CHAPTER – I
INTRODUCTION

Diet is a key factor that influences the adult health and disease. The potential for immense socioeconomic benefit through successful characterization and exploitation of health promoting factors in foods is immense. The day-to-day food one consumes contains in addition to the basic nutrients, innumerable biomolecules which may have the potential to provide solid health benefits. There is an increasing interest in understanding the chemical properties of such biologically active substances and engaging them in promoting health and preventing the occurrence of certain disorders. It has been assumed to be possible for a reduction of 35% in the age standardized incidence of cancer (World Cancer Research, 1997).

1.1. The impacts of diet on health

Diet and nutrition may play important role in the maintenance of health and in the overall prevention of disease. Dietary supplements represent an important source of essential nutrients, since they are widely used. Evidence that diet is a key factor affecting the incidence of many chronic diseases is overwhelming (World Cancer Research, 1997). The food we eat contains thousands of biologically active substances, many of which may have the potential to provide substantial health benefits (Peterson, 1998).

Indeed, several food-derived compounds such as sulforaphane, curcumin, lycopene, and tea polyphenols are among the most promising chemopreventive agents currently being evaluated (Kelloff et al., 2000). The full extent of impacts mediated by biologically active components in our diet is unknown, and our understanding of their mechanisms of action is even more limited. Much of the available data has been derived from in vitro studies with purified compounds in forms and concentrations to which the tissues in our bodies may never be exposed.
1.2. Nutraceuticals

Human quest for alternative medicine or health promoters resulted in identifying nutraceuticals. Nutraceuticals are natural ingredients that exist in food, which beyond their nutritional contributions, are considered as sources of health benefits. A nutraceutical therefore could be defined as “food or any constituent part of food, which provides medical or health benefits, including the prevention and/or treatment of disease” (DeFelice, 2002).

Identification of these foods will allow their inclusion into a number of health-promoting dietary regimes and may enhance the development of new food products. Apart from satisfying basic nutritional requirements, their ingredients exert tremendous impacts on the health care system, possibly by mediating a wide range of effects which may include management, treatment and prevention of disease. Nutraceuticals provide a different approach to medicine, one based on improving the health or wellness of the whole body with the aid of nutrition.

The nutraceutical vary in their chemical nature. They may be “phytochemicals and zoochemicals derived from edible plant and animal products such as Echinacea, St. John’s wort, oils from fish and flax seed, glucosamine and chondroitin, calcium-fortified juices and plant sterol containing substitutes for butter. Although new discoveries are being made almost daily, proving the anti-cancer effects of green tea, the cholesterol-lowering properties of fish oil or the cardiovascular benefits of dietary fiber; reliable knowledge regarding the effects of nutraceuticals is still in its infancy. The specific activities of most of these agents yet remain to be established.

The use of plant extracts and derivatives of plants for healing and preventing disease has been described extensively in traditional and folk medicine literature. Over the centuries, plants have served as a major source of medicines for treating and prevention of diseases affecting mankind. Inspite of new technology for synthesis and design of new drugs, phytomedicines derived from plants still hold a strong position as therapeutic agents in combating ailments. For centuries; specific plants, their extracts or mixtures comprising useful combinations of plant chemicals have been used for treatment of illnesses in the indigenous Indian system of medicine and many of them have been documented as being clinically effective in treating illnesses.
Various plants like Alfalfa, Aloe vera; Eucalyptus, Fenugreek, Garlic, Ginger and Ginseng have been reported to possess hypoglycemic herbal ingredients. Though a multitude of potentially beneficial products is presently available, very few of these have been clinically tested to properly assess their benefits in medical health and risks contained in their usage.

Active compounds are being sought after from both plant and animal sources, in order to be used as pharmaceuticals as well as food ingredients. The primary purpose served in using nutraceuticals is to help prevent disease rather than to cure it. Possible functional components include amino acids, animal pigments and extracts from specific animal species, such as chondroitin.

People are increasingly getting aware of importance of nutraceuticals. According to a study 72% of those surveyed in the USA take supplements to feel better, 67% to prevent illness, 50% to live longer, 37% to build muscle and strength, 12% for weight management; and 33% take these supplements through the guidance of a physician. Significantly, 53% believed that the benefits offered by nutraceuticals could not be matched by conventional drugs, and 56% alleged they offered benefits comparable with drugs, only with fewer side effects. Remarkably, 95% were satisfied with supplements (Dietary Supplement Education Alliance, 2001).

