CHAPTER II
LITERATURE REVIEW

2.1 *Opisthorchis viverrini*

2.1.1 Morphology and biology

O. viverrini is a human liver fluke classified into the phylum Plathyhelminthes, order Opisthorchiida, suborder Distomata, class Trematoda, subclass Digenea, superfamily Opisthorchioidea and family Opisthorchiidae. The adult worms are monoecious, dorso-ventrally flattened, lancet-shaped, thin and transparent. The average size of fresh worms is 7.0(5.4–10.2) × 1.5(0.8–1.9) mm with reddish-bile colored. The oral sucker is subterminal, while the ventral sucker is at approximately anterior one-fifth of the body length. Two testes are deeply lobed, diagonal, situated near the posterior extremity. The long-slightly coiled seminal vesicle terminates in the ejaculatory duct, which open through the genital pore immediately in front of the ventral sucker. Cirrus sac and cirrus are absent. The multilobated ovary is in front of the anterior testis and nearby is the seminal receptacle and Laurer's canal. The vitellaria consist of numerous follicles disposed as several columnous groups in the lateral fields between ventral sucker and testes. Excretory bladder is long, sac-like tubed, S-shaped and runs between the two testes (Sadun, 1955). Egg is embryonated while laying, oval or electric-bulb shaped, yellowish brown colored, 27 μm × 15 μm in average size, operculum well developed. The shoulder, a thickened wall of egg shell surrounding operculum, is prominent. Shell surface is rough or so-called musk-melon pattern and having an aboperculum knob.

2.1.2 Life cycle

The adult worms of *O. viverrini* live in the intra- and extra-hepatic bile ducts, gall bladder, and rarely in the pancreatic duct. They attach to the wall of these ducts by the oral and ventral suckers under the regulatory function of the circular and radius muscles. Embryonated eggs containing ciliated miracidium laid from gravid worms are passed through the bile into the duodenum and excreted with faeces into

the external environment. After reaching freshwater of natural reservoirs, these embryonated eggs do not hatch until they are ingested by *Bithynia* snails into the digestive tracts where hatching occurs and then miracidia transform to sporocysts. Rediae and cercariae are produced by the asexual reproduction of germinal cells in sporocysts and rediae, respectively. Free-living cercariae, after exit the snail will attach, penetrate and transform to metacercariae encysted mainly in the muscle of about 18 susceptible species of fish in the family Cyprinidae (Harinasuta, Harinasuta, 1984). Metacercariae are infective to final hosts including humans, dogs and cats when they ingest raw or inadequately cooked fish. After ingestion, the metacercaria is digested by gastric and intestinal juices, respectively. Excysted juvenile flukes at the duodenum then migrate up through the ampulla of Vater and the common bile duct into the intra-hepatic bile ducts where they mature and fertilize. Some worms are formed in the common bile duct, cystic duct and gall bladder. The life span of *O. viverrini* in human is not known, however, it may be over 25 years as recorded in *C. sinensis* (Attwood, Chou, 1978). *O. viverrini*'s life cycle is shown in Figure 1.

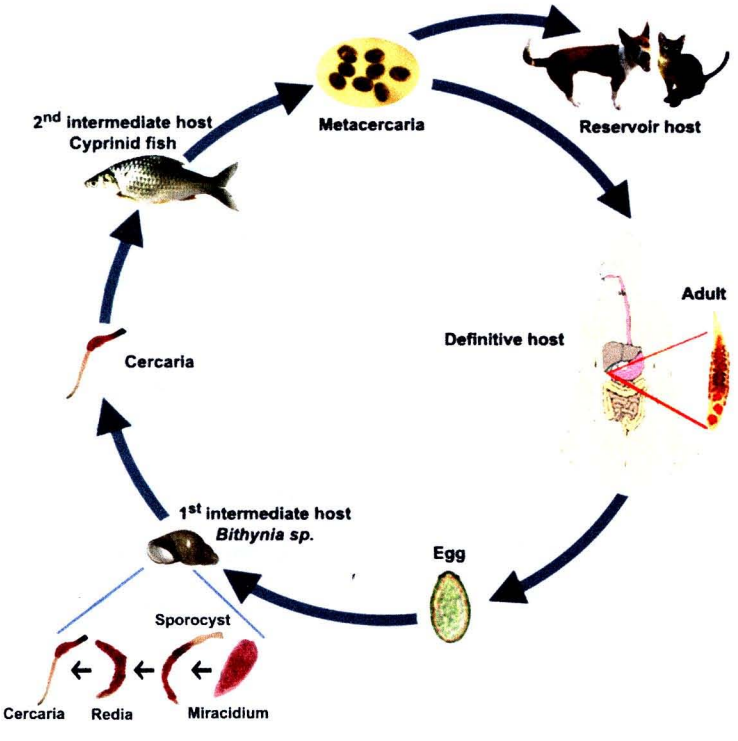

Figure 1 Life cycle of *Opisthorchis viverrini*

2.1.3 Epidemiology

The most recent estimate of the number of people infected with *O. viverrini* in the Mekong region is eight million in Thailand and two million in Laos (Sithithaworn, Haswell-Elkins, 2003). This is a substantial underestimate of its prevalence because no data are available for Cambodia or Vietnam, although opisthorchiasis in known to be common in parts of these countries (Sithithaworn et al., 2006). In Thailand, an average of 9.6% of the population is infected with the liver fluke being distributed mainly in the North (19.3% prevalence) and Northeast (15.7% prevalence) (Jongsuksuntigul, Imsomboon, 2003). There has been a substantial decline in the prevalence of infection in Thailand from 34% (in 1992) to 10% (in 2002), which has been attributed to intensive and continuous control activities. Disturbingly, substantial variation in prevalence ranging from 4% to 33% still remains in affected provinces. Within Khon Kaen province (northeast Thailand), *O. viverrini* infection ranges from 2% to 71% between different districts (Sriamporn et al., 2004). Although data on the prevalence of infection are available, data on incidence are scant (Saowakontha et al., 1993).

2.1.4 Clinical manifestation and pathology

Although *O. viverrini* is regarded as a luminal parasite in biliary bile duct, but it can stimulate both cell mediated and humoral immune responses. In *O. viverrini*-infected hamster, cell-mediated immune responses such as macrophages, lymphocyte, mast cells and eosinophils surrounding the inflamed biliary tissues were observed at the beginning on day 3 post infection (Sripa, Kaewkes, 2000a). A large number of polymorphonuclear, eosinophil and neutrophil, at the acute phase were found whereas mononuclear such as monocyte and lymphocyte were predominated at chronic phase during 45 and 145 days post infection. As described by Sripa (2003), clinical manifestation of *O. viverrini* infection rarely induces acute clinical feature, but trends to be chronic in nature and persist for many years. The intensity, long duration of infection and accumulated flukes from repeated infection are related with the severity of disease. Severe opisthorchiasis is commonly seen in patients with age over 40 years and males are more affected than 10 females (Akai et al., 1994). The clinical features vary from mild to severe manifestations which were classified into 4 types, asymptomatic type; a mild type with irregular episodes of flatulency and

dyspepsia and a 'hot sensation' over the liver area; a moderate type with symptoms of mild cholangitis, dyspeptic flatulence, diarrhea and a severe type with chronic relapsing cholangitis, obstructive jaundice and cholangiocarcinoma (CCA) (Harinasuta, Harinasuta, 1984).

In human, the infection can induce several pathologic changes such as subcapsular bile ducts of heavy infected patients are usually dilated and show prominent fibrotic wall (Riganti et al., 1989). The typical histologic changes of intrahepatic bile ducts in liver fluke infection include inflammation, epithelial desquamation, goblet cell metaplasia, epithelial and adenomatous hyperplasia and periductal fibrosis. These characteristic changes are well established within 7-15 years after *O. viverrini* infection, and they are similar between adult and children. Enlargement of the gall bladder is commonly found in opisthorchiasis both in autopsy and ultrasonographic studies. The abnormalities improve dramatically after treatment with praziquantel (Mairiang et al., 1992). Histologic changes seen in the gall bladder and extrahepatic bile duct in opisthorchiasis are adenomatous hyperplasia, epithelial hyperplasia and chronic inflammation. Chronic inflammation is more frequently observed in *O. viverrini* egg positive than the negative ones. In hamster model, they showed heavy inflammatory cell infiltration, periductal fibrosis, and epithelial bile duct hyperplasia (Pinlaor et al., 2003). The pathological consequences of *O. viverrini* infection occur mainly in the liver, extrahepatic bile ducts and gall bladder and are similar in human. Two phases of pathological change; first and second phase, is divided according to worm development. The early pathological changes consisted of an acute inflammatory reaction and the second phase could induced hyperplasia and adenomatous of the bile duct epithelium. In conclusion, most authors suggested that liver flukes mediate tissue damage directly by mechanical irritation or indirectly through immunological response. The marked pathology in infected hamsters suggests that acute damage may be induced by parasite factors, but the progressive changes are consistent with immunopathologic mechanisms (Bhamarapravati et al., 1978).

