Chapter-1
Introduction

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1.1 INTRODUTION

Earliest evidence available at present is found in the form of tumours in human bones from the prehistoric and early historic periods even though tumours must have acquired at all the times in human and animals at all parts of the world. The Ramayana (one of the greatest epics), a manuscript of ancient India (2000 BC) is the first instant in recorded history through which malignant tumours are evidenced. A reference for tumour treatment has been made in it in which the treatment may be carried out either with knife (Surgery) or with an arsenical compound (Chemotherapy). The Egyptians were the first to realise the tumours originating at different parts of the body differs in their behaviour (signs and symptoms) and should be treated differently. The above said descriptions were evidenced in various Egyptians papyri (1500 BC). In this context Hippocrates of Cos (460-375 BC), the father of medicine, coined the term 'Karkinoma' for solid tumours. The term 'Cancer' appears much later, derived from the Latin word 'Cancrum' denoting 'Crab' (Diamandopoulos and Meissner, 1985).

Carcinogenesis is a multifactorial, multifaceted and multimechanistic disease requires a multidimensional approach for its treatment and it involves fundamental biological process concerning

disorganised cell replication, cell death and disorganisation of tissue structure. Biotic system maintain their species specificity and identity mainly by the unchanged genomic makeup throughout their life period, especially in multicellular organisms, the ratio of cell growth and cell death is strictly balanced through developmental process and homeostasis(Bolsover *et al.*, 1997). When the organism attains adulthood, the cell division is limited and confined to a particular site despite various exogenous and endogenous factors affect the normal pattern of cell growth which may results to cancer.

According to Ponder (2001) the sequence of transformation in a cancerous cell may express the phenotypic responses in six different ways such as

(i) disregardable response of cells to the signal to control proliferation

(ii) the cells to disregard the signal to differentiate

(iii) the possession of the cell for substantial proliferation

(iv) the ability of the cells to evade apoptosis

(v) the ability of the cells to evade angiogenesis and

(vi) the invasive ability of cancer cells.

Certain specific substances or specific condition can have the ability to convert normal cells into a mutant cancerous cell by many factors such as the functional and metabolic status of a presumable cell to become cancerous especially due to reactive oxygen species (ROS). The outcome of cancer may be due to the activation of proto-oncogenes into oncogenes, genetic predisposition (Cancer induced through mutation of hereditary type

gene) and inactivation of the tumour suppressor gene. Occurrence of carcinoma eventually attained by three basic sequential ways such as changes in particular DNA sequence, changes in gene complex and chromosomal aberrations (Prabha *et al.*, 2003). Tumour can be benign (not cancer) or malignant (cancer)-the malignant tumour cells can travel through the blood stream or lymphatic system and can invade and damage the nearby tissues and organs to form new tumour, the spread of cancer is called metastasis.

Nowadays, Cancer stands second for the most common cause of Death, universally. Cancer is ultimately the result of interplay between the environmental (exogenous) and the genetic (host or endogenous) factors. It is generally thought to be caused by the agents external to the body, eventhough it is difficult to determine the exact nature of the carcinogen (Pardee and Reddy, 1982). The cancer inducing substances may be of Biological, Physical and Chemical origin.

Foulds (1975) is of the opinion that cancer is a two step process. The first and initial stage is called Initiation. This must precede the stage of promotion. A single or short term exposure to a chemical by oral, inhalation or dermal route may bring about an irreversible but morphologically imperceptible genetic changes. According to his opinion, some of the cells or tissues may be conditioned for tumour development, if the stage of initiation is followed by repeated exposure to same or different chemical which is a promoter. In the absence of promotion these initiated cells remain dormant and cannot be histologically demonstrated to be anyway different from the surrounding cells. Promotion brings out the phenotypic expression of genotypic alteration brought about by initiation. In this context Slaga *et al*

(1980) demonstrated that the promotion stage can be further subdivided into stage 1 and stage 2, where stage 1 of promotion consists of expansion of altered clones of Initiated cells. Where stage 2 comprised of further proliferation of these altered clones into a full-fledged tumour. If the same chemical is both capable of initiating and promoting the tumour development, is known as carcinogen or complete carcinogen, whereas those chemical which are capable of initiating alone are called initiators and those promoting either the stage 1 or stage 2 are called as promoters.

