anticancer or antimutagenic properties. Majority of dietary supplements and foodstuffs are believed to have preventive effects on chronic diseases due to their radical scavenging or antioxidant property.
Plants are the sources of half of the pharmaceuticals in our modern medicine cabinet. Herbs are known to produce a wide range of secondary metabolites such as alkaloids, terpenoids, polyacetylenes, flavonoids, quinines, phenyl propionates and amino acids and proved for their medicinal properties. Plant based products or Herbalism explains the unmistakable popularity of green magic bullets and is widely accepted in present perspectives. Plant-derived compounds comprise a diverse group with different mechanisms of actions, which seem to have the ability to induce apoptosis. Understanding the modes of action of plant-derived anti-cancer compounds provide useful information for their possible application in cancer prevention. It is thus important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. Medically screened plants which are used as traditional anticancer remedies increase the chance of finding new bioactive principles. Studies on the pharmacological mechanisms and searching for chemical structures from herbal extract for new anticancer drug caught great interest. Epidemiological studies have suggested that the medicinal plants can play a vital role in inhibiting the genotoxicity and carcinogenicity of anticancer drug to normal cells. Considering herbalism as an important strategy for cancer prevention, variety of animal experiments and cell lines culture have been studied. Herbs of pharmacological importance are on the forefront whenever we talk about anticancer remedies. Vinblastine, Vincristine, Taxotere, Navelbine, Etoposide, Teniposide, Topotecan, Irinotecan, Doxorubicin, Dactomycin, Bleomycin and a number of other plant-derived anti-cancer compounds have been approved by United States Food and Drug Administration.

**Phytoproducts and Cancer**

The pharmacological efficacy of plant–derived products has created a revolutionary interest in the mechanism of their action. Attention has been focused towards characterization of the mechanism for herbal action. Several biologically active compounds in a plant work together to produce greater effect than single chemical and take care of other health aspects like short term energy, long term endurance or weight control also.

![Fig. 1.1: Herbalism: Proven efficacy](image-url)
Chemical partnership in the plant extract is the reason to believe that plant constituents might inhibit diseases better when used in combination. The synergistic effect of various components of plant material may enhance the therapeutic effect simultaneously reducing the side effects.

As herbal drugs are a cocktail of active biochemistry rather than a single compound, diseases do not gain resistance easily to such remediation. Reason for accepting herbalism is chemical partnership of secondary metabolites present in the herbs leading to various additive, synergistic and antagonistic effects. This cocktail of bioactive chemicals can be used in two possible ways, firstly, it can be used as therapeutic armamentarium for enhanced bioefficacy and secondly expected to cause delay in resistance by increasing immunity. Thus, synergistic and additive effects make them better therapies as one can get the medicinal effect with smaller doses of each ingredient and simultaneously avoid the side effects that tend to kick in with higher doses.

Green Nanotechnology and Cancer

Recent phytochemical researches have directed attention that mostly single phytochemicals based therapy lacks bioefficacy and highlight the role of combination therapy as new treatment modalities. Modern medicine hooked on the idea of the chemical partnership of chemicals in the plant for designing new generation of pharmaceuticals under synergy research. Experimental breakthrough involving the use of two or more bio agents have been recognized recently, providing enhanced therapeutic bioefficacy with reduced side effects. Multiple active phytochemicals result into synergism in such a way that outcome may not be additive but multiplicative. The recent developments in synergy research have opened highly interesting perspectives for a new generation of phyto pharmaceuticals. The developments in combination therapies involving nanosize enhanced therapeutic activities have shown scope of applications in medical sciences. The bioactive phytochemical embedded with nano particles, particularly; gold nanoparticles have an emerging interdisciplinary area with potential applications of nano composites in therapeutic applications. Recent developments in nanotechnology have witnessed the rapidly evolving power of this interdisciplinary field with myriad of applications in medical sciences. The phytochemical coatings on nanoparticles recently have rendered non toxic features to Green Gold Nanoparticles. Thus the overall synthesis and architecture of gold nanoparticle embedded phytoproduct is highly attractive as it brings an important symbiosis between plant sciences and nanotechnology with the name Green Nanotechnology. The versatile Phytochemicals Mediated Green Nanotechnological process has been shown to be effective in both the generation and stabilization of non toxic nanoparticles for direct application in diagnostic and therapeutic applications. Occlusion of cancer fighting phytochemicals and their future utility in the development of tumor specific nanoparticles will provide unprecedented opportunity towards the design and development of safe nanoparticles through specific anticancer bio agent which can be safely produced and shift worldwide. Thus recent advances in combination therapy suggest many of the current problems related to therapies could be resolved or greatly diminished using drug embedded nanoparticles.