2.1.5 *O. viverrini* infection and host response

Host parasite interaction may involve in the progression of the disease. *O. viverrini* parasite may secrete some molecule to induce host immune response.

These molecules include cysteine proteinase (Sripa, Kaewkes, 2000b) and asparaginyl endopeptidase (Laha et al., 2008). Moreover, abundantly represented protein families included those involved in physiological functions that are essential to parasitism, such as anaerobic respiration, reproduction, detoxification, surface maintenance and feeding have been recently characterized from adult worm (Laha et al., 2007). Parasite molecule may bind to cell surface of host cell via *N*-linked oligosaccharides (Talabnin et al., 2006) induction to host immune response. In addition, IL-12 mediated Th1 cytokine responses to acute infection such as IL-1, IFN-γ, TNF-α and Th2 cytokines such as IL-4, IL-10 and TGF-β responses in chronic infection may inhibit the immune functions, which allows the parasites to evade host immune response (Jittimanee et al., 2007). Thus, the balance response is a key aspect of surviving *O. viverrini*-mediated liver inflammation and minimizing the effect of tissue remodeling on normal liver process. In contrast, *O. viverrini* antigen may induce oxidant molecules including iNOS, heme oxygenase-1, proliferating cell nuclear antigen (Pinlaor et al., 2004b), nuclear transcriptional factor κB and Toll like receptor-2 (Pinlaor et al., 2005) leading to free radical production. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the key free radicals generated by inflammatory cells and act as the major molecules involved in inflammation-mediated pathogenesis and carcinogenesis (Coussens, Werb, 2002). Excess of free radical production induces oxidative and nitrative DNA in animal (Pinlaor et al., 2004a) and oxidative DNA damage in human opisthorchiasis (Thanan et al., 2008). Moreover, ROS can induce lipid peroxidation-mediated DNA adduct in human-infected with *O. viverrini* (Dechakhamphu et al., 2008). In addition, free radical may induce several molecules expression such as proteinase enzymes that involved in tissue fibrosis (Siwik et al., 2001). Fibrosis is the end result of chronic inflammatory reactions induced by the persistent *O. viverrini* infection (Prakobwong et al., 2009). Therefore, host-parasite interaction may alter inflammation-related molecules involved in stress responses, reactants, structural components, and immunity-related proteins. In addition, studying *O. viverrini* molecules may provide basic information to understand host-parasite relationship which may useful for vaccine development in the future.

2.1.6 Opisthorchiasis associated cholangiocarcinoma

Infection with liver flukes has been reported to be associated with bile duct malignancy. A large body of evidence indicates that *O. viverrini* is a definite cause of human CCA. Possible mechanisms of carcinogenesis include chronic inflammation, nitric oxide generation, intrinsic nitrosation and activation of drug-metabolizing enzymes. Early detection of bile duct malignancy is difficult and no sensitive and specific biomarkers are available at present, although CCA-associated soluble antigen has been reported in an experimental study to be a useful early marker of cancer development. Long-term survival after surgical treatment of liver fluke-associated cancer is similar to that reported in patients without liver fluke infestation. Liver fluke-associated CCA is still a health problem in developing countries (Watanapa, Watanapa, 2002).

Chronic inflammation and infection are risk factors for a variety of human cancers. The role of infection in cancer focused on increased cell proliferation, activation of carcinogen metabolism, inactivation of defense enzymes, increased endogenous nitrosation, immunosuppression, and the role of oxygen free radicals generated by phagocytic cells to cause DNA damage and mutagenesis (Ohshima et al., 1994). In Syrian hamster can also be infected with metacercariae of the fluke and heavy loads of parasites cause the development of cirrhotic livers. While the presence of flukes alone does not give rise to neoplasms, large yields of cholangiofibrotic lesions and cholangiocellular carcinomas can be readily induced with additional carcinogenic insult. While removal of the parasite with the antihelminthic drug, praziquantel, can protect against carcinogenesis, this is dependent on the timing of the drug administration and the efficacy of application to the human situation remains to be confirmed (Chaimuangraj et al., 2003).

2.2 Treatment of opisthorchiasis

2.2.1 Praziquantel

This effective drug is developed in the laboratories for parasitological research of Bayer AG in Germany (Elberfeld) 30 years ago (in the mid 1970's). Since it has proven indispensable in more and more indications and is recognized as such by the World Health Organization (WHO). Praziquantel is recommended as the drug of

choice and recommended dose is 40-60 mg/kg body weight for the treatment of opisthorchiasis (Hurych et al., 1981). The cure rate of a single dose of opisthorchiasis is reported as high as more than 90%, and the rate increases to almost 100% when the same dose is repeated (Seo et al., 1983). In *O. viverrini* infected hamster by using the effective dose which shows complete parasite elimination, There is no effect on the pathological change in liver (Thamavit et al., 1992).

2.2.1.1 Chemistry

Praziquantel is 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline-4-one. It is a white to nearly white crystalline powder of bitter taste, melting at 136-140°C with decomposition. It is stable under normal conditions and it is practically insoluble in water, sparingly soluble in ethanol and soluble in organic solvents like chloroform and dimethylsulfoxide.

2.2.1.2 Pharmacokinetics

Praziquantel is well (approximately 80%) absorbed from the GI Tract. Due to extensive first pass metabolization only relatively small amounts enter systemic circulation. Praziquantel has a serum half-life of 0.8 to 1.5h (metabolites 4 to 5h) in adults with normal renal and liver function. In the patients with impaired liver function half-life is increased to 3 to 8 hours in the serum. Praziquantel and its metabolites are mainly excreted in the urine, within 24h after a single oral dose 70 to 80% are recovered in urine, but less than 0.1% is found as the unchanged drug.

2.2.1.3 Mode of actions

Although the mode of action is not exactly known at present, there is experimental evidence that praziquantel increases the permeability of the membranes of parasite cells for calcium ions. The drug thereby induces contraction of the parasites resulting in paralysis in the contracted state. The dying parasites are dislodged from their site of action in the host organism and may enter systemic circulation or may be destroyed by host immune reaction (phagocytosis). Additional mechanisms, focal disintegrations and disturbances of oviposition (laying of eggs) are seen in other types of sensitive parasites. Another hypothesis on the mechanism of action of praziquantel has been recently reported. The drug seems to interfere with adenosine uptake in cultured worms. This effect may have therapeutical relevance given that praziquantel sensitive parasite is unable to synthesize purines de novo.

2.2.1.4 Adverse effect of praziquante

Several evidences from animal and human studies have been demonstrated that praziquantel can cause adverse effects after short-term treatment. For instance, Pinlaor et al. (2008) have reported that short-term praziquantel treatment in *O. viverrini*–infected hamsters induces parasite antigen bursting, recruitment inflammatory cells infiltration and leads to increase inflammation-mediated oxidative and nitrative stress. The author has suggested that this effect might be supported the previous study in clinical data occurring within 24h after praziquantel-treated opisthorchiasis patients (Bunnag, Harinasuta, 1980). In *Schistosoma japonicum*-infected mice, Matsumoto et al. (2002) have reported that praziquantel treatment sudden release of antigens from eggs hatched and accumulation of mast cells infiltration into the intestine in relation to the anaphylactic signs. Praziquantel treatment of schistosomiasis patients in a small community in Maniema and Zaire, heavily infects with *S. mansoni*, direct observations are made of the side effects of praziquantel in the hours immediately after treatment. Intense abdominal discomfort and the production of bloody diarrhoea are observed in more than half of the treated population. These effects are seen both in children and in adults and the onset of the symptoms was registered within 30 min of treatment. The frequency of the side effects is correlated with the intensity of the infection (Polderman et al., 1984). Moreover, a retrospective survey involving 25,693 persons is carried out in four provinces and the city of Shanghai, China document relatively serious side effects of praziquantel used in a mass treatment program for schistosomiasis japonica. Only 122 or 0.47% of those participating in the study had experienced relatively serious side reactions to the drug. Most had only one kind of side effects but two or more are recorded in a few patients. Neuropsychiatric reactions are seen in 39 persons (0.15%,) cardiovascular reactions in 37 (0.14%), hepatic changes in four (0.02%), dermatological reactions in 18 (0.07%) and delay reactions resulting in fatigue and inability to work in 29 (0.11%) (Chen et al., 1983).