Chemical carcinogenesis is a complex, multi step process and cancer usually requires several decades to develop in humans. Most cancers are derived from a single cell, is clonal in origin. The transformation of a normal cell into a malignant cell requires multi step mutations (Barrett., 1993). The carcinogenic process can be induced by both mutagenic and non mutagenic chemicals and is also influenced by non carcinogenic chemicals (Wattenberg., 1993). Thus cancer development involves multi step mutations, multi step causes and multi step mechanisms. Consequently, both mutagens and mitogens can influence the carcinogenic process (Boyd and Barrett., 1990). Tumour promotion is generally considered to involve both the modification of the initiated cells and their progeny, whereas tumour initiation occurs rapidly and largely irreversible. Tumour promotion requires repetitive stimuli and appears to be partially reversible (Vogelstein *et al.*, 1988).

Especially due to the great advances in industrial progress, human population are exposed to various xenobiotics such as mineral oil and other petroleum derivatives, pesticides and fertilizers from food production industries, volatile solvent from textiles, automobiles and metallurgical

industries etc., Recent studies by World Health Organization (WHO) have sent a warning signal that "increased environmental pollutants is responsible for the toxicities in human population world wide" (Murray *et al.*, 2001).

It is reported that perchlorethylene is one among the chemicals released from metallurgy and textile industries, throughout the world This chemical is a colourless volatile halogenated hydrocarbon (chloroalkene). It is the ethylene molecule substituted with chlorine atoms at 1,1 and 2,2 position of the carbon atoms, with a strong lipophilic character and termed as a carcinogen. It has been widely used as a solvents especially in metal industries for degreasing purpose and textile industries for cleaning or bleaching purpose and also at the site of production, acts as a direct contaminant at the point source, when discharged through outlets finds their way to the water sources and serves as a common pollutant and also acts as an indirect dietary carcinogen through food chain by means of bio-accumulation and bio-magnification. Perchlorethylene is an uncharged, non-polar and highly lipophilic compound and consequently can cross the gastrointestinal mucosal barrier by passive diffusion. In human, absorption through gastrointestinal mucosa is extensive, as evidenced by the numerous cases of poisoning by oral ingestion (Defalque, 1961). Although, number of workers have studied the impact of perchlorethylene on cardiac arrhythmias, CNS depression, cytotoxicity and/or carcinogenicity of kidney and lung but there is paucity of information regarding the influence of perchlorethylene on liver toxicity in rats, therefore it is of interest to investigate the effect of perchlorethylene on the liver of rats.

Any practical solution in combating this dreadful disease is bound to be of paramount importance. An alternate solution in this concern is the use of the medicinal plant preparation to arrest the insidious character of the disease. There is a strong belief that the traditional systems of medicine possess drugs for certain conditions, particularly for cytotoxicity that too related with liver diseases, for which modern system offers inadequate or no remedies. Herbal medicines are now in great demand in the developing world for primary health care not only because they are inexpensive but also for their better cultural acceptability, better compatibility with the human body and with minimal contra effects. About 2000 plants of therapeutic value are mentioned in Ayurveda, Siddha and Unani system of medicine. Out of these quite a large number of plants have been claimed to possess hepatoprotective activity. Medicinal plants are one of the important oldest sources of pharmacologically active compounds and used for the treatment of various diseases. The above mentioned property of medicinal use of plants in therapeutics is found in the Rig Veda, one of the oldest, repository of human knowledge had been written between 4500 to 1600 B.C. Ayurveda (the science of life) is a latter production and considered as an Upaveda, which defines the properties of drugs and their uses. Ayurveda, a 5,000 year old healing tradition, rooted in ancient Indian culture. This vast body of healing knowledge sometimes referred to as the "Mother of All Healing".

Herbs or medicinal plants used in the traditional system of medicine contain biologically active secondary metabolites, which are not scientifically screened for its clinical efficacy. Traditional system of medicine, which is the only accessible health care system for most of the population in

rural areas and it should be scientifically evaluated so as to improve the clinical efficacy and safety of medicinal plants.

