CHAPTER V

DISCUSSION AND CONCLUSION

In the past, the drug used for atherosclerosis treatment including statins is believed to be involved in decreasing serum cholesterol which in turn to reduce oxLDL found in atherosclerotic plaque (Miller, 2001). Statins have been found to possess pleiotropic effects such as anti-inflammation, anti-oxidation which have been proposed to be superior to other lipid-lowering drugs (Tandon et al., 2005). However, accumulated evidence demonstrated that statins demonstrated serious adverse effects including rhabdomyolysis, liver failure (Kiortsis et al, 2007). Therefore, *C. comosa*, the medicinal herb, may be one of an alternative to alleviate atherosclerosis because it is natural in origin and has been used in long-term as folk medicines as anti-inflammation. Several studies had been performed to investigate the potential of *C. comosa* to be used in cardiovascular disease and found many related pharmacological effects such as choleretic effect, hypolipidemic effect, anti-inflammatory effect, anti-oxidative effect, and anti-plaque formation effect. Besides investigating pure compounds or various solvent extracts isolated from *C. comosa*, studying of crude pulverized rhizome was also interested because of its pharmacologically effective (Yupin Sanvarinda et al., 2007) and its practically used in traditional medicine. This study primarily focused on the anti-atherosclerotic effects and liver toxicity of *C. comosa* crude pulverized rhizome in rabbits fed with high cholesterol diet by assessing the expression of various inflammatory cytokines in abdominal aorta and liver tissues after 3 months of the treatment.

This study shared the tissues of animals with a previous study of Cheerana Yomchot (2007). The NZW rabbits were fed with 1% cholesterol diet for 1 month then the rabbits were divided into 3 groups. The rabbits in control group were fed with 0.5% cholesterol diet. The rabbits in positive control group were fed with 0.5% cholesterol diet combined with simvastatin at the dosage of 5 mg/day and the rabbits in *C. comosa* group were fed with 0.5% cholesterol diet combined with *C. comosa* at the dosage of 400 mg/kg/day for 3 months. The results showed that *C. comosa* reduced TC and LDL-C levels but TG and HDL-C levels remained unchanged (Cheerana Yomchot, 2007). Not only decreased serum cholesterol, but *C. comosa* was

also found to reduce atherosclerotic plaque. Treatment with 100 mg/kg/day of *C. comosa* hexane extract for 3 months reduced atherosclerotic plaque formation and attenuated platelet aggregation in NZW fed with high cholesterol diet. Similarly, simvastatin at the dosage of 5 mg/day attenuated atherosclerotic plaque progression in rabbit fed with high-cholesterol diet for 3 months (Piyanee Rattanachamnong, 2008; Yupin Sanvarinda et al., 2007). This is consistent with the study of Nicholls et al. (2007) who demonstrated that simvastatin directly attenuated progression of coronary atherosclerotic plaque in patients with angiographic coronary disease receiving simvastatin for 18 months. NZW rabbits used in the present study were a good model for studying effects of *C. comosa* on the expression of pro- and anti- inflammatory cytokines in hypercholesterolemic conditions with many reasons. Unlike mouse model which was resistant to develop atherosclerosis (Potteaux, Ait-Oufella, and Mallat, 2007), rabbits were the animal model that had the tendency to exhibit hypercholesterolemia within a few days of the administration of high cholesterol diet. Lipid parameters can be increased by up to 8 times after the administration of diet enriched with 0.1-2% cholesterol within the first 20 days (Bocan et al., 1993). The atherosclerotic plaque which was supposed to resemble to human atherosclerotic plaque at last stage (Yanni, 2004) could be readily assessed by an increase of intimal area of aorta (Piyanee Rattanachamnong, 2008) which was resulted from the phenotype of rabbit that could not increase the excretion of sterols (Kolodgic et al. 1996). Lastly, treatment with simvastatin and *C. comosa* which reduced atherosclerotic plaque (Piyanee Rattanachamnong, 2008; Yupin Sanvarinda et al., 2007) can be readily detected by MRI, CT and nuclear imaging techniques (Rudd and Fayad, 2008).