2.2.2 Other antihelminthic drug for treatment of *O. viverrini* infection

There are others effective drugs, but low efficiency than praziquantel, for treatment of *O. viverrini* infection such as mebendazole and albendazole.

2.2.2.1 Mebendazole

Mebendazole is a benzimidazole drug. It is used to treat infestations by worms including pinworms, roundworms, tapeworms, hookworms, and whipworms. 30 mg/kg of mebendazole for 21 and 28 days gives an eradication rate of 94 and 89%, respectively (Jaroonvesama et al., 1981). Mebendazole is thought to kill worms by selectively inhibiting the synthesis of microtubules, impairing the parasite's ability to utilise glucose.

2.2.2.2 Albendazole

Albendazole is a benzimidazole drug. It is a broad spectrum anthelmintic, effective against: roundworms, tapeworms, and flukes of domestic animals and humans. A total of 52 adult patients with opisthorchiasis with or without concomitant intestinal helminthic infections are treated with albendazole at dosage regimens of 400 mg twice daily for 3 days (group I with 25 patients) and 7 days (group II 27 patients). By concentration method with four examinations from two fecal specimens of each patient the cure rates and percentage egg reduction on day 30 in group I and group II were 12% and 33%, 94 and 95 respectively. Albendazole is shown to be effective against *O. viverrini* infection as well as other concomitant intestinal helminthic infections; but the optimal dosage and duration of treatment have not yet been achieved (Mairiang et al., 1992).

2.3 Free radicals

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Major types of free radicals include:

2.3.1 Reactive oxygen species

Reactive oxygen species (ROS) is a term collectively describing radicals and other non-radical reactive oxygen derivatives. These intermediates may participate in reactions giving rise to free radicals or that are damaging to organic substrates. Radicals derived from oxygen represent the most important class of radical species generated in living systems. The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen ($^3O_2$). This process is mediated by

enzymes such as NAD(P)H oxidases and xanthine oxidase or nonenzymatically by redoxreactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain. Superoxide dismutase (SOD) convert superoxide enzymically into hydrogen peroxide (Deby, Goutier, 1990; Fridovich, 1978). In biological tissues superoxide can also be converted nonenzymically into the nonradical species hydrogen peroxide and singlet oxygen ($^1O_2$) (Steinbeck et al., 1993). In the presence of reduced transition metals (e.g., ferrous or cuprous ions), hydrogen peroxide can be converted into the highly reactive hydroxyl radical ($^{\bullet}OH$) (Chance et al., 1979). Alternatively, hydrogen peroxide may be converted into water by the enzymes catalase or glutathione peroxidase. In the glutathione peroxidase reaction glutathione is oxidized to glutathione disulfide, which can be converted back to glutathione by glutathione reductase in an NADPH-consuming process. Because superoxide and NO are readily converted by enzymes or nonenzymic chemical reactions into reactive nonradical species such as singlet oxygen ($^1O_2$), hydrogen peroxide, or peroxynitrite ($ONOO^-$), i.e., species which can in turn give rise to new radicals. Most of the regulatory effects are indeed not directly mediated by superoxide but rather by its ROS derivatives. Frequently, different reactive species coexist in the reactive environment and make it difficult to identify unequivocally which agent is responsible for a given biological effect. ROS in living organisms is presented in Table 1.

Table 1 ROS in living organisms

Radicals	Symbols	**Non-Radicals**	Symbols
Hydroxyl	$HO^{\bullet}$	Peroxynitrite	$ONOO^-$
Superoxide	$O_2^{\bullet -}$	Hypochloric acid	HOCl
Nitric Oxide	$NO^{\bullet}$	Hydrogen peroxide	H_2O_2
Thyl	$RS^{\bullet}$	Singlet oxygen	$^1\Delta_g$ ($^1O_2$)
Peroxyl	$RO_2^{\bullet}$	Ozone	O_3
Lipid peroxyl	$LOO^{\bullet}$	Lipid peroxide	LOOH

(Novo, Parola, 2008)

2.3.2 Reactive nitrogen species

Reactive nitrogen species (RNS) are radical nitrogen-based molecules that can act to facilitate nitrosylation reactions. The NO radical ($NO^{\bullet}$) is generated by NO synthase (NOS) isoforms through the conversion of L-arginine to citrullin (Palmer et al., 1988). Three types of NOS have been identified: endothelial NO synthase (eNOS), which is bound to plasma membranes and known to be strongly activated by the entry of calcium through membrane-bound receptors; inducible NO synthase (iNOS), which was first identified in macrophages and then in other cells, including hepatocytes, is known to be up-regulated by pro-inflammatory cytokines and/or lipopolysaccharide (LPS), and is able to generate low levels of NO compared with the other NOS isoforms; and neuronal NO synthase (nNOS). Depending on the microenvironment, NO can be converted to various other RNS such as nitrosyl cation (NO^{+}), nitroxyl anion (NO^{-}) or peroxynitrite ($ONOO^{-}$) (Stamler et al., 1992). Some of the physiological effects may be mediated through the intermediate formation of *S*-nitroso-cysteine or *S*-nitroso-glutathione (Gow, Stamler, 1998). RNS in living organisms is shown in Table 2.

Table 2 RNS in living organisms

Radicals	Symbols	**Non-Radicals**	Symbols
Nitrous oxide Nitrogen	$NO^{\bullet}$	Nitroxyl anion	NO^{-}
dioxide	$NO_2^{\bullet}$	Nitryl chloride	NO_2Cl
		Peroxynitrite	$OONO^{-}$
		Peroxynitrous acid	ONOOH NO^{+}
		Nitrosyl cation	N_2O_3 HNO_2
		Dinitrogen trioxide	
		Nitrous acid	

(Novo, Parola, 2008)

2.4 Oxidative stress and nitrative stress

ROS and RNS are produced as by-products of normal metabolic processes in all aerobic organisms. In physiological conditions, the oxidant defense systems in the body protect the cells and tissues against these species. When the generation of ROS/RNS exceeds the ability of antioxidant defense systems to remove them, such an

imbalance can cause oxidative/nitrative damage to cellular constituents (DNA, proteins, lipids), which is defined as oxidative/nitrative stress.

Since ROS/RNS themselves are very reactive and have an extremely short half-life, direct determination of them in tissue or body fluids is generally impracticable. Therefore, measurement of oxidatively/nitrosatively modified DNA, proteins, and lipids in biological samples has been expected to detect appropriate biomarkers for diseases in which ROS/RNS are involved.

2.5 Nuclear factor-erythroid 2-related factor-2 (Nrf2)

Nrf2 is a transcription factor which in humans is encoded by the *NFE2L2* gene (Moi et al., 1994). Nrf2 is a master regulator of the antioxidant response (Li, Kong, 2009; Nguyen et al., 2009). The antioxidant response is important for the amelioration of oxidative stress. Oxidative stress can result in cancer, cardiovascular diseases, inflammation, neurological diseases, and renal disease. Because Nrf2 is able to induce genes important in combating oxidative stress, thereby activating the body's own protective response, it is able to protect from a variety of (oxidative stress)-related complications, even in situations where the administration of exogenous antioxidants (such as Vitamin C and Vitamin E) have failed.

2.5.1 Molecular mechanisms underlying up-regulation of Nrf2/ARE-dependent genes

Under the basal resting condition, Nrf2 is sequestered in the cytoplasm by the cytoskeleton-associated protein, Kelch-like ECHassociated protein 1 (Keap1) (Figure 2) (Itoh et al., 1999). Keap1 functions as a negative regulator of Nrf2 by promoting ubiquitination and proteasomal degradation of Nrf2 (Furukawa, Xiong, 2005). When liberated from its repressor Keap1, Nrf2 translocates into the nucleus and forms a heterodimer with a small Maf (sMaf) protein (Itoh et al., 1995; Kensler et al., 2007)). The Nrf2–sMaf dimer then binds to ARE, a cis-acting DNA regulatory element with a core nucleotide sequence of 5'-GTGACNNNGCN-3', localized in the promoter region of many genes whose products have a cellular defensive function (Kensler et al., 2007). Many xenobiotics undergo oxidative metabolism in cells, and form or generate reactive species, such as electrophiles or ROS that can interact with thiol residues present in the functionally critical motif of many proteins (Lee,

Johnson, 2004). A widely accepted model for nuclear accumulation and activation of Nrf2 involves alteration of the Keap1 structure by oxidation or covalent modification of critical cysteines present in Keap1 (Lee, Johnson, 2004) as illustrated in Figure 2.