Biomolecules that are tested for its preventive role is given in Table 1.1. Herbal medicines have great potential and are often considered as foods as well as medicines and are used in preventative and curative treatments throughout the world. Although there is a large amount of anecdotal evidence supporting its use, the scientific studies to support these claims is in its infancy stage in many cases. Research of this kind could highlight that a particular plant may have active constituents.

1.3. Diet and modulation of gene expression

A complete knowledge of the biologically active components available through our diet is not yet fully available. Our understanding of their mode of action in modifying the physiological and biochemical function is very much limited. To organize our understanding of the relevance of these bioactive molecule, what is needed is a physiologically and biochemically relevant model system. At least two
Table - 1.1: Nutraceuticals tested
(Kelloff et al., 2000; Liu et al., 2002; Etminan et al., 2005; Dietary Supplement Education Alliance, 2001).

<table>
<thead>
<tr>
<th>Nutraceutical</th>
<th>Disease / Condition</th>
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<tbody>
<tr>
<td>Soy protein (genistein)</td>
<td>Coronary heart disease (reduces low-density lipoproteins)</td>
</tr>
<tr>
<td>Stanol/sterol esters</td>
<td>Coronary heart disease (reduces low-density lipoproteins)</td>
</tr>
<tr>
<td>Omega-3 oil</td>
<td>Coronary heart disease</td>
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<tr>
<td>B-glucans</td>
<td>Coronary heart disease</td>
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<tr>
<td>Cranberry</td>
<td>Urinary tract infections</td>
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<tr>
<td>Soy isoflavones</td>
<td>Breast, prostate and bone cancer</td>
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<tr>
<td>Phytoestrogens</td>
<td>Cognition in post-menopausal women</td>
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<tr>
<td>Black cohosh</td>
<td>Menopausal hot flashes (serotonin modulation)</td>
</tr>
<tr>
<td>Chinese mushrooms</td>
<td>Prostate and bladder cancer</td>
</tr>
<tr>
<td>Black haw (<em>Virburnum prunifolium</em>)</td>
<td>Premenstrual tension</td>
</tr>
<tr>
<td>Chasteberry (vitex)</td>
<td>Menstrual disorders</td>
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<tr>
<td>Polyphenols</td>
<td>Cancer</td>
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<tr>
<td>Isothiocyanates</td>
<td>Cancer</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Cancer</td>
</tr>
<tr>
<td>Green tea</td>
<td>Cancer</td>
</tr>
<tr>
<td>Lutein</td>
<td>Muscular degeneration</td>
</tr>
<tr>
<td>Cucurmin</td>
<td>Cancer</td>
</tr>
<tr>
<td>Tea polyphenols</td>
<td>Cancer</td>
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</table>
broad areas of research may be required to interpret the relevance and importance of these biomolecules, first is the characterization of the extent and rate of absorption, tissue dispersal and site specific targeting of metabolically relevant compounds. And the second is a comprehensive study of time and dose effects of these biomolecules. The components of the diet or its derivatives can modulate the physiological processes even at a molecular level. Figure 1.1 presents a scheme of the dietary modulation by influencing gene expression.

1.4. Dietary proteins

There is a dearth of scientific data over the role of dietary protein and amino acids in the prevention of various diseases. A limited number of protein studies investigating only the absorption rate of amino acids from specific protein sources such as casein, whey, milk, pea, egg, soy and meat have been conducted. Most of the studies carried out so far did not provide clear data on dietary protein intake and its mechanism of disease prevention.

Therefore, to study the possible preventive/protective mechanism of action of selected protein isolates, the following proteins are chosen for study. The present study addresses the health-protective effects of selected protein isolates from five different sources, namely soy, garlic, coconut, whey and casein.

Scientific studies offer evidence suggesting that consumption of soy protein in the diet is associated with reduction in the risks leading to coronary heart disease. Soy isoflavonoids, especially genistein and daidzein, exist as components of soy protein that possess antioxidant properties and are involved both in the regulation of circulating lipid levels (Van et al., 2000) as well as in lowering cancer risk (Lamartiniere, 2000).

