Introduction

1. INTRODUCTION

Oxygen is the essential molecule for all aerobic organisms, and plays predominant role in ATP generation, namely, oxidative phosphorylation. During this process, reactive oxygen species (ROS) are produced as by-products, which seem indispensable for signal transduction pathways that regulate cell growth and reduction-oxidation (redox) status (Fang *et al.*, 2009; Desai *et al.*, 2010).

Due to the highly reactive nature of the ROS, which can damage DNA, proteins and lipids, cells utilize antioxidative or defense systems to balance these toxic products to keep the cells in a state of redox homeostasis. However, under the situation of imbalance in redox status, depending on the magnitude of ROS encountered, high levels of ROS can induce apoptosis (Lau *et al.*, 2008).

Oxidative and antioxidative processes are associated with electron transfer influencing the redox state of cells and the organism. The changed redox state stimulates or inhibits activities of various signal proteins, resulting in a changed ability of signal pathways to influence the fate of cells. At present, the opinion that oxidative stress is not always harmful has been accepted. Depending on the type of oxidants, intensity and time of redox imbalance and the type of cells, oxidative stress can play a role in the regulation of many important processes. They exert their action by modulating the signaling pathways, influencing synthesis of antioxidant enzymes, repair processes and inflammation. They also manipulate the process of apoptosis and cell proliferation, and thus malignancy (Durackova, 2009).

If the increase in free radicals is greater than the ability to neutralize them, the radicals will attack cellular components, especially lipids. The attack on lipids initiates a chain reaction called lipid peroxidation, which leads to the generation of more radicals and ROS that can harm other cellular components (Brambilla *et al.*, 2008).

The harmful effects of ROS are counterbalanced by the antioxidant action of both antioxidant enzymic and non-enzymic antioxidants. Antioxidants are chemical compounds that give an electron to free radical moieties and convert them to a harmless configuration, avoiding damaging chain reaction, which can involve lipids, proteins, enzymes, carbohydrates, DNA and nuclear membranes up to the cell death. Being either

exogenous or endogenous, they prevent the oxidation-induced damage, a process that causes damage in all tissues through the chemical reactivity of free radicals (Iannitti and Palmieri, 2009). However, despite the presence of the cell's antioxidant system, oxidative damage accumulates during the life cycle. Such accumulated damage has been proposed to play a pivotal role in the development of age-dependent diseases such as atherosclerosis, arthritis, neurodegenerative disorders and cancer (Balsano and Alisi, 2009).

Growing evidence suggests that reactive oxygen species (ROS) within cells act as second messengers in intracellular signaling cascades, which induce and maintain the oncogenic phenotype of cancer cells. ROS are tumorigenic by virtue of their ability to increase cell proliferation, survival, cellular migration, and also by inducing DNA damage leading to genetic lesions that initiate tumorigenicity and sustain subsequent tumor progression (Storz, 2005).

ROS have also been proposed as common mediators for apoptosis. Recent studies have demonstrated that the mode of cell death depends on the severity of the oxidative damage. Apoptosis (or programmed cell death) is a series of genetically controlled events that result in the removal of unwanted cells. Apoptosis is an important method of cellular control. Deregulation of apoptosis has been implicated in numerous pathological conditions including cancer (Bensassi *et al.*, 2009).

Cancer initiation and progression have been linked to oxidative stress, a condition in which the balance between the production and the disposal of reactive oxygen or nitrogen species is altered. Oxidative stress has several protumorigenic effects, such as increasing DNA mutation rate or inducing DNA damage, genome instability and cell proliferation (Visconti and Grieco, 2009).

Chemoprevention, a relatively new and promising strategy to prevent cancer, is defined as the use of natural dietary compounds and/or synthetic substances to block, inhibit, reverse, or retard the process of carcinogenesis. The chemopreventive effects elicited by the natural dietary compounds are believed to include antioxidative and anti-inflammatory activities, induction of phase II enzymes, apoptosis, and cell cycle arrest (Pan and Ho, 2008).

Cancer chemopreventive agents block the transformation of normal cells and/or suppress the promotion of premalignant cells to malignant cells. Certain agents may achieve these objectives by modulating xenobiotic biotransformation, protecting cellular elements from oxidative damage, or promoting a more differentiated phenotype in target cells. Conversely, various cancer chemopreventive agents can encourage apoptosis in premalignant and malignant cells *in vivo* and/or *in vitro*, which is conceivably another anticancer mechanism (Hail *et al.*, 2008).

One can anticipate lots of changes in the metabolism of cancer due to a fight between a normal cell and a cancer cell and also due to logistic suppression of normal cells by cancer cells (Kiruthika *et al.*, 2009). A major complication of chemotherapy is toxicity to normal cells, which is due to the inability of drugs to differentiate between normal and malignant cells. This often impacts the efficacy of the treatment and even makes it impossible to cure the patients. One of the requisite of cancer chemopreventive agent is elimination of damaged or malignant cells through cell cycle inhibition or induction of apoptosis without or with less toxicity in normal cells.

Thus, the search for natural products represents an area of great interest in which the plant kingdom has been documented as the most important source to provide many antioxidant and cancer chemopreventive agents with novel structures and unique mechanisms of action. The traditional knowledge, with its holistic and systems approach, supported by experimental base can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines (Tian *et al.*, 2007).

There is great scope for overcoming the moral dilemma inherent in experiments using live animals by reducing the numbers of animals required, minimizing any suffering caused, and, in many cases, replacing them altogether through the development, validation and application of alternative methods. *In vitro* assays are increasingly being used in drug metabolism studies to screen novel chemicals. Their advantages are two fold: first, they allow testing early in the drug discovery phase, providing important information on chemical characteristics; second, human cells or cell constituents can be utilized, increasing the relevance to man (Balls, 2009; Rose *et al.*, 2009). The alternative systems that have been popular include tissue slices, primary culture, cell lines and lower organisms like *Saccharomyces cerevisiae, Drosophila melanogaster* and many more (Kniewald *et al.*, 2005; Marin and Vallejo, 2005).

Medicinal plants constitute an ever-expanding goldmine of medicinal preparations, with the added advantage of minimal or no side effects. The identification and scientific validation of rich sources of valuable pharmaco-phytochemicals among the rich biodiversity, as prevailing in India, can provide a cure for many dreaded diseases and disorders. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage but also to rationalize the use of natural products in health care.

With this backdrop, the present study was formulated to analyze the antioxidant potential of *Zea mays* leaves. *Zea mays* is commonly known as makkacholam or maize. It belongs to the family Gramineae. Although it has a history of usage in treating various disorders, no systematic study has been undertaken on analyzing the extracts of *Zea mays* leaves on oxidant challenged events *in vitro*. Hence, the study was formulated with the following objectives:

- To assess and compare the antioxidant status of *Zea mays* leaves at different time periods of growth
- To conduct *in vitro* studies to establish the antioxidant activity against potential target biomolecules
- To analyze the antioxidant effects of *Zea mays* leaf extracts in simulated *in vitro* systems challenged with a standard oxidant
- To identify the active principle rendering the protective effects of *Zea mays* leaves

The vast literature available pertaining to the study was collected and scrutinized. A very brief review of the same is presented in the next chapter.