Murine Keap1 has 25 cysteine residues, and its human homologue has 27 (Dinkova-Kostova et al., 2005). Since Keap1 has highly reactive sulfhydryl groups in its cysteine residues, it is considered as a sensor for electrophilic compounds as well as oxidants. Upon stimulation of cells with ROS, the reactive cysteine residues within Keap1 undergo oxidation and form an intramolecular disulfide bond (Wakabayashi et al., 2004). A compound with high electron with drawing potency can also function as an Nrf2 activator through modification of cysteines in Keap1 (Dinkova-Kostova et al., 2005). It is quite likely that the sites of Keap1 modification may vary depending on the type of reactive chemicals and also the intracellular redox environment (Dinkova-Kostova et al., 2005). Use of radiolabeled or biotinylated inducers of Nrf2 activation revealed that among the many cysteine residues in Keap1, the most reactivity was found at Cys257, Cys273, Cys288 and Cys297 and these multiple cysteines in Keap1 can be modified in a similar pattern (Hong et al., 2005). For example, sulforaphane, a well-known strong inducer of Nrf2 activation, forms the sulforaphane-Keap1 thionoacyl adduct and modifies the tertiary structure of Keap1 most readily at the cysteine residues localized at the Kelch domain thereby stabilizing Nrf2. The selection of compounds which do not show cellular toxicity and yet modify Keap1 structure may provide important clues for the development of therapeutically applicable drugs targeting Nrf2. Alternatively, oxidative/electrophilic stress can activate protein kinase C, mitogen activated protein kinases (MAPKs) or phosphatidylinositol 3-kinase (PI3K) which, in turn, phosphorylates Nrf2, facilitating Nrf2 release from Keap1 and allowing its translocation into nucleus (Lee, Johnson, 2004).

Overexpression of Nrf2, either using expression vector or virus, has been shown to up-regulate ARE-dependent genes. Adenoviral delivery of Nrf2 gene to rat ventricular cardiomyocytes resulted in high-level expression of Nrf2 in both cytosol and nucleus (Purdom-Dickinson et al., 2007). Such overexpression of Nrf2 caused an increased ARE-transcriptional activity, thereby augmenting expression of several ARE-dependent antioxidant and cytoprotective enzymes including HO-1,

GCL, GPx, and NQO1 (Purdom-Dickinson et al., 2007). Delivering Nrf2 gene to target cells or tissues may hence fortify cellular antioxidant and cytoprotective capacity by increasing the expression of ARE dependent genes (Kim et al., 2010).

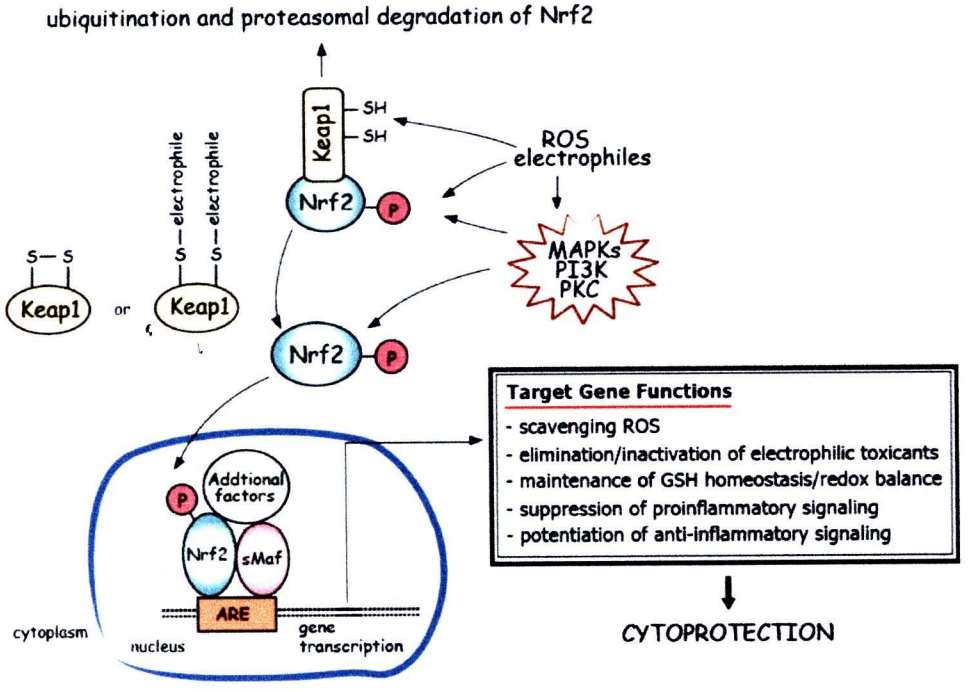

Figure 2 A proposed mechanism responsible for activation of Nrf2-ARE signaling (Kim et al., 2010)

Tissue distribution: Nrf2 is ubiquitously expressed with the highest concentrations (in descending order) in the kidney, muscle, lung, heart, liver, and brain (Moi et al., 1994).

2.5.2 Nrf2 regulate stress response

Nrf2 binds to antioxidant response elements (ARE) located in the promoter region of genes encoding many phase II detoxifying or antioxidant enzymes and related stress-responsive proteins (Lee, Johnson, 2004). These include NAD(P)H:quinone oxidoreductase (NQO1), glutathione S-transferase (GST), heme oxygenase-1 (HO-1), glutathione peroxidase (GPx), glutamate cysteine ligase (GCL), and peroxiredoxin I (Prx I) that play key roles in cellular defense by enhancing the removal of cytotoxic electrophiles or ROS (Lee, Johnson, 2004). Induction of these

cytoprotective enzymes via Nrf2–ARE signaling hence provides an effective means for achieving cellular protection against a variety of electrophilic carcinogens and other reactive toxicants as well as ROS (Kensler et al., 2007). Since ROS- or electrophile-induced mutations are critical for carcinogenesis, Nrf2 has been recognized as one of the most important and promising molecular targets for chemoprevention. In addition to protection against oxidative and electrophilic stresses, recent studies have demonstrated that Nrf2 responds to pro-inflammatory stimuli and rescues cells/tissues from inflammatory injuries (Braun et al., 2002; Cho et al., 2004). Thus, Nrf2 knockout mice develop complex pathogenic manifestations. These include lupus-like autoimmune syndrome characterized by multi-organ inflammatory lesions, intravascular deposition of immunoglobulin (Ig) complexes and premature death due to rapidly progressing glomerular nephritis (Del Brutto et al., 2006). Oligonucleotide microarray analysis has revealed that Nrf2 regulates the expression of acute phase proteins in the lung (Kwak et al., 2003), whose plasma concentrations vary depending on the stage of inflammation, suggesting that Nrf2 may act as a critical mediator of cellular adaptation in response to pro-inflammatory as well as other noxious stimuli. Therefore, understanding the defense mechanism by which the Nrf2 activation confers protection against inflammation can provide rationale to develop therapeutic and preventive strategies for the management of inflammation-associated disorders.

2.5.3 Target Genes of Nrf2: Activation of Nrf2 results in the induction of many cytoprotective proteins. These include, but are not limited to, the following:

2.5.3.1 NAD(P)H quinone oxidoreductase 1 (Nqo1)

Nqo1 is a prototypical Nrf2 target gene that catalyzes the reduction and detoxification of highly reactive quinones that can cause [redox cycling] and oxidative stress (Venugopal, Jaiswal, 1996).

2.5.3.2 Glutamate-cysteine ligase, catalytic (Gclc) and glutamate-cysteine ligase, modifier (GCLM) subunits form a heterodimer

Which is the rate-limiting step in the synthesis of glutathione (GSH), a very powerful endogenous antioxidant? Both Gclc and Gclm are characteristic Nrf2 target genes, which establish Nrf2 as a regulator of glutathione, one of the most important antioxidants in the body (Purdom-Dickinson et al., 2007).