Supplementation of animal protein with vegetable protein has been reported to reduce lipid levels in experimental animals and humans. Coconut kernel contains 5-6% proteins, globulins being the major fraction, showed cholesterol lowering activity and found to reduce myocardial infarction (MI). Coconut kernel contains various enzymes, fats and carbohydrates. The milk of fresh coconut forms a valuable food for children suffering from nutritional deficiency. In addition it contains a high quality protein which is valuable for growth and repair of the body (Mini et al., 2002).
Figure 1.1: A schematic representation of the steps involved in gene expression, and the stages at which diet can modulate these processes (Ruan and Teng, 2002)

(a) Regulation by diet

Gene expression process

- DNA
  - Transcription, RNA processing and stability
  - RNA
  - Translation, Modification, and stability
  - Protein

Cell

- Metabolites
  - Health

(b) Earliest markers of food bioactivity

- Cascade of changes in gene expression with scope for return to healthy state through dietary modification
- Reduced scope for return to healthy state through dietary modification

Diet

- ‘Pivotal’ changes in gene expression
  - Abnormal gene
  - Cellular dysfunction
  - Disease
Garlic protein may protect the blood vessels from deleterious effects of free radicals and exerts a positive influence on blood lipids and thereby increases capillary flow and lowers elevated blood pressure and prevents the development of arteriosclerosis. Several studies have shown that garlic and its constituents have a wide array of biological activities, which includes antioxidant properties, inhibition of tumorigenesis and suppression of platelet aggregation (Gulizar, 2004).

Whey protein contains a multitude of components namely lactalbumin, lactoglobulin, immunoglobulin, lactoferrin, lactoperoxidase and many other biologically active components. It is also found to contain high concentrations of glutathione. Glutathione has been shown to have both antioxidative and xenobiotic binding functions (Hakkak et al., 2001).

Dietary casein protein is known to enhance the immune system and promote host protection against the development of cancer in laboratory animals. Like whey proteins, casein proteins are composed of biologically active peptides, particularly α and β casein fractions which have immunomodulatory functions. Peptide residues derived from the pepsin/trypsin hydrolysis of αs1-caseins were found to significantly suppress mitogen-induced proliferation of human peripheral blood mononuclear cells in vitro (Kayser and Meisel, 1996). Although epidemiological data and preclinical studies are virtually promising, clinical studies to ascertain the effect of these milk peptides have not yet been carried out (Groziak and Miller, 2000).

1.5. Scope of the Work

The study was conducted in order to assess the effects of dietary regimes, especially proteins in the diet in preventing the incidence of hepatocellular carcinoma (HCC) and cardiovascular disease (CVD) in mice. The assessment was carried out by monitoring the changes in biochemical parameters by comparing the incidence of cancer and myocardial infarction specific enzymes and antioxidant enzyme levels in serum and respective tissues (liver and heart). To ascertain the suitability of these concentrates in diet, additionally the study included the antibacterial activity and allergic effects of these proteins.
1.5.1. Objectives and Hypotheses
A) The high incidence of i) Cancer, in particular; liver cancer and ii) Cardiovascular diseases when considered in the global and Indian perspective, is alarming.

B) Although treatment and curative measures in both these diseases are available, the cost is beyond the reach of common man and the side-effects of allopathic regimens are painful.

C) Therefore, the need for proper nutraceutical intervention as a preventive/curative measure for these diseases is the felt need. Hence, the objective of the work is to address this situation and seek a remedy for these disorders through common nutraceuticals. The protein from the common food soy, garlic, milk (casein and whey) and coconut are the candidates of the present study.

1.5.2. Hypotheses

On the basis of the observations given above, and the review of literature available, the following hypotheses were formulated for approaching the study:

i) The prevention/cure of cancer and cardiovascular disease through dietary regimes is a better approach in tackling cancer and cardiovascular disease; as both these do not have specific cures.

ii) Proteins would act not only as nutrient but also would act as nutraceuticals in preventing/curing cancer.

The study consists of two broader aspects

Part I. A study of the candidate proteins
A) 1. A comparison of the aminoacid profile of the candidate proteins.
2. Analysis of the peptide fragments by MALDI-TOF MS and comparison using bioinformatic tools.

B) 1. Antimicrobial activities of the proteins.
2. Dietary safety of the proteins-allergic activity.

Part II. The effect of the candidate proteins in disease control
1. The preventive role of selected protein isolates on induced hepatocellular carcinoma.
2. The protective role of selected protein isolates on induced myocardial infarction.
PART-I

A STUDY OF THE CANDIDATE PROTEINS
**PART-I** is subdivided into **Part-IA** (Chapter-2) and **Part-IB** (Chapters 3 and 4)

**Part-IA** deals with two experiments with reference to the Candidate proteins.

*Experiment -Ia-1:*
A comparison of the amino acid profile of the candidate proteins

*Experiment -Ib-2:*
Analysis of the peptide fragments by MALDI-TOF MS and comparison using bioinformatic tools

**Part-IB** deals with two experiments.

*Experiment –II*
Antimicrobial activities of the proteins

*Experiment –III*
Dietary safety of the proteins-allergic activity

Each experiment has its introduction, materials and methods, results and discussion.