CHAPTER I

INTRODUCTION

Cardiovascular diseases (CVD) is one of the cause of morbidity and mortality in the United States and worldwide (American Heart Association, 2007). World Health Organization (WHO) estimates that 17.5 million people around the world died from CVD in 2005. This is representing 30 percent of all global deaths. If appropriate action is not taken, by 2015, an estimated 20 million people will die from CVD every year. In Thailand, CVD are the leading causes of death for 1 in 5 of all deaths (World Health Organization, 2007). Atherosclerosis is a major cause of CVD. Epidemiologic studies have identified a multitude of risk factors for atherosclerosis, among these risk factors might be associated with lipid factors and nonlipid factors such as hypertension, diabetes, cigarette smoking, menopause and male (Expert panel NCEP-ATP III, 2001). For lipid factors, high levels of low density lipoprotein-cholesterol (LDL-C) have emerged as one of the strongest risk factor of atherosclerosis. The oxidation of LDL is the key factor of atherosclerosis which oxidized LDL (Ox-LDL), a chemo-attractant monocytes interact with the endothelial layer, attach firmly to the endothelium, migrate into the subendothelial space, and then accumulated into the macrophage after that macrophage become foam cells which is an early state of atherosclerosis is process (Keaney, 2000; Stocker and Keaney, 2004). In contrast, high density lipoprotein (HDL) is known as good cholesterol containing anti-atherogenic properties because it has beneficial effect in removing cholesterol from blood stream, hydrolyze lipid peroxides and mediate cholesterol efflux from macrophage foam cell (Mertens and Holvoet, 2001; Nofer et al., 2002; Zuliani et al., 2007). Interestingly, paraoxonases (PONs) which are a group of enzyme that associated with HDL now believed to be related to the anti-atherogenic properties of HDL.

The paraoxonase (PON) gene family consists of three members: paraoxonase1 (PON1), paraoxonase2 (PON2), and paraoxonase3 (PON3), located adjacent to each other on the long arm of chromosome 7 in humans and on chromosome 6 in mouse between q22.3 and q23.1 (Aviram and Rosenblat, 2004). Of the PONs family, PON1 is the most investigated and best understood member whereas PON2 is the oldest in

the evolution. PONs family shares approximately 65% similarity at the amino acid level and approximately 70% similarity at the nucleotide level (Draganov and La Du, 2004). PON1 is primarily synthesized in the liver and excreted in the blood where associated with the HDL particle (James and Deakin, 2004). It is highly conserved in mammals but is absent in fish, birds and invertebrates (Durrington et al., 2001). PON1 is a glycoprotein of 354 amino acids with a molecular mass of 43-45 kDa (Ng et al., 2005) and contains as many as three carbohydrate chains, three cysteine (Cys) residues, a single disulfide bond between Cys-42 and Cys-353 (Mackness et al., 1998; Aviram, 1999). A single free cysteine only at position 284 which has a free sulfhydryl group (-SH) is essential for the action of PON1 to inhibit of both LDL and HDL oxidation. Thus, one function of PON1 may be to act as an antioxidant in the prevention of atherosclerosis (Draganov and La Du, 2004). The information regarding PON2 is scarce. PON2 is a widely expressed intracellular protein with a molecular mass of approximately 44 kDa, which is widely distributed in a number of tissues, including heart, liver, kidney, lung, placenta, small intestine, spleen, stomach and testis as well as in the cells of the artery wall, including endothelial cells, smooth muscle cells, and macrophage cells (Ng et al., 2005). PON2 is not detectable in serum, so it is likely that PON2 play a role in intracellular to inhibit LDL lipid peroxidation, reverse the oxidation of mildly oxidized LDL, and inhibit its ability to induce monocyte chemotaxis (Ng et al., 2001; Shamir et al., 2005). PON3 shows a high similarity in structure and functions with PON1 which primarily synthesized in the liver and associated with HDL in plasma. However, significant PON3 mRNA level is also detectable in the kidneys (Lu et al., 2005). Indeed, PON3 shares three conserved cysteine residues: Cys-42, Cys-353 and Cys-284, and possesses similar properties in structure and activities with PON1 (Reddy et al., 2001). In contrast to PON1, PON3 has very limited arylesterase activity and no activity towards paraoxon, but it can hydrolyze lactones rapidly (Draganov et al., 2000; La Du, 2001). Even through, the information of PON3 is scarce but it has a promising evidence of anti-atherosclerotic properties. Both rabbit PON3 and PON1 have the ability to protect LDL against *in vitro* copper-induced oxidation while rabbit PON3 is approximately 100 times more potent than rabbit PON1 in protecting LDL against oxidation (Draganov et al., 2000). Recently, it was found that over-expression of human PON3 in mice reduced atherosclerotic lesion (Ng et al., 2007). Thus, PONs might play a role

in atherosclerotic process. Furthermore, some clinical data suggest that treatment with hypolipidemic drugs such as simvastatin modulates PON1 activity (Tomas et al., 2000; Balogh et al., 2001).