Hence the search for an effective hepatoprotective drug still continues. For the above mentioned reasons it is necessary to take steps to evaluate, to develop, to validate and to promote such natural medicines with standard of safety and efficacy can certainly revitalise the treatment for cytotoxicity.

Since, most of the synthetic drugs used to cure the liver diseases may produce other complications such as cytotoxicity and side effects, it is of interest to investigate the cytoprotective effect of *Terminalia chebula* against perchlorethylene induced cytotoxicity. In this context *T. chebula* has been studied for its anticancer effect on *in vitro* cytotoxicity evaluation on cultured human tumour cell lines including A-549, SK-OV-3, SK-MEL-2, XF-389, HCT-15 (Lee *et al.*, 1995), methanolic extract of *Terminalia chebula* is reported to have a high potential for inhibiting the growth of leukemia cells, attributed to arjunglucoside I and arjungenin (Creencia *et al.*, 1996), a tannin fraction from the dried fruit pulp of *Terminalia chebula* is reported to have antimutagenic activity *in vitro* (Kaur *et al.*, 1998). Ammar Saleem *et al* (2002) reported that *T. chebula* fruit extract decreases the number of cells in immortalized and cancer cell lines by inhibiting the rate of cell proliferation and inducing cell death. At low concentration of *T. chebula* extract is able to initiate cellular pathways that lead to apoptosis whereas at high concentration the extract has direct toxic effects, which leads to rapid necrotic cell death but there is no information available regarding the efficacy of *T. chebula* in *in vivo* anticancer effect against perchloroethylene induced cytotoxicity in rats.

One of the most attractive approaches to disease prevention involves the use of specific nutrients to protect tissues against cytotoxicity, carcinogenicity and acid degenerative diseases (Ames, 1983). The most widely studied protective agents are the antioxidant nutrients i.e., Vitamin E, Vitamin C, Vitamin A and numerous poly phenolic compounds (Pascoe and Reed, 1989). In liver cytotoxicity, vitamin E (tocopherol) – a lipophilic vitamin will render protection by preventing the lipid peroxidation in biological membranes (Tappel, 1973).

As stated above, polychlorinated hydrocarbons are well known to cause cytotoxicity in industrial concern. The previous literature evidence showed that some natural antioxidants like vitamin E and some nutritional supplements like α-deoxy D-glucose has the potential to protect the living system against perchlorethylene at the same time with a minimal side effects (Ebrahim *et al.,* 1996), but the protectivity rendered by botanicals against the perchlorethylene toxicity has not been studied so far. So, it paves the way to trigger my mind to explore the ameliorating efficacy of *Terminalia chebula,* with reference to the protectivity offered by Vitamin-E, a natural antioxidant. Even though there are more than 50,000 chemicals arose in the market nowadays and liver being the main organ of metabolism, easily getting affected by the chemical exposure, surprisingly there are only a few hepatoprotective agents available, that too were mainly of herbal formulations, it is necessary to study the botanicals to screen out their protective potential, without causing any side effects to the other vital organs for the development and promotion of one of the best hepatoprotective agents.

Hence the present study takes a closer look at one group of common pollutants, perchloroethylene, belongs to the category of polychlorinated hydrocarbons (PCHs) and to evaluate protective potential rendered by the Botanical, *Terminalia chebula,* with reference to α-tocopherol.

Keeping the above said lacunae in mind, the present investigation was performed with the following objectives.

Objectives of the present study

- To induce the cytotoxicity using perchlorethylene and to evaluate the protective and modulatory potency of ethanolic fruit rind extract of *Terminalia chebula,* during its simultaneous treatment.

- To compare the protectivity of ethanolic fruit rind extract of *Terminalia chebula,* with reference to α-tocopherol with the same conditions followed as that of the previous objective.

- To investigate the histopathological changes and biochemical changes, if any upon the administration of perchlorethylene, ethanolic fruit rind extract of *Terminalia chebula* and α-tocopherol.