# CHAPTER V

## DISCUSSION AND CONCLUSION

It is believed that one of the underlying mechanisms of atherosclerosis is oxidized low density lipoprotein (Ox-LDL). Thus, lowering cholesterol especially LDL and oxidative stress are the key of atherosclerosis treatment. However, the lipid lowering agent such as simvastatin has adverse effects. *C. comosa*, the herbal medicine, may be one of an alternative for the reduction of atherosclerosis because it is natural in origin and have been used in long-term as folk medicines. This study primarily focused on the anti-atherosclerotic effects of *C. comosa* rhizome in rabbits fed with high-cholesterol diet by monitoring lipid parameters, oxidative stress parameters and paraoxonases (PONs) activities at 4 months of treatment. At 4 months of treatment, the levels of biomarkers for liver function and kidney function of *C. comosa* treatment group were not significantly different from those in the control group. However, the biomarker of liver function in the cholesterol-fed with simvastatin was significantly higher than those in the control group (Appendix D). In order to develop *C. comosa* for a medicinal purpose in CVD, many studies had been performed and found many related pharmacological effects such as choleretic effect, hypolipidemic effect, anti-inflammatory effect and anti-oxidative effect. In this study, effects of *C. comosa* rhizome on oxidative stress and PONs activities that play an important role in cardiovascular diseases were also investigated.

In this study, twelve male New Zealand White (NZW) rabbits were treated with 1.0% cholesterol for 1 month and subsequently treated with either 0.5% cholesterol or 0.5% cholesterol combined simvastatin at the dosage of 5 mg/day or 0.5% cholesterol combined *C. comosa* at the dosage of 400 mg/kg/day for 3 months. Simvastatin decreased TC and LDL-C but TG and HDL-C levels remained unchanged. These findings were consistent to the observation found in human (Tomas et al., 2000; Balogh et al., 2001). Thus, the finding in this study supported the anti-atherosclerotic role of simvastatin, of which the mechanism was to inhibit hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting step in *de novo* cholesterol synthesis. In this study, long term treatment with *C. comosa* rhizome in rabbits fed with high-cholesterol diet also caused a decrease of both TC

and LDL-C levels which was consistent to the result of Piyachaturawat et al. (1999), who reported the plasma cholesterol lowering effect of *C. comosa* extract in hypercholesterolaemic hamsters. The results in that study showed that intragastric administration of ethyl acetate extract of *C. comosa* caused a decrease in plasma cholesterol, LDL-C and TG levels with a dose-dependent manner (Piyachaturawat et al., 1999). In addition, cholesterol and LDL-C lowering effects of *C. comosa* rhizome in the present study was consistent to the effects of phloracetophenone in hypercholesterolemic hamsters which reported by Piyachaturawat et al. (2002a). In that study phloracetophenone caused a decrease in plasma cholesterol, LDL-C, TG and very low density lipoprotein (VLDL) levels in hypercholesterolemic hamsters (Piyachaturawat et al., 2002a). The different finding between using rabbits and hamsters was that *C. comosa* extracts caused an increase of HDL-C and caused a decrease of serum lipid parameters such as TC, TG, VLDL and LDL-C in hamsters (Piyachaturawat et al., 1999, 2002a). However, using rabbits in this study, long term treatment with *C. comosa* rhizome did not decrease TG, did not increase HDL-C but decreased TC and LDL-C. The mechanism of *C. comosa* on lipid parameters was still unknown. It might be associated with interference with the synthesis, secretion of lipoprotein into plasma and/or acceleration of removal of the circulating cholesterol for excretion. Actually, NZW rabbits in the present study seem to be a good animal model for studying a lipid-lowering effect of the compounds because the supplement feeding of high cholesterol diet to rabbits successfully raised the level of lipid parameters at 4 months as consistent to the result of Bocan et al. (1993), who reported that rabbit is the only one that has the tendency to exhibit hypercholesterolemia within a few days of the administration of a high cholesterol diet (Bocan et al., 1993). Lipid parameters can be increased by up to 2 to 8 times after the administration of a diet enriched with 0.1-2% cholesterol within the first 20 days (Bocan et al., 1993).

To date, the information regarding the efficacy of statins such as simvastatin in lowering serum cholesterol levels is well documented. Besides an effect on HMG-CoA reductase inhibition, statins and their metabolites were also found to have additional antioxidant effects (Aviram and Rosenblat, 2005). There are extensive evidences showing that hypercholesterolemia is associated to increased lipid peroxidation and increased oxidative stress (Morrow, 2005). The oxidative modification of lipoproteins particularly LDL has emerged as a fundamental process