2.5.3.3 Heme oxygenase-1 (HO-1)

HO-1 is an enzyme that catalyzes the breakdown of heme into the antioxidant biliverdin, the anti-inflammatory agent carbon monoxide, and iron. HO-1 is a Nrf2 target gene that has been shown to protect from a variety of pathologies, including sepsis, hypertension, atherosclerosis, acute lung injury, kidney injury, and pain (Jarmi, Agarwal, 2009).

2.5.3.4 The glutathione S-transferase (GST) family

The GST family includes cytosolic, mitochondrial, and microsomal enzymes that catalyze the conjugation of GSH with endogenous and xenobiotic electrophiles. After detoxification by [GSH] conjugation catalyzed by GSTs, the body can eliminate potentially harmful and toxic compounds. GSTs are induced by Nrf2 activation and represent an important route of detoxification.

2.5.3.5 The UDP-glucuronosyltransferase (UGT) family

Catalyze the conjugation of a glucuronic acid moiety to a variety of endogenous and exogenous substances, making them more water soluble and readily excreted. Important substrates for glucuronidation include bilirubin and acetaminophen. Nrf2 has been shown to induce UGT1A1 and UGT1A6 (Yueh, Tukey, 2007).

2.5.3.6 Multidrug resistance-associated proteins (Mrps)

Mrps are important membrane transporters that efflux various compounds from various organs and into bile or plasma, with subsequent excretion in the feces or urine, respectively. Mrps have been shown to be upregulated by Nrf2 and alteration in their expression can dramatically alter the pharmacokinetics and toxicity of compounds (Maher et al., 2007; Reisman et al., 2009).

2.6 Antioxidants and antioxidant-related enzymes

Defense mechanisms against free radical-induced oxidative damage include the following: (i) catalytic removal of free radicals and reactive species by factors such as catalase (CAT), superoxide dismutase (SOD), peroxidase, and thiol-specific antioxidants; (ii) binding of proteins (e.g., transferrin, metallothionein, haptoglobins, caeroplasmin) to pro-oxidant metal ions, such as iron and copper; (iii) protection

against macromolecular damage by proteins such as stress or heat shock proteins; and (iv) reduction of free radicals by electron donors, such as GSH, vitamin E (α tocopherol), vitamin C (ascorbic acid), bilirubin, and uric acid (Halliwell, Gutteridge, 1986).

Animal catalases are heme-containing enzymes that convert hydrogen peroxide (H_2O_2) to water and O_2, and they are largely localized in subcellular organelles such as peroxisomes. Mitochondria and the endoplasmic reticulum contain little CAT. Thus, intracellular H_2O_2 cannot be eliminated unless it diffuses to the peroxisomes (Halliwell, Gutteridge, 1986). Glutathione peroxidases (GSH-Px) remove H_2O_2 by coupling its reduction with the oxidation of GSH. GSH-Px can also reduce other peroxides, such as fatty acid hydroperoxides. These enzymes are present in the cytoplasm at millimolar concentrations and also present in the mitochondrial matrix. Most animal tissues contain both CAT and GSH-Px activity. SODs are metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation. Three isoforms have been identified, and they all are present in all eukaryotic cells. The copper-zinc SOD isoform is present in the cytoplasm, nucleus, and plasma. On the other hand, the manganese SOD isoform is primarily located in mitochondria. Dietary micronutrients also contribute to the antioxidant defense system. These include β-carotene, vitamin C, and vitamin E (the vitamin E family comprises both tocopherols and tocotrienols, with α-tocopherol being the predominant and most active form). Water-soluble molecules, such as vitamin C, are potent radical scavenging agents in the aqueous phase of the cytoplasm, whereas lipid soluble forms, such as vitamin E and β-carotene, act as antioxidants within lipid environments. Selenium, copper, zinc, and manganese are also important elements, since they act as cofactors for antioxidant enzymes. Selenium is considered particularly important in protecting the lipid environment against oxidative injury, as it serves as a cofactor for GSH-Px (Deneke, Fanburg, 1989; Lauterburg et al., 1984). The most abundant cellular antioxidant is the tripeptide, GSH (l-γ-glutamyl-l-cysteinyl glycine). GSH is synthesized in two steps. First, γ-glutamyl cysteine synthetase (γ-GCS) forms a γ-peptide bond between glutamic acid and cysteine, and then GSH synthetase adds glycine. GSH prevents the

oxidation of protein thiol groups, either directly by reacting with reactive species or indirectly through glutathione transferases (Deneke, Fanburg, 1989; Lauterburg et al., 1984).

2.7 Phase I and phase II enzyme

2.7.1 Phase I enzyme

Phase I oxidative reactions include oxidation, hydrolysis and reduction. Depending on the chemical nature of a xenobiotic, the former is characterized by oxidative metabolism resulting in either: pharmacological inactivation or activation, facilitated elimination, and/or addition of reactive groups for subsequent phase II conjugation (McCarver, Hines, 2002).

2.7.2 Phase II enzyme

Phase II conjugative reactions including glutathione conjugation, glucuronidation, sulfation, acetylation, and methylation, generally result in pharmacological inactivation or detoxification, although instances of bioactivation are known. Conjugation products also can be substrates for specific transport enzymes, thus facilitating elimination from the body (Hines, McCarver, 2002). The phase II detoxification proteins are characterized by their ability to conjugate xenobiotics using small molecular weight, organic donor molecules such as glutathione, UDP-glucuronic acid, or acetyl coenzyme A. There also exits the antioxidase in the cell, they can eliminate the active oxygen species procuced by exogenous toxin or produced during the endogenous detoxification processes, and finally transform the ROS into H_2O and oxygens (Limon-Pacheco, Gonsebatt, 2009).

2.8 Curcumin

Turmeric, derived from the plant *Curcuma longa*, is a gold-colored spice commonly used in the Indian subcontinent, not only for health care but also for the preservation of food and as a yellow dye for textiles (Aggarwal et al., 2007b). It has been shown to possess anti-inflammatory, antioxidant, and antitumor properties (Thangapazham et al., 2006).

Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa* has a wide spectrum of biological and pharmacological activities.

Chemically, curcumin is a bis-α, β -unsaturated *β*-diketone (commonly called diferuloylmethane, which exhibits keto–enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium. Commercial curcumin contains approximately 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin (figure 3) (Anand et al., 2007).

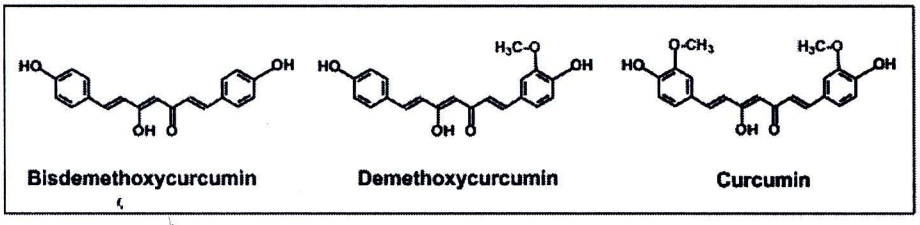

Figure 3 Major curcuminoids in *Curcuma longa* (Anand et al., 2007)

2.8.1 Chemical

Curcumin incorporates several functional groups. The aromatic ring systems, which are polyphenols are connected by two α,β-unsaturated carbonyl groups. The two carbonyl groups form a diketone. The diketone form stable enols or are easily deprotonated and form enolates, while the α, β-unsaturated carbonyl is a good Michael acceptor and undergoes nucleophilic addition. The structure was first identified in 1910 by Kazimierz Kostanecki, J. Miłobędzka and Wiktor Lampe.

