Introduction

1. INTRODUCTION

Oxygen, an essential element for the life, can also be a reason for the destruction of tissue and impair its ability to function normally. Oxidants or free radicals or oxygen–free radicals (OFR), more generally called as reactive oxygen species (ROS), are formed due to various exogenous and endogenous factors. In some cases, ROS are produced specifically to serve essential biological functions, whereas in other cases, they are the products of metabolic processes (Krishnaiah *et al.,* 2007; Luhua *et al.*, 2008).

Oxidative stress is an imbalance between the factors that exert oxidative stress and those that possess potential antioxidants. Oxidative damage is the consequence of excess oxidative stress, inadequate antioxidant potential or the combination of both (Chow and Chang, 2007; Chen *et al.*, 2008). Free radicals are very reactive chemical species, can cause oxidative injury to living beings by attacking the macromolecules like lipids, nucleic acids, proteins and carbohydrates. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and the antioxidant defense systems. Impairment in the oxidant / antioxidant equilibrium creates a condition known as oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue damage in a wide spectrum of human diseases (Ramakrishna and Jailkhani, 2007).

Oxidative stress is initiated by reactive oxygen species such as superoxide anion, perhydroxy radical and hydroxyl radical. These radicals are formed by one electron reduction process of molecular oxygen. ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage to cell membrane phospholipids and lipoproteins by propagating a chain reaction cycle. Thus, antioxidant defense systems have evolved with aerobic metabolism to counteract oxidative damage from Reactive Oxygen Species (Sundararajan *et al.*, 2006).

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. Current life style is causing the overproduction of free radicals and reactive oxygen species in our body and decreasing physiological antioxidant capacity (Lopez *et al.*, 2007; Orrell *et al.*, 2008).

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 and other harmful by-products. These products are closely related to carcinogenesis and have been suggested as modulators of signaling pathways related to proliferation and apoptosis, two processes implicated in cancer development (Marquez *et al.*, 2007).

Hydrogen peroxide (H_2O_2) is a non-radical reactive oxygen species and the most stable intermediate in the four-electron reduction of oxygen to water. Since H_2O_2 is uncharged, it easily passes through the cell membranes by diffusion, and when inside the cells, it can react with transition metals liberating hydroxyl radicals. At high concentrations, these radicals induce peroxidation of lipids and proteins affecting cell integrity (Da Rosa *et al.*, 2008).

Cells, tissues and body fluids are equipped with powerful defense systems that help to counteract oxidative challenge. To maintain a steady state of metabolites and functional integrity in the aerobic environment, an antioxidant defense is organized at three principal levels of protection. They are prevention, interception and repair (Sies, 2007). Cells are equipped with elaborate defense systems that act in concert to detoxify ROS.

Free radicals can be generated in biological systems in the form of reactive oxygen species and these are removed by the antioxidant system in the body. There are several antioxidants including superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase. However, when the level of free radicals exceeds the ability of the antioxidant system, lipid peroxidation and DNA and protein damage occurs, which results in aging and various diseases, including inflammation, cancer, Parkinson's disease, cardiovascular diseases, multiple sclerosis and lupus (Lee *et al.*, 2007a).

Antioxidant potential is the sum of a large number of interrelated and interdependent antioxidant systems. Antioxidants are physiological substances that are derived from both endogenous and exogenous sources and that act against oxidative stress (Rai and Phadke, 2006). The balance between antioxidants and oxidation is believed to be a critical concept of maintaining a healthy biological system. Due to the deletion of many bioactive compounds in food with possible antioxidant activity, there has been increased interest in the relationship between antioxidants and diseases (Tarhan *et al.*, 2007).

Apoptosis is critical to the development and homeostasis of the immune system. In mammals, there are two distinct apoptotic cell-death pathways that require aspartate-specific cysteine proteases termed caspases. The intrinsic pathway is mediated by the mitochondria and functions in response to certain stimuli such as cytokine deprivation, Reactive Oxygen Intermediates (ROIs), genotoxic drugs, DNA damage and calcium overload. The extrinsic pathway of apoptotic cell death is mediated by death receptors interacting with their ligand. There are several death receptors including Fas, tumor necrosis factor receptor (TNFR) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) (Weant *et al.*, 2008).

Although the signaling cascades that initiate the two death pathways are different, they ultimately converge at the executioner caspases. It is imperative that these processes be tightly controlled because excess apoptosis occurs in degenerative diseases such as Alzheimer's and Parkinson's diseases, and lack of apoptosis is critical in cancer and autoimmunity (Goodnow, 2007).

