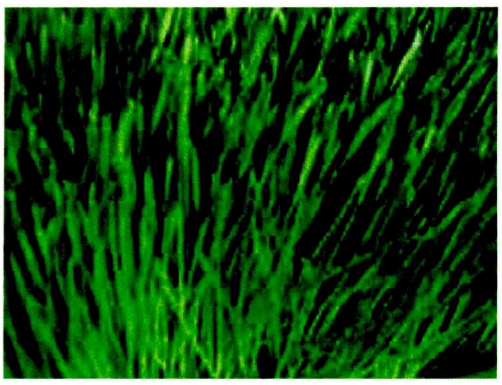

Introduction

1. INTRODUCTION

Plants have provided man with all his needs in terms of shelter, clothing, food, medicines, flavours and fragrances. Plants have formed the basis of sophisticated traditional medicine systems, among which are Ayurveda, Unani and Chinese systems of medicine. These systems have given rise to some important drugs in use today (Gurib-Fakim, 2006). The use of herbal medicines in the developed world is widespread and is increasing. Herbal medicines, which include a wide spectrum of substances ranging from home made teas to the national regulatory bodies-approved medicinal substances, are defined as plant-derived products that are used for medicinal and/or nutritional purposes (Kuczkowski, 2006). Medicinal plants are most important source of life saving drugs for a majority of the world's population (Debnath *et al.*, 2006).

Oxygen free radicals, or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems (Alexandratou *et al.*, 2005).

Overproduction of ROS (arising either from mitochondrial electron transport chain or excessive stimulation of NAD(P)H) results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins and DNA. In contrast, the beneficial effects of ROS/RNS (e.g., superoxide radical and nitric oxide) occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as seen in the defense against infectious agents, the function of a number of cellular signaling pathways, and the induction of a mitogenic response. Ironically, various ROS-mediated actions in fact protect the cells against ROS-

induced oxidative stress and re-establish or maintain "redox balance" also termed as "redox homeostasis" (Valko *et al.*, 2007).

Reactive oxygen species (ROS) play a central role as second messengers in many signal transduction pathways, where they can post-translationally modify proteins via the oxidation of redox sensitive cysteine residues. The range of cellular processes under redox regulation is extensive and includes both the proliferative and apoptotic pathways. Control of the cellular redox environment is essential for normal physiological function and perturbations to this redox balance are characteristic of many pathological states. Oxidative stress is particularly prevalent in cancer, where many malignant cell types possess an abnormal redox metabolism, involving down-regulation of antioxidant enzymes and impaired mitochondrial function (Giles, 2006).

Oxidants play a significant role in the pathogenesis of a number of diseases. Oxidative stress may be defined as an imbalance between cellular production of ROS and antioxidant defense mechanisms. ROS (e.g., superoxide radical, peroxynitryl, hydroxyl radical and hydrogen peroxide) are constantly produced as a result of metabolic reactions in living systems (Geronikaki and Gavalas, 2006). Nitric oxide (NO) reacts with superoxide ($O_2^{\bullet-}$) forming peroxynitrite (PXN) ($ONOO^{\bullet-}$), a strong oxidant which reacts with several biomolecules leading to enormous implications in biological process (Kamat, 2006).

Loads of reactive oxygen species (ROS), including superoxide anion and nitric oxide, that overburden antioxidant systems induce oxidative stress in the body. Major cellular targets of ROS are membrane lipids, proteins, nucleic acids and carbohydrates (Tsukahara, 2007).

Lipid peroxidation is an important process in oxygen toxicity. Free radicals inflict this damage by attacking polyunsaturated fatty acids, thus setting off a deleterious chain reaction that ultimately results in their disintegration into

malondialdehyde (MDA), 4 hydroxy-2-nonenal (HNE) and other harmful by-products (Kurien and Scofield, 2006). These products are closely related to carcinogenesis and have been suggested as modulators of signal pathways related to proliferation and apoptosis, two processes implicated in cancer development (Marquez *et al.*, 2007).