in the development of atherosclerosis. Oxidative modification of LDL that is an initiating factor in atherosclerosis, possesses numerous unfavorable biological effects, including induction of endothelial dysfunction, activation of endothelial adhesiveness, monocyte differentiation and adhesion, and smooth muscle cell proliferation. Several studies suggest a relationship between Ox-LDL and severity of atherosclerosis in coronary arteries (Heinecke, 2006). Only a few studies have shown the ability of statins to reduce the levels of circulating Ox-LDL or other measures of LDL oxidation, such as circulating conjugated dienes (CD), malondialdehyde (MDA) or total peroxide (TP). Aviram and colleagues demonstrated that oxidized metabolites of atorvastatin but not the parent compound exerts inhibitory effect on lipoprotein oxidation *in vitro* (Aviram et al., 1998). Their results were consistent to the study done by Thomas and collaborates who reported a decrease of lipid peroxide level after treatment familial hypercholesterolemic patients with simvastatin (Tomas et al., 2000). In the present study, effect of *C. comosa* rhizome on oxidative stress parameters was investigated. The results showed that both *C. comosa* and simvastatin significantly increased levels of TP levels and oxidative stress index (OSI) while only simvastatin significantly decreased of total antioxidant status (TAS) levels at 4 months after treatment. Interestingly, similar results were also found in the long term treatment with atorvastatin in hypercholesterolemic patients (unpublished data). However, results from this study were inconsistent to the results which were obtained from short term treatment of patients with simvastatin as previously reported (Tomas et al., 2000; Kural et al., 2004). Unlike simvastatin, *C. comosa* did not significantly decrease TAS levels in the long term treatment in this study suggested the information that long term treatment of *C. comosa* may be advantageous than simvastatin in term of antioxidant status which may related to the protective effect against atherosclerosis.

Although the clinical trials have provided strong evidence that lowering plasma LDL-C and raising HDL-C reduce the risk of CVD, information on the contribution of antioxidant system on atherosclerosis-related diseases are growing. Since paraoxonase1 (PON1) is the most potent HDL-associated antioxidant enzyme, and PON1 activity has been shown to correlate negatively to cardiovascular risk. Thus, in the past few years, there were many evidences provided important functions of paraoxonases (PONs) family in the reduction of the risk and/or prevention of CVD. Normally, PONs family consists of three members, PON1, paraoxonase2 (PON2) and

paraoxonase3 (PON3) (Primo-Parmo et al., 1996). These three member genes share approximately 65% similarity at the amino acid level and approximately 70% similarity at the nucleotide level (Mackness et al., 2002). Accumulated data indicated that both PON1 and PON3 are closely associated with HDL and are involved in the prevention of atherosclerosis (Draganov et al., 2000). Hence, the factors influence PON1 activity has been intensively investigated including lipid lowering agents. In this study, long term treatment with *C. comosa* rhizome and simvastatin in rabbits fed with high-cholesterol diet did not affect PON1 activity. This result was consistent to a previous finding that found an insignificantly decreased in PON1 activity after one month of simvastatin treatment in patients with types IIa and IIb hyperlipidemia (Balogh et al., 2001). Also, this finding was consistent to a previous observation that simvastatin did not influence the activity of PON1 at 3-months treatment in hyperlipidemic patients (Paragh et al., 2004). In contrast, some evidences demonstrated that short term treatment with simvastatin increased PON1 activity, thereby improving the anti-atherosclerotic effects of simvastatin (Tomas et al., 2000). This short term treatment of simvastatin on PON1 activity was consistant to a study which indicated that simvastatin increased paraoxonase concentration and activity in cell culture model (Deakin et al., 2003).

As mention before, the information of PON3 is scarcely but has a promising evidence of anti-atherosclerotic properties (Draganov et al., 2000; Ng et al., 2007). Hence, an investigation on modulation of PON3 was available. PON3 has been found to be unchanged in the oxidative stress (Reddy et al., 2001). In the present study, we also found unchanged level of PON3 after long term treatment with either simvastatin or *C. comosa* rhizome. Where OSI of both treatment were significantly higher than the control. Thus, oxidative stress may not influence PON3 as it does on many other antioxidant enzymes such as superoxide dismutase (SOD) and/or catalase (Lin et al., 2007)

PON2 is a member of PONs family, which expressed intracellular and found in various tissues and cells especially the cells of the artery wall and macrophages (Ng et al., 2001). Recently data demonstrated the physiological role of cellular PON2. PON2 possesses antioxidant property by defense cellular oxidative stress, thus protect atherosclerosis via cell mediated (Ng et al., 2001). So, the investigation on expression and regulation of PON2 has started in the past few years. Macrophage PON2 has been

shown to be up-regulated under high oxidative stress and down-regulated under low oxidative stress (Shiner et al., 2006). This study demonstrated for the first time that long term treatment with either *C. comosa* rhizome or simvastatin had no influence on PON2 activity in abdominal aorta of rabbits fed with high-cholesterol diet. However, the tendency of PON2 activity was lowest in the simvastatin treated rabbits which was also observed to have highest oxidative stress. These may be due to the possibility that compensatory up-regulated PON2 mechanism was not adequate for oxidative-induced PON2 inactivation (Shiner et al., 2006).

The results obtained from PONs activities study were not associated with significantly increased of the TP levels and OSI of both *C. comosa* rhizome and simvastatin. One explanation may be that long term used of both *C. comosa* rhizome and simvastatin did not influence indirectly to antioxidant property or may not be potent enough to cope with the oxidative burden caused from high-cholesterol diet or agents themselves. Unfortunately, we did not investigate the direct effect of antioxidant property of both agents. Also, our data were limited, thus, true difference between treatments that should present in larger samples might be missed in our study group of 4 rabbits or modulation of PON1, PON2 and PON3 might not be associated with the hypolipidemic effect and oxidative stress of the both *C. comosa* rhizome and simvastatin.

In conclusion, this study demonstrated that long term treatment with *C. comosa* rhizome decreased total cholesterol and LDL-C in rabbits fed with high-cholesterol diet similarly to simvastatin. *C. comosa* and simvastatin significantly increased TP levels and OSI which seemed to be correlated with the unchanged effect of both compounds on PON1, PON2, and PON3 activities. Further study to explore another mechanistic pathway to explain the lipid lowering effect of *C. comosa* was suggested.