Scope of the study

Colorectal cancer (CRC) is the second most common cancer in developed countries and its incidence is apparently increasing in many developing countries like India. The incidence of new colon cancer patients in India is increasing day by day by westernized life style. A diet rich in high fat is associated with greater risk of colon cancer than the low fat diet. The aim of cancer control is to reduce the incidence of the disease, decrease the morbidity and mortality, as well as to ensure better quality of life through early detection, treatment, rehabilitation and palliation.

The three most accepted therapies for cancer include surgery, chemotherapy and radiotherapy. Each of these are restricted by their own set of limitations owing to factors which include, location, size, stage of malignancy present, along with the age and medical condition. Use of just one mode of treatment in a targeted approach is not sufficient to restrain the malignant growth, as it utilizes multiple pathways to bring about the whole process of carcinogenesis. Chemotherapy being a systemic approach impacts on wide range of tissues, whereas radiotherapy is comparatively more localized.

The goal of radiation therapy is to destroy or inactivate cancer cells while preserving the integrity of normal tissues within the treatment field. This modification is important to achieve the maximum effect of ionizing radiation on tumour tissue, while at the same time minimizing the effect on normal tissue. The side effects caused by radiotherapy to the normal dividing cells can be minimised if therapeutic dose given could be reduced, but the desired tumour suppression still should be achieved. With respect to the potential application of ionizing radiation in radiotherapy, the development of effective radio modifiers is of great importance. The use of naturally occurring compounds as radiation modifiers has become an important strategy in the field of radiotherapy.

Plant derived chemopreventive agents exhibit limited side effects, less toxicity and at the same time protect the normal cells against radiation. Over the past few decades, there has been a growing body of interest in

Combined therapeutic efficacy of CVC and X-radiation
Chapter 2: Scope of the Study

identifying naturally occurring chemopreventive agents, particularly those present in our diet. Carvacrol (2-methyl-5-(1-methylethyl-phenol; CVC), used in this study, is a monoterpenoid phenol which is present in many essential oils of the family Labiatae including Origanum, Satureja, Thymbra, Thymus, and Coridothymus species, has potent pharmacological properties such as antioxidant, antiproliferative, antiinflammatory and anticarcinogenic effects.

Based on the above knowledge, we wanted to test the hypothesis

- Whether CVC acts as a radioprotector to normal cells
- Whether CVC acts as a radiosensitizer to cancer cells
- Whether the combined therapeutic effect of CVC and radiation is effective against DMH induced colon cancer

To achieve this, the whole study was carried out in three phases and the following aspects were evaluated

**Phase I: Using human blood lymphocytes (in vitro study)**

**Specific objectives**

- To study the percentage cell survival by MTT assay and Dose Modifying Factor (DMF).
- To analyze chromosomal abnormalities by micronuclei (MN) formation assay and dicentric chromosome (DC) aberration assay.
- To evaluate DNA protective effect by comet assay and DNA fragmentation assay.
- To analyze the apoptotic changes by Acridine orange/Ethidium bromide (AO/EtBr) dual staining method.
- To evaluate the levels of lipid peroxidative byproducts such as thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH) and conjugated dienes (CD).
- To evaluate the status of endogenous antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH).

**Combined therapeutic efficacy of CVC and X-radiation**
Phase II: Using HT-29 and SW480 colon cancer cell lines (*in vitro* study)

**Specific objectives**

- To study the cell cytotoxicity by MTT assay.
- To visualize cell morphology using AO/EtBr dual staining and Hoechst 33258 staining.
- To evaluate the mitochondrial membrane potential using 5,5’, 6,6’-tetrachloro-1,1’, 3,3’-tetraethylbenzimidazolocarbocyanine iodide (JC-1) staining.
- To study the generation of reactive oxygen species (ROS) by 2-7-diacetyl dichlorofluorescein (DCFH-DA) assay.
- To investigate the extent of DNA damage by performing single cell gel electrophoresis (comet assay).
- To elucidate the level of lipid peroxidation end product TBARS.
- To study the enzymic antioxidants such as SOD, CAT and GPx and the non enzymic antioxidant, GSH.
- To study the expression of proteins associated with apoptosis and cell proliferation: Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2) and cyclin D1.

Phase III: Using male albino Wistar rats induced colon cancer by 1,2-dimethylhydrazine (DMH) (*long term – in vivo* study)

**Specific objectives**

- To observe the body weight and growth rate of the animals throughout the experimental period.
- To evaluate the incidence of colonic polyps/tumours and preneoplastic changes such as aberrant crypt foci (ACF), dysplastic aberrant crypt foci (DACF) and β-catenin accumulated crypts (BCAC).
To analyze the activities of lipid peroxidation by products (TBARS, LOOH, and CD) in the liver and colon of rats.

To assay the activities of enzymatic like SOD, CAT, GPx and glutathione reductase (GR) and non enzymatic antioxidants like GSH, vitamin C and vitamin E in the liver and colon of rats.

To examine the activities of hepatic and colonic xenobiotic metabolizing enzymes such as phase I and phase II enzymes.

To analyze the bacterial enzymes viz., β-glucuronidase, β-glucosidase, β-galactosidase, mucinase, nitroreductase and sulphanase in the colonic mucosa and faeces of experimental animals.

To visualize the effects on mucin screening goblet cells and mast cell counts.

To examine the cell proliferation markers such as argyrophillic nucleolar organizing region associated proteins (AgNORs), proliferating cell nuclear antigen (PCNA), and cyclin D1.

To study the expressions of Bax, Bcl-2, p53, homeobox protein (CDX-2), cytochrome C, caspase-3 and caspase-9 as indices of apoptosis.

To study the expressions of inflammatory markers such as interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2).

To determine the angiogenesis markers such as hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF).

To analyze the expressions of transcriptional factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), transforming growth factor-β (TGF-β) and Smad-4.

To investigate the radiation/stress markers such as p38 and c-Jun N-terminal kinase (JNK).

To analyze the gene expressions of DNA repairing genes such as human 8-oxoguanine DNA glycosylase 1 (hOGG1) and X-ray cross-complementation group 1 protein (XRCC1).

To visualize the histological changes of the liver and colon.