To date, inflammation has emerged as a fundamental process in the development of atherosclerosis (Steffens and Mach, 2004). Many cytokines are involved in inflammation process. The prominent roles of IL-1β (Kirii et al., 2003), MCP-1 (Lu et al., 1998), and TNF-α (Sprague and Khalil, 2009) to be pro-inflammatory cytokines as well as IL-10 (de Waal Malefyt et al., 1991) and TGF-β (Maeda et al., 1995) to be anti-inflammatory cytokines in atherosclerotic lesion progression are well documented. In inflammation process involved in atherosclerosis, pro-inflammatory cytokine expressions were increased (Tipping and Hancock, 1993) while anti-inflammatory cytokine expressions were decreased

(Grainger et al., 1995). In addition, the reduction of pro-inflammatory cytokine expression has been found to decrease atherosclerotic plaque (Brånén et al., 2004; Gosling et al., 1999; Kirii et al., 2003). In the other hand, the reduction of anti-inflammatory cytokine expression resulted in increased atherosclerotic plaque (Mallat et al., 1999; Robertson et al., 2003). Therefore, expression of pro- and anti-inflammatory cytokines was associated to inflammation and progression of atherosclerosis. The information regarding the efficacy of statins such as simvastatin to reduce atherosclerosis plaque by lowering serum cholesterol levels is well documented (Nicholls et al., 2007). Especially, the convincing evidences showed that hypercholesterolemia is associated with increased plaque inflammation (Rudd and Fayad, 2008) and several studies suggested that anti-inflammatory effects of statins to reduce pro-inflammatory cytokines and increased anti-inflammatory cytokines are involved in regression of atherosclerosis (Blake and Ridker, 2000). In the present study, the results showed that in the abdominal aorta simvastatin significantly decreased the expression of pro-inflammatory cytokine, IL-1β but did not alter pro-inflammatory cytokines including MCP-1 and TNF-α and anti-inflammatory cytokines including IL-10 and TGF-β. Similar results were also found that there was no alteration in serum level of pro-inflammatory cytokine, TNF-α, in hypercholesterolemic patients treated with 20 mg/day of simvastatin for 3 months (Zubelewicz-Szkodzińska et al., 2003). In addition, the decrease of the expression of IL-1β was consistent to the results which were obtained from long term treatment in patients that reported the decreased in IL-1β (Ferro et al., 2000). However, the finding that there was no alteration of the expression of MCP-1 in this study was inconsistent to Ferro et al. (2000) who reported the decreased in MCP-1. Although the expression of MCP-1, and TNF-α in abdominal aorta tended to be decreased by simvastatin but it did not achieve statistical significance. These may be due to the limitation of number of animals and the encompassed various cell types located in the abdominal aorta. Interestingly, the present study demonstrated that *C. comosa* significantly decreased the expression of pro-inflammatory cytokines including IL-1β, MCP-1, and TNF-α which was consistent to a previous finding that there was significant decrease in IL-1β and TNF-α releasing from cultured human mononuclear cells treated with purified compounds from *C. comosa* (Amorntus Sodsai et al., 2007). Therefore, long term

treatment of *C. comosa* may be advantageous than simvastatin in term of anti-inflammation which may attribute to the preventive effect against atherosclerosis.

Nonalcoholic fatty liver disease (NALFD) is the most common chronic liver disease in the western world. It encompasses a wide spectrum of disease ranging from simple steatosis characterized by hepatic TG accumulation to nonalcoholic steatohepatitis (NASH) characterized by the association of lipid accumulation with evidence of hepatocyte injury and inflammation (Neuschwander-Tetri and Caldwell, 2003). New evidence suggests that histological severity of liver injury and inflammation is strongly associated with an increased cardiovascular risk and an atherogenic lipid profile (Alkhouri et al., 2009). Generally, ALT is an important enzyme in amino acid metabolism and gluconeogenesis (Ozer et al., 2008). This enzyme is generally used as the gold standard of hepatotoxicity detection (Amacher, 1998) because of its specificity to hepatocyte damage. AST is also an enzyme in amino acid metabolism and gluconeogenesis. However, AST is a less specific biomarker because it is also leaked from many organs such as liver, heart, and skeletal muscle (Rej, 1989). Besides ALT and AST, other biomarkers of liver function include total bilirubin, alkaline phosphatase, gamma-glutamyl transferase (GGT), and bile acid but they failed to be specific indicators of hepatocyte cell damage. Despite the specificity of ALT to hepatocyte damage, ALT is failed to be used to predict NAFLD because there is no relationship between ALT levels and histological severity in NAFLD patients (Wong et al., 2009). An appropriate method to distinguish the extent of chronic hepatitis and liver fibrosis in NAFLD is liver biopsy (Saleh and Abu-Rashed, 2007). Nevertheless, strong association between cardiovascular disease and elevated serum ALT was reported (Ioannou et al., 2006). