CHAPTER I
INTRODUCTION

1.1 Background

Liver fluke, *Opisthorchis viverrini*, is endemic in Southeast Asia including Thailand, Lao PDR, Vietnam and Cambodia (IARC, 1994). Approximately 6 million people are estimated to be infected with the liver fluke in Thailand (Jongsuksuntigul, Imsomboon, 2003). *O. viverrini* infection is the major risk factor for development of cholangiocarcinoma (CCA) in Northeast Thailand, where the incidence of CCA is the highest in Thailand (Sripa, Pairojkul, 2008). The infection occurs after eating raw cyprinid fish containing infective stage, metacercaria of the parasites. Praziquantel is the current drug of choice for treatment of *O. viverrini* infection and its cure rate is more than 90% (Jongsuksuntigul, Imsomboon, 2003). However, after the treatment, praziquantel could induce bursting of the parasite antigen and resulting in inflammation, leading to an increase in oxidative and nitrative stress (Pinlaor et al., 2008). Nuclear factor-erythroid 2-related factor-2 (Nrf2) is a key transcription factor that is activated by electrophilic agents and plays a central role in response to oxidative and nitrative stress (Kim et al., 2010; Surh et al., 2009). Protection against oxidative/nitrative stress involves not only enhanced expression of Nrf2 and also its regulated gene products including Kelch-like ECH-associated protein 1 (Keap1), NAD(P)H:quinine oxidoreductase 1 (NQO1), glutamate cysteine ligase (GCL) and heme oxygenase-1 (HO-1) (Jeong et al., 2006; Zhang, Gordon, 2004). Activation transcription factor 3 (ATF-3) is a novel repressor of Nrf2-regulated stress pathway (Brown et al., 2008). Alternatively, activation of transcription factors such as nuclear factor-kappaB (NF-κB), which acts as a modulator linked to inflammation and carcinogenesis (Karin, 2006). Persistently activated NF-κB is implicated in the expression of various genes involved in inflammation [cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS)]. Recently, Pinlaor et al. had shown that NF-κB-mediated iNOS expression might lead to the formation of oxidative and nitrative DNA lesions [8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-

nitroguanine, respectively] in *O. viverrini*-infected hamsters (Pinlaor et al., 2004b). Thanan et al. also observed an increase in urinary excretion of 8-oxodG in *O. viverrini*-infected patients compared with normal subjects. The findings suggest that inflammation-mediated DNA damage plays a key role in the onset and development of cancers (Kawanishi, Hiraku, 2006; Pinlaor et al., 2004a; Thanan et al., 2008). Curcumin or a diferuloylmethane is derived from turmeric (*Curcuma longa*) and is a pharmacologically safe agent. Curcumin has been shown to suppress the activation of transcription factors, such as NF-κB (Aggarwal, Sung, 2009) and activated the expression of Nrf2-regulated pathways (Balogun et al., 2003). Curcumin had also been reported to, reduce oxidative and nitrative DNA damage, inhibits cell proliferation (Pinlaor et al., 2009) and reduces periductal fibrosis in *O. viverrini*-infected hamsters (Pinlaor et al., 2010). Therefore, curcumin might be used as protective agent to reduce oxidative/nitrative stress in *O. viverrrini*-infected hamsters induced by a short-term praziquantel treatment.

The aim of this study was to clarify the protective effects of curcumin against oxidative/nitrative stress in relation to adverse effects after praziquantel treatment in *O. viverrrini*-infected hamsters. The expression of Nrf2, a key transcriptional factor responsed to stress, and Nrf2-related stress molecules including Keap1, NQO1, GCL, HO-1 and ATF-3 were assessed in liver tissues. The expression of oxidant molecules (NF-kB, COX-2 and iNOS), antioxidant-generating genes (SOD2, CAT, Prdx3 and Prdx6) and proinflammatory cytokines were also investigated. The level of 8-oxodG, an oxidative DNA damage marker, was measured in urine samples. Several biochemical parameters such as NOx, MDA, FRAP and ALT were determined in the plasma. In addition histopathological changes were performed by staining with hematoxylin and eosin. The outcome of the study might encourage the curcumin application not only for the prevention or the reduction adverse effect in praziquantel-treated opisthorchiasis patients but also in the treatment of inflammation-mediated diseases.

1.2 Research questions

1.2.1 Can curcumin prevent adverse effect after a single dose of praziquantel treatment in *O. viverrini*-infected hamster?

1.2.2 How are the stress response molecules affected by curcumin in praziquantel treated *O. viverrini*-infected hamster?

1.3 Objectives of the study

1.3.1 To evaluate the preventive effects of curcumin on oxidative and nitrative stress in *O. viverrini*-infected hamsters and with praziquantel treatment.

1.3.2 To investigate the possible actions of curcumin to reduce oxidative and nitrative stress in *O. viverrini*-infected hamsters and with praziquantel treatment.

1.3.3 To examine the protective effects of curcumin on histopathological changes in *O. viverrini*-infected hamsters and with praziquantel treatment.

1.4. Location of research conducting

This experiment was performed at the Departments of Parasitology, Biochemistry and Pathology, Faculty of Medicine, Khon Kaen University, Thailand.

1.5 Anticipated outcomes

This study will provide further information about the effects of curcumin on oxidative and nitrative stress in hamster infected with *O. viverrini* after a short-term praziquantel treatment, which lead to better understanding in the biochemical and cellular events of host defense mechanism in praziquantel induced inflammation. The results of these prevention effects of curcumin may have implication for utilizing as a chemopreventive agent to reduce the adverse effects of praziquantel in *O. viverrini* infection.