2.8.2 Biosynthesis

The biosynthetic route of curcumin has proven to be very difficult for researchers to determine. In 1973 Roughly and Whiting proposed two mechanisms for curcumin biosynthesis. The first mechanism involves a chain extension reaction by cinnamic acid and 5 malonyl-CoA molecules that eventually arylized into a curcuminoid. The second mechanism involves two cinnamate units being coupled together by malonyl-CoA. Both mechanisms utilize cinnamic acid as their starting point, which is derived from the amino acid phenylalanine. This is noteworthy because plant biosyntheses employing cinnamic acid as a starting point are rare compared to the more common use of p-coumaric acid (Kita et al., 2008). Only a few identified compounds, such as anigorufone (Schmitt et al., 2000) and pinosylvin (Rosemann et al., 1991), use cinnamic acid as their start molecule. It wasn't until 2008 in which an experimentally backed route was presented. This propose

biosynthetic route follows both the first and second mechanisms suggested by Roughley and Whiting. However, the labeling data supported the first mechanism model in which 5 malonyl-CoA molecules react with cinnamic acid to form curcumin (Kita et al., 2008). However, the sequencing in which the functional groups, the alcohol and the methoxy, introduce themselves onto the curcuminoid seems to support more strongly the second proposed mechanism (Kita et al., 2008). Therefore it is concluded that the second pathway proposed by Roughly and Whiting was correct.

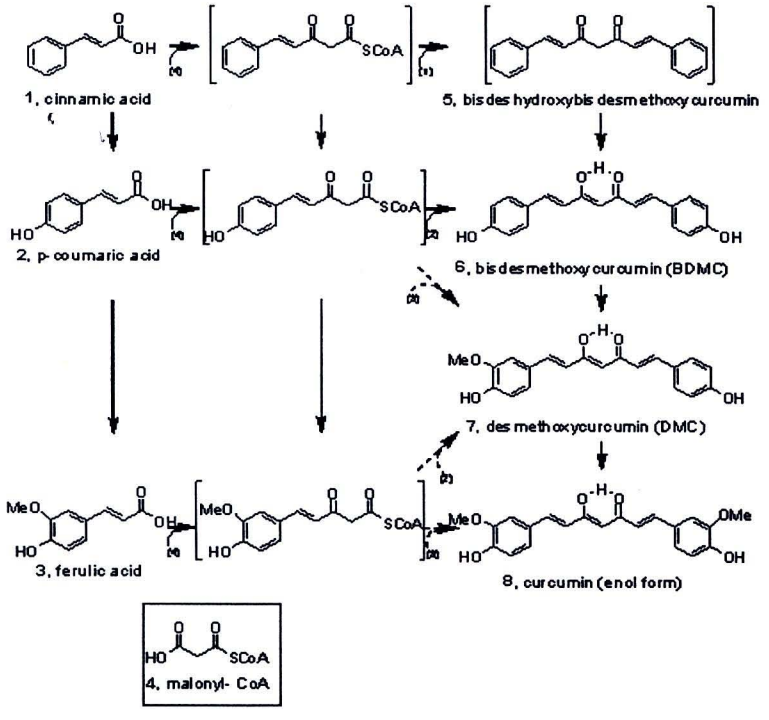

Figure 4 Biosynthetic pathway of curcumin in *Curcuma longa* (Kita et al., 2008)

2.8.3 Bioavailability

Little curcumin, when eaten, is absorbed (Anand et al., 2007) from 2 to 10 grams of curcumin eaten alone resulted in indetectable to very low serum levels (Shoba et al., 1998). Curcumin is unstable in the gut, and the traces that pass through the GI tract rapidly degrade or are conjugated through glucuronidation.

There are several commercial products developed to provide an alternate route to curcumin. For example, curcumin supplements with piperine are readily available. But curcumin in a non-solubilized pill form can limit

bioavailability. Other products, such as Nutmeric, provide curcumin in an oil-solubilized form similar to Indian curry preparations.

Co-supplementation with 20 mg of piperine (extracted from black pepper) significantly increased the absorption of curcumin by 2000% in a study funded by a prominent manufacturer of piperine (Shoba et al., 1998). However, the increase in absorption only occurred during the first hour, after which the difference between the piperine curcumin and the regular curcumin is almost the same as far as absorption. Due to its effects on drug metabolism, piperine should be taken cautiously (if at all) by individuals taking other medications.

2.8.4 Problems of Curcumin bioavailability

The reasons for reduced bioavailability of any agent within the body are low intrinsic activity, poor absorption, high rate of metabolism, inactivity of metabolic products and/or rapid elimination and clearance from the body. Problems of curcumin bioavailability such as low serum levels, limits tissue distribution, apparent rapid metabolism and short half-life (Anand et al., 2007).

2.8.4.1 Serum concentration

One of the major observations related to curcumin studies involves the observation of extremely low serum levels. The first report study to examine the uptake, distribution, and excretion of curcumin was by Wahlstrom and Blennow in 1978 using Sprague–Dawley rats. Negligible amounts of curcumin in blood plasma of rats after oral administration of 1 g/kg of curcumin showed that curcumin was poorly absorbed from the gut. In 1980, Ravindranath et al. have showed that after oral administration of 400 mg of curcumin to rats, no curcumin was found in heart blood, whereas a trace amount (less than 5μg/ml) is found in the portal blood from 15 min to 24 hours after administration of curcumin (Ravindranath, Chandrasekhara, 1980). In another study using tritium-labeled curcumin, the same group has been shown detectable amounts of curcumin in blood with doses ranging from 10 to 400 mg of curcumin per animal (Ravindranath, Chandrasekhara, 1981b). When curcumin is given orally at a dose of 2 g/kg to rats, a maximum serum concentration of 1.35 ± 0.23 μg/mL is observed at time 0.83 hours, whereas in humans the same dose of curcumin resulted in either undetectable or extremely low (0.006 ± 0.005 μg/mL at 1 hour) serum levels (Shoba et al., 1998).

2.8.4.2 Tissue distribution

Uptake and distribution of curcumin in body tissues is obviously important for its biological activity, yet only a limited number of studies have been addressed this issue. Ravindranath et al. (1980) have show that after oral administration of 400 mg of curcumin to rats only traces of unchanged drug were found in the liver and kidney. At 30 min, 90% of curcumin is found in the stomach and small intestine, but only 1% was present at 24 hours. In an *in vitro* study, when averted sacs of rat intestine are incubated with 50–750 μg of curcumin in 10 mL of incubation medium 30–80% of the curcumin disappeared from the mucosal side and no curcumin is found in the serosal fluid. Less than 3% of the curcumin is found in the tissues at the highest curcumin concentration (Ravindranath, Chandrasekhara, 1981a). Another study evaluated the tissue distribution of curcumin using tritium-labeled drug. They found that radioactivity is detectable in blood, liver, and kidney following doses of 400, 80, or 10 mg of [^{3}H] curcumin. With 400 mg, considerable amounts of radio labeled products are present in tissues 12 days after dosing. The percentage of curcumin absorb (60–66% of the given dose) remain constant regardless of the dose indicating that administration of more curcumin does not result in higher absorption (Ravindranath, Chandrasekhara, 1981b). That is, in rats there is a dose-dependent limitation to bioavailability.

2.8.4.3 Metabolites

Various studies have evaluated the metabolism of curcumin in rodents and in humans. Once absorbed, curcumin is subjected to conjugations like sulfation and glucuronidation at various tissue sites. The very first biodistribution study have been reported the metabolism of major part of curcumin orally administered to rats. Liver was indicated as the major organ responsible for metabolism of curcumin (Garcea et al., 2004; Hoehle et al., 2006; Wahlstrom, Blennow, 1978). Holder et al. (1978) have reported that the major billiary metabolites of curcumin are glucuronides of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC) in rats. A minor biliary metabolite was dihydroferulic acid together with traces of ferulic acid. In addition to glucuronides, sulfate conjugates are found in the urine of curcumin treated rats (Ravindranath, Chandrasekhara, 1980). Hydrolysis of plasma samples with glucuronidase by Pan et al. showed that 99% of curcumin in plasma is

present as glucuronide conjugates. This study also reveals curcumin–glucuronoside, dihydrocurcumin–glucuronoside, THC-glucuronoside, and THC are major metabolites of curcumin *in vivo* (Pan et al., 1999).

2.8.4.4 Half-life

Systemic elimination or clearance of curcumin from the body is also an important factor, which determines its relative biological activity. Early studies by Wahlstrom and Blennow (1978) have reported that when 1 g/kg curcumin is given orally to rats, 75% of it is excreted in the feces and negligible amounts are found in the urine. Intravenous (i.v.) and i.p. administration of curcumin result in biliary excretion of drug from cannulated rats (Holder et al., 1978). Another study using radiolabeled curcumin shows that when drug is administered orally to rats at a dose of 400 mg/rat, nearly 40% of curcumin in unchanged form is found in the feces (Anand et al., 2007).