Among the secondary metabolites, flavonoids, a large group of natural polyphenolic compounds, are ubiquitously present in the green plants world. Remarkable beneficial pharmacological activities of dietary flavonoids like antioxidant, anticancer and cardio protective properties have been established. The chemopreventive properties of flavonoids are believed to reflect their ability to scavenge endogenous reactive oxygen species (ROS). Their antioxidant action is extended towards an important mechanism for anticancer and apoptosis-inducing properties. Structure activity relationship studies on flavonoids have shown that structural moieties, associated with enhanced
ROS scavenging and cytotoxicity, are the 2, 3 double bonds, appropriate number of hydroxyl groups and ortho hydroxylation in ring B of flavonoids. *In vitro* and *in vivo* studies on different types of flavonoids with special reference to anticancer bioefficacy highlights that keen attention has been paid on apigenin, catechin, epicatechin, genistein, kaempferol, luteolin, myricetin, naringenin, orientin, quercetin, rutin and silymarin. Structurally related cytotoxic effects of flavonoids on variety of cancer cells have highlighted that 3,6-dihydroxyflavone is most important bioactive compound worthy of development for cancer chemotherapy.

Further, several compounds have been found to sensitize the path way of cancer chemotherapy. Selenium compounds are reported to affect numerous cellular events of cancer therapy following different molecular pathways leading to apoptosis. It has been reported that organically bound selenium therapeutically enhances the efficacy and selectivity of anticancer drug against tumor. An anticancer drug in presence of selenium methyl selenocysteine (MSC), a naturally occurring selenium compound, has been found to be active against malignant tumors.

**Fig. 1.2: 3,6-dihydroxyflavone**

**Selenium methyl selenocysteine** (MSC) is non toxic compared to any other selenium form and is completely bioavailable compound. It has strong antioxidative property. MSC exhibits significant protection against an oxidative insult. It facilitates cell damaging free radical in the body. MSC showed strongest chemoprevention against cisplatin induced loss of viability and ROS formation. MSC inhibits cell proliferation and induces apoptosis in several tumor cell lines, ovarian cancer, lung cancer, leukemia, mammary and breast cancer. It has been found to support five different genes (Trap 53, p21, Gadd 45 α & CMyc) in breast cancer. MSC is reported to be an effective growth inhibitor of mammary cell. Selenium enriched diet significantly reduced the expression of Per 2 and DBP m-RNA in mammary tumor in rats. MSC acts as a modulator of docetaxel efficacy against prostate cancer. MSC induces DNA fragmentation and performs better tumor vascular function. It decreased cdk2 kinase activity by delaying cell cycles and therapeutically enhances the efficacy and selectivity of irinotecan against human tumor xenografts. The combination effect has shown inhibition of tumor growth and increase apoptosis in tumor tissues. MSC is the most efficient among the other studied selenoamino acids and deemed to be a promising sensitizer.
Antioxidants also have anticancer or antimutagenic properties. Antioxidants being free radical scavengers are reported to enhance apoptosis pathway and inhibit mutation. Among various naturally occurring antioxidants, lutein is an oxy-carotenoid and powerful antioxidant. It is found in green leafy vegetables and characterized as primary nutrient. Lutein may prevent cellular damage by quenching singlet oxygen. In vitro and in vivo studies support for chemo preventive role of luteins against carcinogenesis. The mechanisms for a potential protective role of lutein against carcinogenesis include selective modulation of apoptosis, inhibition of angiogenesis, enhancement of gap junction intercellular communication, induction of cell differentiation, prevention of oxidative damage and modulation of the immunity system. Lutein among other carotenoids becomes more important because of its bioavailability, metabolism and dose response relationships with intermediate biomarkers having excellent clinical outcomes. The hydrophilic properties of lutein allow it to react with singlet oxygen generated in water phase more efficiently than other carotenoids. A detailed study on the chemo preventive role of the lutein in carcinogenesis is strongly recommended.

Fig. 1.4: Lutein

The present thesis deals with the nanotech enforcement and combination therapeutic exploitation of dietary chemicals (dietary flavonoid: 3,6-dihydroxyflavone; sensitizer: Selenium methyl selenocysteine and antioxidant: Lutein) for the enhancement in antioxidative (DPPH, Fenton H₂O₂ and NO free radical scavenging), antitumor activity in vitro (MCF-7 and MDA-MB-468 breast cancer cell lines) and in vivo (Female Balb/c mice induced with sarcoma 180 cancer cell line) experiments.