CHAPTER I

INTRODUCTION

According to the oxidative stress hypothesis of aging, the senescence-associated loss of functional capacity is due to the accumulation of molecular oxidative damage (Sohal, Mockett, and Orr, 2002) by toxic free radicals produced during normal respiration. Oxidative damage may contribute to the aging process and to the neuropathogenesis of several diseases including Stroke, Parkinson's disease and Alzheimer's disease (Harman, 1993). Free radicals have previously been reported to be capable of damaging many cellular components such as proteins (Dean et al., 1997), lipids (Cai, Tian, and Wei, 1996) and DNA (Hamilton et al., 2001) and cause glyco-oxidation (Saxena et al., 1999). Brain is particularly vulnerable to oxidative damage due to various factors like (a) high utilization of inspired oxygen, (b) high susceptibility of large amount of oxidizable polyunsaturated fatty acids that are prone to lipid peroxidation, (c) the abundance of redox-active transition metal ions, and (d) the relative dearth of antioxidant defense systems (Subbarao and Richardson, 1990; Ogawa, 1994)

Nitric oxide is a physiological messenger in the central nervous system that is formed from L-arginine and O_2 in a reaction catalyzed by the NO synthase (Knowles and Moncada, 1994). Activation of this enzyme forms equimolar concentrations of NO and citrulline, and NO then exerts physiological actions through elevations in cyclic GMP levels (Knowles et al., 1989; Bredt and Snyder, 1989). NO seems to be implicated in important functions in the CNS such as regulation of cerebral blood flow or memory. The excess NO may bind to several requisite metalloproteins necessary for the function of adjacent cells and inactivate them, whereas under normal oxygen tensions in the brain, NO can react with oxygen in its final deactivation step (Snyder, 1992; Lancaster, 1992). In neurodegenerative diseases such as Alzheimer's and Huntington's Chorea and in vascular strokes, a large majority of neurons are destroyed due to the postulated inability of the brain to inactive excess NO production (Snyder, 1992).

To protect cells against oxidative damage by oxidants, produced during the oxygen metabolism, an antioxidant system has presumably evolved in aerobic organisms (Cebalas-picot et al., 1992). Major antioxidants like superoxide dismutase

(SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione, ascorbic acid, and α-tocopherol are important for cellular protection due to their ability to detoxify free radicals, such as reactive oxygen species (ROS) (Young and Woodside, 2001). Various synthetic antioxidants have been used, which restricted the use of natural antioxidants as in food (Madavi and Salunkhe, 1995).

Phytochemicals have long been recognized to possess many properties including antioxidant, antiallergic, anti-inflammatory, antiviral, antiproliferative and anticarcinogenic effects (Youdim and Joseph, 2001). *Centella asiatica* (L) urban (synonym *Hydrocotyle asiatica*) belongs to the family Apiaceae and is found almost all over the world. In Ayurveda, an Indian system of medicine, *Centella asiatica* was used in the management of central nervous system, skin and gastrointestinal disorder. The major principles in the plant are the polyphenols (Abdul-Hamid et al., 2003) and triterpenes (Inamdar et al., 1996). *Centella asiatica* has been shown to improve memory, general mental ability of mentally retarded children (Kuppurajan, Srinivasan, and Janaki, 1978). It was also shown to have wound healing property (Suguna, Sivakumar, and Chandrakasan, 1996), anticancer property (Babu, Kuttan, and Padikkala, 1995), antioxidant property (Abdul-Hamid et al., 2003) and was also shown to have antileprotic property (Sahu, Roy, and Mahato, 1989).

Centella asiatica has been used as a traditional herbal medicine in Asian countries for hundreds of years. It is a perennial, herbaceous creeper growing to 50 cm with fan shaped leaves. The whole plant is collected and dried for use. It contains triterpene saponins, mainly asiaticoside and sapogenin asiatic acid (Singh and Rastogi, 1968). The plant and its extracts were incorporated into the Indian Pharmacopia in 19^{th} century (Srivastra, Shukla, and Kumar, 1997) with indications for inflammation and epidermal wound healing, e.g. leg ulcers and leprosy. Its wound healing effects may be due to its up-regulation of human collagen I expression (Bonte et al., 1994, 1995) and an increase in tensile strength of the wounds (Suguna et al., 1996). A dermal product containing ingredients of *Centella* is also available in Europe for the treatment of wounds and ulcers (Maquart et al., 1990). Even though this precious herb is surrounded with various claims, the underlying mechanisms involved in its physiological effects are lacking. More scientific data are required before recommendation for increase in its consumption/utilization can be given with confidence.

With few exceptions, CNS neurons cannot divide, nor can they regenerate when their axons are interrupted. Thus, any pathological process causing neuronal loss generally has irreversible consequences. At first sight, this appears to be very unpromising territory for pharmacological intervention, and indeed drug therapy currently has rather little to offer, except in the case of Parkinson's disease. Nevertheless, the incidence and social impact of neurodegenerative brain disorders in ageing populations has resulted in a massive research effort in recent years, and the advances made may be translated into therapeutic progress in not-too-distant future (Rang, Dale, and Ritter, 1999).

Several lines of recent studies suggest the potential of antiinflammatory compounds and antioxidative agents for modulation of chronic inflammatory processes as a therapeutic approach to a neurodegenerative disease (Schwartz et al., 1998; Van Eldik, 2001; Gonzalez-Pelez et al., 2002). As a consequence, search for natural antioxidants, especially of plant origin, has notably increased in recent years (Loliger, 1991). Antioxidative compounds obtained from natural sources, such as grains, oilseeds, beans, leaf waxes, bark, roots, spices, fruits and vegetables, have been investigated (Chen et al., 1996). As evidenced by previous unpublished observations that *Centella asiatica* extract and asiaticoside might be useful in slowing down neurodegenerative disease and prevention by their antioxidant properties or reducing free radical activity. The present study was designed to investigate the potential ability of asiaticoside and *Centella asiatica* extract to prevent or attenuate the process of neurodegeneration in the *in vitro* model of nitric oxide-induced injuries in neuronal cell line cultures.