2.8.5 Health benefits of curcumin

2.8.5.1 Antioxidant effects

Water- and fat-soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity, comparable to vitamins C and E (Toda et al., 1985). Incubation (18 hours) with curcumin result in enhance cellular resistance to oxidative damage. Curcumin's antioxidant role in down-regulating nitric oxide formation, a key element in inflammation and possibly in the process of carcinogenesis (Brouet, Ohshima, 1995).

2.8.5.2 Anti-inflammatory effects

In numerous studies, curcumin's anti-inflammatory effects have been shown to be comparable to the potent drugs with anti-inflammatory effect. Unlike the drugs, which are associated with significant toxic effects (ulcer formation, decreased white blood cell count, intestinal bleeding), curcumin produces no toxicity. *Curcuma longa* significantly reduce inflammatory swelling and inhibiting pro-inflammatory arachidonic acid, as well as neutrophil function during inflammatory action (Arora et al., 1971; Chandra, Gupta, 1972; Mukhopadhyay et al., 1982; Srivastava, 1989).

2.8.5.3 Anticarcinogenic effects

Curcumin's ability is to inhibit carcinogenesis at three stages: tumor promotion, (Kawamori et al., 1999) angiogenesis, (Thaloor et al., 1998) and tumor growth (Limtrakul et al., 1997). In two studies of colon and prostate cancer, curcumin is found to inhibit cell proliferation and tumor growth (Hanif et al., 1997). Turmeric and curcumin are also capable of suppressing the activity of several common mutagens and carcinogens in a variety of cell types. The anticarcinogenic effects of turmeric and curcumin are due in part to direct antioxidant and free-radical scavenging effect but they also enhance the body's natural antioxidant system, increasing glutathione levels, thereby aiding in hepatic detoxification of mutagens and carcinogens, and inhibiting nitrosamine formation.

2.8.5.4 Cardiovascular effects

Turmeric's protective effects on the cardiovascular system include lowering cholesterol and triglyceride levels, decreasing susceptibility of low density lipoprotein (LDL) to lipid peroxidation, and inhibiting platelet aggregation.

2.8.5.5 Other turmeric applications

Natural remedies such as turmeric, have been used topically for the avoidance and treatment of many conditions. For example, turmeric is used in many countries as treatment of wound, burns, including sun burns and psoriasis as well as in body lotions, moisturizers, antiseptic agents, beauty aids, allergic reaction formulations, anti-inflammatory products, anti-cancer products, anti-aging products, anti-oxidant products and osteoporosis products, including vitamin D. Turmeric extract inhibits the growth of a variety of bacteria, parasites, and pathogenic fungi.

2.8.6 Adverse effects of curcumin

Though curcumin is demonstrably bioactive and nontoxic, there are rare anecdotal reports of its deleterious side effects under certain conditions. Frank et al. (2003) have reported that copper-bound curcumin loses its ability to inhibit liver and kidney tumors in Cinnamon rats. Others have noted that curcumin can exhibit some blood-thinning properties such as suppression of platelet aggregation, although it remains to be established whether curcumin interacts in any way with blood-thinning drugs. Although several published studies suggest that curcumin may beneficially induce apoptosis in part through its induction of p53 expression

(Aggarwal et al., 2007a), at least two other studies suggest that curcumin may instead have a deleterious, antiapoptotic effect by downregulating p53 (Lauterburg et al., 1984; Tsvetkov et al., 2005). Similarly, although dozens of studies indicate that curcumin potentiates the effect of chemotherapeutic agents, at least one study has been done in mice suggests that a curcumin supplemented diet may inhibit the antiproliferative effects of cyclophosphamide on breast cancer growth (the investigators in that study, however, monitored tumor growth for only 3 days) (Somasundaram et al., 2002).

2.8.7 Effect of curcumin on oxidative and nitrative stress

Curcumin counteracts with the ROS by increasing ornithine decarboxylase, glutathione, antioxidant enzymes, and phase II metabolizing enzymes and therefore protects the kidney from oxidative damage (Okazaki et al., 2005). Curcumin is found to be a superior chemopreventive agent in both initiation and postinitiation stages of 4-nitroquinoline 1-oxide–induced oral carcinogenesis when compared with beta-carotene and hesperidin (Tanaka et al., 1994). HO-1, the rate-limiting enzyme of heme catabolism, has been found to counteract oxidative stress (Motterlini et al., 2000), modulate apoptosis, and inhibits proliferation in rat and human breast cancer cells (Hill et al., 2005). Curcumin has been found to induce HO-1 expression by signaling through (NF-E2)-related factor 2 (Nrf-2) and NF-κB and thereby has the potential to reduce oxidative stress (Balogun et al., 2003). Nrf2 is a transcription factor that regulates the expression of conjugating enzymes like glutathione S-transferase (GST) via an antioxidant response element (ARE) (Itoh et al., 1997). Nrf2 activity is regulated by Nrf2's sequestration in the cytoplasm by the kelch-domain-containing protein Keap1 (Kelch-like ECH associated protein 1). Keap1 releases Nrf2 in the presence of oxidants and chemoprotective agents, thereby leading to the activation of ARE and the expression of phase II enzymes (Pool-Zobel et al., 2005). In renal epithelial cells, curcumin has been shown to promote dislodging of Nrf2 from Nrf2-Keap1 complex, leading to increased Nrf2 binding to the resident HO-1 AREs, resulting in upregulation of HO-1 expression (Balogun et al., 2003). Curcumin prevents initiation of tumors either by curtailing the proinflammatory pathways or by inducing phase II enzymes (Thangapazham et al., 2006).

2.8.8 The effect of curcumin on *O. viverrini* infection

In animal model, Pinlaor et al. (2009) have reported that curcumin can reduce oxidative and nitrative DNA damage by suppression of oxidant-generating genes and enhancement of antioxidant genes, leading to inhibition of oxidative and nitrative stress. Moreover, 1% curcumin (w/w) supplement in normal diet can reduce periductal fibrosis at the long-term treatment in *O. viverrini*-infected hamster.

These results suggest that curcumin reduces periductal fibrosis by tissue resorption via inhibition of TIMPs expression and enhancement of MMPs expression mediated by cytokines (Pinlaor et al., 2010). In addition, curcumin can prevent cholangiocarcinogenesis induced by the combination of *O. viverrini* infection and *N*-nitrosodimethylamine (NDMA) administration using a hamster model. Curcumin inhibits CCA development through the suppression of NF-κB-related gene products involved in inflammation, DNA damage, apoptosis, cell proliferation, angiogenesis and metastasis and improves the survival rate of hamsters (Prakobwong et al., 2010).

2.8.9 The effect of curcumin on others parasitic disease

In *Leishmania donovani*-infected mice, Adapala and Chan (2008) have studied the bioavailablity of a well-established dietary antiinflammatory, curcumin, and examine its effect on adaptive immunity. The results showed that curcumin activates PPARs in mice, modulates the type 1/type 2 immune balance, and influences adaptive immunity to *L. donovani*. The study also suggests that curcumin has the potential to attenuate or exacerbate inflammatory diseases of the liver and spleen depending on whether inflammation and type 1 immune response are protective or detrimental. In agreement, Chan et al. (2005) have found that curcumin protects promastigotes and amastigotes of the visceral species, *L. donovani*, and promastigotes of the cutaneous species, *L. major*, against the actions of S-nitroso-N-acetyl-D, L-penicillamine (SNAP) and DETANONOate, which releases NO, 3-morpholino-sydnonimine hydrochloride (SIN-1), which releases NO and superoxide, and peroxynitrite. Also, Shahiduzzaman et al. (2009) have studied the effects of curcumin on infectivity and development of *Cryptosporidium parvum* in a recently established *in vitro* system combining infection of human ileocecal adenocarcinoma cell cultures with quantification of intracellular parasites by real-time PCR. Curcumin has been found to be effective (>95% inhibition of parasite growth) at 50 microM for 24 hours

when infected cultures are exposed for more than 12 hours. Withdrawal of curcumin after 24 hours of exposure does not result in a significant resumption of *C. parvum* growth. The invasion of host cells by sporozoites (infectivity) is found to be inhibited at least 65% in the presence of 200 microM curcumin. No significant reduction of viability of *C. parvum* oocysts after incubation with curcumin is recorded. Altogether, curcumin has showed promising anticryptosporidial effects under *in vitro* conditions.