Deoxyribonucleic acid is another particularly important target for oxidation, as its damage may lead to heritable alterations. Increased ROS production may lead to an increase in the oxidative damage to DNA (Wikman *et al.*, 2000). ROS-induced DNA damage involves single- or double-stranded DNA breaks, purine, pyrimidine or deoxyribose modifications and DNA cross-links. DNA damage can result in either an arrest or an induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with several pathogenetic processes including cancer (Valko *et al.*, 2006).

Oxidative imbalance is considered an important factor in the etiology of several conditions like myocardial infarction, rheumatoid arthritis, autoimmune disorders, haemorrhagic shock, cataracts, aging and cancer (Duthie *et al.*, 2006). In epidemiologic studies, statistically significant correlations have been described between the incidence of the diseases and low levels of the antioxidant molecules (Tucker and Townsend, 2005). Antioxidants are one of the key players in tumorigenesis. Several natural and synthetic antioxidants have been shown to have anticancer effects (Ramakrishnan *et al.*, 2006). Antioxidants, in general, play an important role in mitigating the damaging effects of oxidative stress (Rao *et al.*, 2006a).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. They can, thus, prevent or repair damage done to the body's cells by

oxygen. They act by one or more of the following mechanisms, such as, reducing activity, free radical-scavenging potential, complexing of pro-oxidant metals and quenching of singlet oxygen. The consumption of natural antioxidant phytochemicals might protect the human body against damage by ROS and has been reported to have potential health benefits (Sumino *et al.*, 2002).

Ayurveda and plant-based remedies for herbal care through day-to-day life experiences are part of the cultural heritage in India. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies on traditional medicine for their primary healthcare needs. In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their back bone (Mukherjee and Wahile, 2006).

The plant kingdom has wider biochemical diversity than the animal kingdom. Atleast four-fifths of the secondary metabolites come from the vegetable world. To date, about 40% of modern drugs derive directly or indirectly from the plants. Phytotherapy constitutes the most popular medical practice of complementary medicine, in many countries its increase has been continuous (Giachetti and Monti, 2005).

Natural products either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Natural compounds possess highly diverse and complex molecular structures compared to small molecule synthetic drugs and often provide highly specific biological activities likely to be derived from the rigidity and high number of chiral centers. Ethnotraditional use of plant-derived natural products has been a major source for the discovery of potential medicinal agents (Gonzales and Valerio, 2006).

Plants have been the source of medicines in pharmacopoeia. Herbs have been utilized to treat acute and chronic disorders for thousands of years. In the past

few decades, significant advances in experimental methodology and molecular biology have enabled researchers to investigate the potential use of phytochemicals to treat or manage a plethora of chronic diseases including cancer, inflammatory diseases and cardiovascular abnormalities (Issa *et al.*, 2006).

Increasing evidence indicates that wheat and wheat-based food products contain significant levels of natural antioxidants, which may provide health benefits to consumers in addition to general nutrients and energy (Zhou *et al.*, 2004). The plant selected for the present study is *Triticum aestivum*, which is commonly called as wheat grass. It belongs to the family Poaceae. Wheat grass juice is an extract squeezed from the leaves of the mature sprouts of wheat seeds (*Triticum aestivum*). Wheat grass juice has been reported to be helpful in curing diseases such as thalassemia (Marwaha *et al.*, 2004), distal ulcerative colitis (Ben-Arye *et al.*, 2002), osteoporosis and cancer. However, no systematic study has been undertaken on analyzing the fresh extracts of *Triticum aestivum* leaves on oxidant challenged events *in vitro* and *in vivo*. Hence the present study was formulated with the following objectives.

- To assess and compare the antioxidant status of *Triticum aestivum* leaves at different stages of growth
- To establish the free radical quenching potential and biomolecule protective effects of *Triticum aestivum* leaves *in vitro*
- To analyze the antioxidant effect of the leaf extracts in simulated *in vitro* systems challenged with a standard oxidant
- To confirm the effects observed *in vitro* using *in vivo* studies
- To screen the *Triticum aestivum* leaves for their phytochemical constituents to understand the nature of the active components.

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.