CHAPTER I
INTRODUCTION

Reactive oxygen species (ROS) are chemically reactive oxygen containing molecules that produced in biological systems. The common ROS are hydroxyl radical ($HO^{\cdot}$)superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO). ROS are produced during processes of cellular metabolism such as mitochondria respiratory chain reaction [1]. Activity of enzymes, for example, xanthine oxidase yields $O_2^{\cdot -}$ and nitric oxide synthase (NOS) yields NO. The most harmful ROS are hydroxyl radical ($HO^{\cdot}$) which is produced from iron catalyzed reaction of H_2O_2 and $O_2^{\cdot -}$ via Fenton reaction, and peroxynitrite ($ONOO^{\cdot}$) which is a product from reaction of $O_2^{\cdot -}$ and NO. ROS involve in several biological processes including cell proliferation, cell adaptation, immune response and also cell damage and cell death [1]. Inducible ROS generation has been shown following many triggers including viral infection [2]. Therefore, ROS are crucial for pathogenesis and pathology of several diseases including cancer, cardiovascular diseases, neurodegenerative diseases and infectious diseases [3]. Modification of ROS production by mean of antioxidant therapy is believed to delay or reduce the disease severity and complication.

Japanese encephalitis virus (JEV) is an emerging arthropod-borne virus from *Flaviviridae* family. It is the one of the most important causative agents for viral encephalitis in human. JEV infection is endemic to the entire East and Southeast Asia. The mortality rate of JEV infection is estimated at about 30%. Dengue virus (DENV) belongs to the same family of JEV. There are 4 serotypes of dengue viruses (DENV1-4) causing a broad range of severity from dengue fever, dengue hemorrhage fever and dengue shock syndrome. Recently, increased numbers of dengue encephalitis have been reported in worldwide [4]. Dengue infection is endemic in Thailand, therefore dengue encephalitis should not be ignored.

Microglia cells are the resident macrophage presenting in the central nervous system responsible for homeostasis regulation and defense against injury. Microglia cells are usually beneficial and support neuron survival through secretion of growth factors and anti-inflammatory cytokines. Activated microglia cells produce various pro-inflammatory mediators, NO and ROS. Prolonged activation of microglia cells can be a hallmark of many neurodegenerative disorders [10]. In addition, roles of microglia activation and ROS have been reported in Japanese Encephalitis (JE). Molecular mechanisms of JEV induced neuronal damage have been elucidated [11], but not in the case of DENV.

In order to evaluate whether DENV has capability in the induced neuroinflammatory process, the pathway involving with ROS production was investigated in microglia cells. JEV was used as a reference pathogen and lipopolysaccharide (LPS) was used as a standard compound for microglia activation. LPS activate microglia/macrophages leads to morphological changes and to induce harmful factors to cells [8]. Exposing HAPI cells to LPS can induces production of NO and secretion of tumor necrosis factor-alpha (TNF-α) that lead the microglia to initiate neuronal damage [9].

Inducible nitric oxide synthase (iNOS) and apoptosis are well documented pathways that relate to the change in redox status of the cells. Therefore the effects of DENV on the alteration of these pathways were also evaluated. Two serotypes that circulate in Thailand with differences in clinical severity, DENV-2 and DENV-4, were compared. The results may provide basic information for evaluate the risk and for further study of molecular mechanism of DENV induced encephalitis.