In *Angiostrongylus cantonensis*-infected mice, Shih et al. (2007) have found that curcumin alone does not interfere with MMP-9 expression or action, and so may be not useful for larvicidal effect. The possible reasons include low level of curcumin across the blood–brain barrier and the larvae that survive stimulate MMP-9 production which in turn promotes blood–brain barrier damage, with leukocytes then crossing the blood–brain barrier to cause meningitis. Benificial effect of curcumin on parasite infection may be explained by immunomodulatory effects. In curcumin treatment on murine schistosomiasis mansoni, the study is designed by using curcumin at a dose of 400 mg/kg body. Curcumin treatment modulates cellular and humoral immune responses of infected mice and leads to a significant reduction of parasite burden and liver pathology in acute murine schistosomiasis mansoni (Allam, 2009).

2.8.10 The cytotoxicity of curcumin to kill various parasites

Curcumin has not only reduces the severity of the parasite diseases but also its may affect on parasites directly. For example, in vitro schistosomicidal activity of curcumin (doses ranging from 5 to 100 μM) was carried out against *Schistosoma mansoni* adult worms. Curcumin (at 50 and 100 μM) caused death of all worms. When tested at the doses of 5 and 20 μM, it decreased the worm viability in comparison with negative (Roswell Memorial Park Institute (RPMI) 1640 medium alone or RPMI 1640 medium with 10% dimethyl sulfoxide) and positive (heat-killed worms at 56°C or praziquantel 10 μM) control groups. All pairs of coupled adult worms were separated into individual male and female by the action of curcumin at the doses of 20 to 100 μM. When tested at 5 and 10 μM, curcumin reduced egg production by 50% in comparison with the positive control group. It is the first time that the schistosomicidal activity has been reported for curcumin (Magalhaes et al., 2009). In addition curcumin can inhibit *Giardia lamblia* trophozoite, growth and

adhesion in more than 50% in dose and time dependent manner in *in vitro*. Morphological changes are described as protrusions formed under the cytoplasmic membrane, deformation due to swelling and cell agglutination. Curcumin can also induce apoptosis-like nuclear staining in dose and time dependent manner (Perez-Arriaga et al., 2006). Likewise, curcumin treatment in *Plasmodium falciparum* culture, this nutraceutical agent can damage of both mitochondrial and nuclear DNA, probably due to the elevation of intracellular ROS and that curcumin is potent against both chloroquine (CQ)-susceptible (CQS) and -resistant (CQR) *P. falciparum* strains. The parasiticidal effect is at least partially due to the generation of ROS, and down-regulation of the PfGCN5 HAT activity (Cui et al., 2007). In *in vitro* leishmanicidal activity, it has been found that the leishmanicidal activity of curcumin is shown as 50% growth inhibitory concentration (GI_{50}), 100% growth inhibitory concentration (TGI) and LD_{50} after incubation with leishmania for 24 hours. A 100% growth indicates a leishmania mass equal to untreated control and 0% indicates no increase in leishmania mass over zero (Koide et al., 2002).

2.8.11 The effect of curcumin to prevent adverse effect of various conditions

In addition to reduce the disease and cytotoxicity effect of curcumin, it can use as chemopreventive agent to prevent inflammation-induced adverse effect of various condition. Awasthi et al. (1996) have studied efficacy of curcumin, an antioxidant present in the commonly used spice turmeric, in preventing cataractogenesis in an *in vitro* rat model. The animals in the experimental group are administered 1 ml corn oil containing 75 mg curcuminl kg body wt by oral gavage daily for 14 days. At this dose, no apparent adverse effects are observed in the animals. These studies suggest that curcumin may be an effective protective agent against cataractogenesis induced by lipid peroxidation. *In vivo* studied role of curcumin in protecting the liver against injury and fibrogenesis caused by carbon tetrachloride (CCl_4) in rats by treated with curcumin at 200 mg/kg. The result shows that curcumin protects the rat liver from CCl_4-caused injury and fibrogenesis by suppressing hepatic inflammation, attenuating hepatic oxidative stress and inhibiting HSC activation (Fu et al., 2008). In a human breast cancer xenograft model, dietary administration of curcumin significantly decrease the incidence of breast cancer

metastasis to the lung and suppresses the expression of NF-κB, cyclooxygenase 2, and matrix metalloproteinase-9 (Aggarwal et al., 2005). Curcumin activates the HO-1 gene via regulation of Nrf2 and the antioxidant-responsive element in renal epithelial cells. This effect is associated with a significant increase in HO-1 protein expression and haem oxygenase activity. From several lines of investigation report that curcumin (and, by inference, CAPE) stimulates *HO-1* gene activity by promoting inactivation of the Nrf2–Keap1 complex, leading to increased Nrf2 binding to the resident *ho-1* AREs (Balogun et al., 2003). Also, Samuhasaneeto et al. (2003) have studied the mechanism of curcumin-attenuated inflammation and liver pathology in early stage of alcoholic liver disease in rat model. Curcumin treatments results in improving of liver pathology, decreasing the elevation of hepatic MDA, and inhibition of NF-κB activation. The 400 mg/kg body wight of curcumin treatment reveals only a trend of decreased hepatocyte apoptosis.

2.9 Conceptual framework

O. viverrini-infected hamster induces immunosuppression at chronic infection. However, praziquantel treatment induces a parasite antigen bursting and stimulates inflammatory cell migration for host defense mechanism. This response leads to inflammation-mediated oxidative and nitrative stress via free radicals production including reactive oxygen species (ROS) and reactive nitrogen species (RNS) during short-term treatment. Therefore, intervention by chemopreventive agents such as curcumin may be prevents oxidative and nitrative stress induced by praziquantel treatment in *O. viverrini*-infected hamsters.

Curcumin may reduce oxidative/ nitrative stress via activation of the transcription factor Nrf2, and expression of relate gene products including HO-1, Keap1, NQO1, and GCL, leading to an increase in the level of the ferric antioxidant capacity in the plasma. In contrast, curcumin may inhibit NF-κB, and related gene products involved in inflammation-associate oxidative/nitrative stress. This nutraceutical agent may also inhibit proinflammatory cytokines, in relation to oxidative and nitrative stress markers, and reduce urinary 8-oxodG level, plasma levels of MDA and NOx, and prevents liver injury.

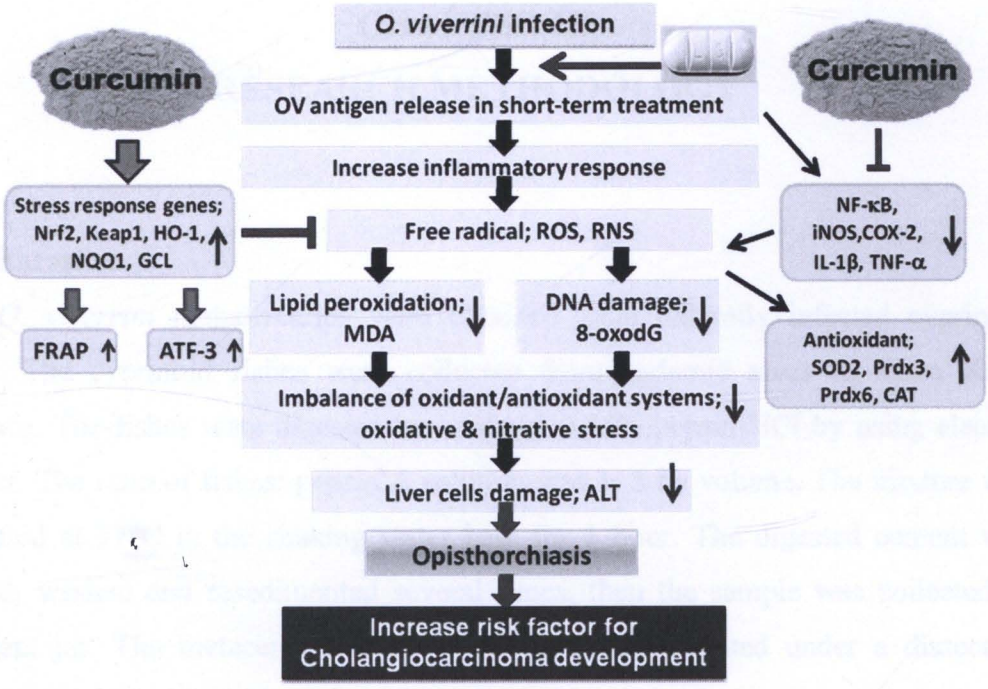

Figure 5 Conceptual framework of the study