CHAPTER-1

INTRODUCTION

The failure of a cell to undergo its desired fate that is, either to divide or to die is an abnormality that allows a cell to multiply infinitely without any restriction, which ultimately leads to progression of a disease called cancer [1]. In colon cancer, the normal colonic epithelial cells undergoes immeasurable divisions to form polyps, these polyps are slow-growing benign tumors that carry a small risk of becoming malignant, eventually leading to cancer [2].

Colon cancer is a major killer worldwide and as per the Globocan report of the International Agency for Research on Cancer (IARC), the specialized cancer agency of the World Health Organization, it accounts for 10% of all cancers in men and 9.2% in females, worldwide. In India, 64,000 people were diagnosed with colon cancer, out of which 49,000 died in 2012 [3]. These alarming statistics of colon cancer incidence and mortality coupled with low survival rates has raised serious concerns on the use of current treatment options such as, radiotherapy and chemotherapy. This could be attributed to either the resistance developed by tumor cells or the associated adverse effects of conventional treatments [4]. Therefore, screening of newer therapeutic strategies is urgently required for successful management of this disease.

A decline in mainstream conventional therapies and adoption of unproven, unconventional, unorthodox and questionable treatments mainly termed as CAM (complementary and alternative medicine) therapies against cancer has opened up a new field where people across the world have started using the traditional systems for the treatment of various ailments, including cancer. According to the WHO, around 80% of the world’s population relies upon CAM therapy for their primary health care [5]. A survey conducted by Indian Council of
Medical Research revealed that 34.3% of cancer patients opted for traditional medicines before using allopathic medicines [6].

A variety of therapeutic or preventive health care practices followed worldwide are Homeopathy, Naturopathy, Unani, Siddha, Chinese traditional medicine and Indian herbal medicine [7]. Their popularity stems from their easy availability, cost effectiveness and the fact that they have been used for centuries. Over the years, several surveys have shown the potential role of various CAM therapies in cancer treatment [8-10].

The rationale behind this study was based on the belief that although several chemotherapeutic drugs were available in the market against cancer, but they failed to discriminate between the normal cells and the cancer cells, thereby leading to the various side effects, which many times prove to be fatal. Therefore, CAMs which have been around for centuries and whose therapeutic potential is known could selectively induce toxicity to cancer cells while at the same time sparing normal cells. Hence the identification of natural agents, either in form of plant extracts or homeopathic remedies which could successfully exhibit apoptotic and cell cycle modulating properties and at the same time show limited toxicity to normal cells is the need of the hour [11].

The aim of the study was to evaluate possible attributes in several well documented sources of CAM therapies ( shoots of Triticum aestivum (Wheatgrass), Achyranthes aspera roots, fruiting bodies of mushroom species- Pleurotus ostreatus, Macrolepiota procera and Auricularia polytricha and homeopathic drug- Ruia graveolens), which could render them as effective therapeutic agents. For this we explored various parameters against colon cancer derived COLO-205 cells, such as free-radical scavenging potential, cytotoxicity, anti-proliferative activity, clonogenicity, migration, apoptosis and changes in cell cycle. This is probably the first time that these CAM sources are being tested against COLO-205 cells. In order to understand the mechanism associated with cell death, we analysed the effect of most potent extract/homeopathic potency from each CAM source on apoptosis and cell cycle by performing semi-quantitative reverse transcriptase PCR of pro-survival and anti-survival
genes such as caspase-9, caspase-8, caspase-3, Bax, Bcl-2, p16, p21 and p27, followed by
determination of cell cycle changes by flow cytometric analysis.

To further verify whether these CAM sources had any significant effect on normal cells, we
analysed the role of these therapies (shoots of *Triticum aestivum* (Wheatgrass), *Achyranthes
aspera* roots, fruiting bodies of mushroom species- *Pleurotus ostreatus*, *Macroleptora
procera* and *Auricularia polytricha* and homeopathic drug- *Ruta graveolens*) on normal
epithelial cells (NRK-52E). The parameters studies were cytotoxicity, morphological
alterations, proliferation and apoptosis and we compared the effects with those of 5-FU (5-
Fluorouracil- A standard chemotherapeutic drug against colon cancer) treated NRK-52E
cells.

Combinatorial treatment of the most potent extract and homeopathic potency from each CAM
source in terms of apoptosis induction and cell cycle arrest, were assessed in order to
determine whether there was enhancement of cytotoxicity to the COLO-205 cells. A cocktail
mix was prepared using IC$_{50}$ concentrations of most potent extract and homeopathic drug
against COLO-205 cells. Moreover, the effect of cocktail mix was also checked on normal NRK-52E cells to emphasize the potential use of cocktail mix as an effective anti-cancer
therapy that selectively eliminates cancer cells only.

The experimental observations from this study could throw light upon the effectiveness of CAM sources as valuable chemotherapeutic agents.

**AIM OF THE STUDY**

1. Investigation of anti-cancer activities in various extracts of *Triticum aestivum*
   (wheatgrass) (HWE-hexane wheatgrass extract, CWE- chloroform wheatgrass extract,
   MWE- methanol wheatgrass extract and AWE- aqueous wheatgrass extract).

2. Exploration of anti-cancer properties of *Achyranthes aspera* root extracts (EAA-
   ethanolic *Achyranthes aspera* roots extract and AAA- aqueous *Achyranthes aspera*
   roots extract).


5. Cytotoxicity screening of CAM sources on normal epithelial (NRK-52E) cells.

6. Evaluation of stimulatory or inhibitory effect of cocktail mix (combination of most potent extract/potency from each CAM source) on colon cancer (COLO-205) and normal cell (NRK-52E) lines.