Introduction

1. INTRODUCTION

Aerobic organisms are dependent of oxygen, which plays an important role in energy production. Activated oxygen that functions as an oxidant may be represented as a free radical. Free radicals are continuously produced in the human body as they are essential for energy supply, detoxification, chemical signaling and immune function. A large body of evidence has been accumulated that living systems have not only adapted to a coexistence with free radicals, but have also developed various mechanisms for the advantageous use of free radicals in various physiological functions (Metelitza and Karasyova, 2007; Ali *et al.*, 2008a).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism and are well recognized for playing a dual role in living systems. Beneficial effects of ROS occur at low or moderate concentrations and involve physiological roles in several cellular responses. Damage to cellular lipids, DNA and proteins are considered as harmful effects of free radicals (Valko *et al.*, 2006).

The harmful effects of ROS are counteracted by the host's antioxidant defense system. A certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increases during ageing and other pathological events, as the efficiency of antioxidative and repair mechanisms decrease, leading to the condition of oxidative stress (Pandey and Rizvi, 2010). The high concentration of ROS is responsible for oxidative stress-induced diseases, where mitochondria play a major role in oxygen metabolism and subsequently become a major source of ROS formation (Pallardó *et al.*, 2010).

ROS is a collective term used for radicals that are generally produced by endogenous and exogenous factors. They possess the ability to cause far-reaching oxidative damage to healthy cells by reacting with their nucleic acids, lipids, proteins, enzymes and other small cellular molecules (Vinayak *et al.*, 2010). Free radicals inducing oxidative damage of cellular lipids, nucleic acids and proteins are thought to be one of the major risks for diseases such as cancer, atherosclerosis, diabetes mellitus, coronary heart disease, inflammation, cerebral ischemia, skin damages and various other degenerative diseases (In-Ja *et al.*, 2009; Wang *et al.*, 2010a).

Lipid peroxidation is considered as one of the basic mechanisms of tissue damage caused by free radicals, which disturb the normal structure and functions of the membrane (Balakrishna *et al.*, 2009; Seal *et al.*, 2010). Growing research suggests that the LPO products (like lipid hydroperoxides and malondialdehyde) have been shown to be mutagenic and carcinogenic (Greenberg *et al.*, 2008). It is evident that LPO products as well as ROS/RNS exert various biological functions *in vivo* such as regulators of gene expression, signaling messengers, activators of receptors, nuclear transcription factors and inducers of adaptive responses (Niki, 2009).

Essential cellular functions, such as oxidative respiration, create ROS that can damage DNA (Dinant *et al.*, 2008). Hydroxyl radical plays a major role in ROS-induced DNA damage, which causes mutation. The mutated DNA loses its control in cell division, which ultimately leads to cancer (Irigaray and Belpomme, 2010).

A large body of evidence shows that proteins are significant targets for reactive oxygen and nitrogen species *in vivo*. Increased levels of protein carbonyls and nitrated amino acids are early markers of damage in biological

systems subjected to oxidative stress, even in the presence of endogenous antioxidants (Domazou *et al.*, 2009).

Research also suggests that oxidative stress and a high concentration of ROS induce apoptotic cell death in various cell types (Abedelahi *et al.*, 2010). Apoptosis is a fundamental cellular process for the self-destruction of cells, essential for tissue development and for maintaining homeostasis. Recent studies have demonstrated that apoptosis plays an important role in the pathogenesis of tumors (Kim *et al.*, 2007).

Chemoprevention of cancer is a novel and more effective means of cancer control, where naturally occurring agents are considered to be less toxic and more effective in controlling various human malignancies (Zhou *et al.*, 2008a). There are a number of alternative medicine systems (like Unani, Ayurveda and Siddha) based on traditional theories and philosophy that have originated in specific geographical areas and evolved over the years. These medicinal systems are claiming to treat and/or prevent several diseases including cancer (Shafi *et al.*, 2009).

It is well documented that ROS are involved in the etiology of several chronic diseases. Antioxidants counteract the effects of free radicals. Hence, over the last decades, numerous methods have been developed to detect antioxidants, which are easy to perform and largely used in screening trials (Hoelzl *et al.*, 2005). Several model organisms are used in oxidative stress related studies, like *Podospora anserine*, *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* (Muller *et al.*, 2007).

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 (Al-Duais *et al.*, 2009). An aerobic cell possesses

different antioxidant defense systems to counteract the deleterious effects of ROS (Zamora *et al.*, 2010).

Endogenous antioxidants are insufficient; hence, dietary antioxidants are required to countermeasure excess ROS. Recently, significant attention has been paid to antioxidants in food as well as additives to cosmetic and pharmaceutical products (Wang *et al.*, 2009).

Numerous natural free radical scavengers and antioxidants can protect biomolecules against the attack of free radicals and/or suppress the resultant injury. Natural antioxidative substances usually have phenolic moiety in their molecular structures. Phenolics/flavonoids are groups of naturally occurring phytochemicals predominantly synthesized by higher plants. The antioxidant effects of phenolics/flavonoids are supposedly due to their free radical scavenging activities. Evidence from *in vivo* and epidemiological studies suggests that these compounds, through their antioxidant properties, may exert myriad health benefits, thus ameliorating chronic diseases associated with oxidative damage (Etcheverry *et al.*, 2008; Adebiyi *et al.*, 2009; Beevi *et al.*, 2010; Shahidi and Chandrasekara, 2010).

It is a well-known fact that free radicals/ROS are responsible for oxidative stress-induced diseases, which occur when ROS concentration exceeds the antioxidant capacity. As a consequence, much research has focused on antioxidants and on their action mechanisms. Several plant extracts or secondary metabolites have been found to show strong antioxidant activity and protection against oxidant-induced damage (Moura *et al.*, 2007).

With this backdrop, the present study was formulated to analyze the antioxidant potential of *Artemisia vulgaris* leaves. *Artemisia vulgaris* is commonly called as mugwort or masipatchilai in Tamil. It belongs to the

family Asteraceae. The plant is used in traditional medicine to treat various ailments like gastric diseases, malaria and menstrual problems. But there is no systematic study on analyzing the antioxidant effect of *Artemisia vulgaris* leaves. Hence, the present study was formulated to assess the effects of the extracts of *Artemisia vulgaris* leaves on oxidant challenged *in vitro* events.

The objectives laid out for the study were:

- To study the antioxidant content of *Artemisia vulgaris* leaves and to test the free radical quenching ability of the leaf extracts
- To determine the biomolecule protective effects and to analyze the antioxidant effects evoked by the leaf extracts in an *in vivo*-simulated *in vitro* system subjected to oxidative stress
- To study the effect of the plant extracts on events associated with apoptotic death induced by oxidative stress
- To analyze the active moieties in *Artemisia vulgaris* leaves rendering the antioxidant effects.

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