Previous studies have shown that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins effectively reduce risk for major coronary heart disease (CHD) and atherosclerosis (Liao, 2002; Rosenson, 2004; Almuti et al., 2006; Rallidis, 2007). The National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) guidelines have been expanded to recognize the importance of both LDL and HDL levels to be major risk factors of CHD. The recommended LDL-C goal is <100 mg/dL but in high risk persons, an LDL-C goal of <70 mg/dL is a therapeutic goal. Goal of HDL-C is >40 mg/dL but if >60 mg/dL which negative one risk point from the major risk factor list (Grundy et al., 2004). Following the NCEP-ATP III, the results from clinical trials showed the benefit of statins for lipid lowering, reducing the risk of CHD and atherosclerosis. Moreover, statins decreased LDL-C by 18-55% and increased HDL-C by 5-15% (dependent on types and dosages of drugs treatment) (Expert panel NCEP-ATP III, 2001) and associated to an increase of PON1 activity (Tomas et al., 2000; Deakin et al., 2003). As these reasons, statins might play a role in reduction risk of CHD and atherosclerosis through PON1. Currently, statins in clinical use include lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, pitavastatin and rosuvastatin (Davidson, 2002). Simvastatin is widely used as lipid lowering drug in patients with disturbed lipid metabolism, CHD and atherosclerosis because it is unexpensive as compared to other statins. Several clinical studies were conducted to explore whether patients would benefit most from simvastatin such as the Scandinavian Simvastatin Survival Study (4S), Heart Protection Study (HPS) and Phase Z of the A to Z trial (Scandinavian Simvastatin Survival Study Group, 1994; Heart Protection Study Collaborative Group, 2002; De Lemos, 2004; Ong, 2005). It was shown that simvastatin may cause some adverse effects such as myalgias, myopathy and rhabdomyolysis (Bays, 2006). Therefore, herbal medicine namely *"Curcuma comosa* Roxb. (*C. comosa)"* might be one of an alternative instead of statins / synthetic drugs to reduce the risk of CVD because it has been claimed to be natural origin and has been used long-term as folk medicines.

C. comosa Roxb. is a plant in family Zingiberaceae. It is an indigenous plant of Thailand with a common name in Thai as Waan Chak Mod Look (Smitinand, 2001). Rhizomes of *C. comosa* have been used extensively in Thai traditional medicine as an anti-inflammatory agent particularly for the treatment of postpartum uterine bleeding, peri-menopausal bleeding and uterine inflammation. A number of different active principles of *C. comosa* include: (1) Diarylheptanoids: *trans*-1,7-diphenyl-5-hydroxy-1-heptene, *trans*-1,7-diphenyl-6-hepten-3-one-5-ol, *trans*-1,7-diphenyl-3-acetoxy-6-heptene, *trans*-1,7-diphenyl-6-heptene-3-one, *trans,trans*-1,7-diphenyl-1,3-heptadien-5-ol, *trans,trans*-1,7-diphenyl-4,6-heptadien-3-one, 1,7-diphenyl-1(*1E,3E,5E*)-heptatriene, 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(*1E*)-1-heptene), 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene. (2) Acetophenones: 4,6-dihydroxy-2-*O*-(*β*-D-glucopyranosyl) acetophenone (Suksamrarn et al., 1994, 1997). Many studies have revealed pharmacological effects of *C. comosa* which related to CVD besides its estrogenic effects. The choleretic effect of *C. comosa* rhizome extract had been recently investigated. It remarkably stimulated bile secretion and enhanced biliary excretion of bile salt and cholesterol which consequently led to a decrease in plasma cholesterol (Piyachaturawat et al., 1996). The hypolipidemic effect of *C. comosa* from ethyl acetate extract has been shown to effectively decreased LDL, triglycerides but increased HDL (Piyachaturawat et al., 1999, 2002a). Also, several studies reported that *C. comosa* possessed a strong anti-inflammatory activity (Jantaratnotai et al., 2006; Sodsai et al., 2007) and anti-oxidative effect (Niumsakul et al., 2007).

As mentioned above, *C. comosa* is an interesting plant with many pharmacological effects related to CVD such as choleretic effect, hypolipidemic effect, anti-inflammatory effect and anti-oxidative effect. Thus, *C. comosa* may be potential to be developed for medicinal purposes in the area of CVD. However, effects of *C. comosa* on PONs and oxidative stress have never been investigated. In addition, there is no information on the effect of simvastatin on activities of PON2 and PON3. Therefore, the aim of this study was to investigate the effect of *C. comosa* rhizome on PONs activities and oxidative stress in rabbits fed with high-cholesterol diet, compared to a lipid lowering drug, simvastatin (simvastatin, the known medicine using in cardiovascular disease).

Hypothesis

C. comosa rhizome and simvastatin increased or/and decreased paraoxonase activities and oxidative stress in rabbits fed with high-cholesterol diet.

Study design and process

1. Animal treatment: *in vivo* study
2. Sample collecting
3. Determination of lipid parameters in blood samples
4. Determination of paraoxonase activities
5. Determination of oxidative stress parameters
6. Data analysis

Anticipated benefits from the study

An information regarding the effects of *Curcuma comosa* rhizome on PONs and oxidative stress compared to simvastatin, the known medicine using in cardiovascular diseases, will provide an information on the potential of this plant to be used in cardiovascular diseases in the future.