Tumorigenesis is a complex process involving the activation of oncogenes, inactivation of tumor suppressors, and deregulation of cell death programs. The findings that pro-apoptotic genes might act as tumor suppressors and that anti-apoptotic genes can serve as oncogenes suggested that the balance between pro-apoptotic and anti-apoptotic genes modulates tumor growth. How to selectively activate apoptosis in transformed cells remains a primary strategic problem in cancer therapy, and extensive studies are being performed to find efficient mechanisms of apoptosis induction in tumor cells (Gogvadze *et al.*, 2008).

Normal cells continually audit their viability by assessing the balance of survival (anti-apoptotic) and death (pro-apoptotic) signals that they receive. In normal cells, DNA damage leads to a block in proliferation (cell cycle arrest) while the potential for repair is assessed. If the level of damage exceeds the capacity for repair, the balance of anti- and pro-apoptotic signals tips and the cell undergoes programmed cell death (apoptosis). This prevents the persistence of DNA damage and avoids the risk that mutations will be passed to the progeny of cell division. As such, this mechanism represents a very powerful barrier to the development of cancer (Harrington, 2007).

The process of programmed cell death or apoptosis is generally characterized by morphological characteristics and energy-dependent biochemical mechanisms. Inappropriate apoptosis is a factor in many human disease conditions, including neurodegenerative disease, ischemia, autoimmune disorders and many types of cancer. Therefore, research continues by elucidating the analysis of the cell cycle machinery and signaling pathways that arrest or induce apoptosis (He *et al.*, 2008).

There has been a worldwide trend in the reassessment of animal experiments and a rise in interest in alternative methods, leading to a reduced number of animals being used in experiments. There are several alternative approaches, such as tissue, cell and organ culture, survey of human studies, replacement with less sentient organisms, use of discarded human placentas for microsurgery, chromatography and mass spectrometry, computer simulation, audio visual aids, centralization of existing data with easy access. The alternative systems that have been popular include tissue slices, primary culture, cell lines and lower organisms like *Drosophila melanogaster*, *Saccharomyces cerevisiae* and many more (http://en.wikipedia.org/wiki/model_organism).

The efficacy and safety of medicinal plants naturally represent the object of interest for the pharmacologist and this aspect gives the most important information on herbal medicine (Calapai and Caputi, 2007). Herbal medicine is the use of medicinal plants for the prevention and treatment of diseases; it ranges from traditional and popular medicines of every country to the use of standardized herbal extracts (Firenzuoli and Gori, 2007).

Medicinal plants are sources of important therapeutic aids for alleviating human ailments (Ingale, 2006). Medicinal herbs are known to contain a variety of antioxidants (Gupta *et al.*, 2007). Thus, the search for crude drugs of plant origin with antioxidant activity has become a central focus of research. In tune with this effort, the present study centered around analysing the antioxidant activity of the candidate plant *Rhinacanthus nasutus.*

Rhinacanthus nasutus, is commonly called as Nagamalli (Tamil). *Rhinacanthus nasutus.* Kurz. (Family *Acanthaceae*) is a valuable plant that is widely distributed and cultivated in South China, Taiwan, India and Thailand. *Rhinacanthus nasutus* is well known as a source of flavonoids, steroids, terpenoids, anthraquinones, lignans and especially naphthoquinone analogues.

Various parts of this plant have also been used for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases (Siripong *et al.*, 2006).

There is an increased quest to obtain natural antioxidants with broad-spectrum action. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. The aim of this research effort was to characterize the antioxidant capacity of one such under-exploited plant, *Rhinacanthus nasutus*. Antioxidant features of the leaf extracts encompassing its protective effect on induced oxidative damage were measured using various *in vitro* systems that are the alternatives to the use of live animals in research.

The objectives of the present investigation were as follows:

- To assess and compare the antioxidant status of the leaves of *Rhinacanthus nasutus*
- To determine the free radical scavenging ability and the DNA- and lipid-protective effects of different solvent extracts of *Rhinacanthus nasutus* leaves against oxidative damage induced under *in vitro* conditions
- To analyse the antioxidant response evoked by the leaf extracts in various *in vivo* simulated *in vitro* systems challenged with a standard oxidant
- To study the effect of the plant extracts on morphological changes and nuclear events associated with apoptotic death induced by oxidative stress in different alternative models
- To identify the phytochemical constituents present in the leaves in order to understand the nature of the bioactive component responsible for its therapeutic value.

A very brief review of the vast literature collected and scrutinised relevant to the present study is reviewed and presented in the next chapter.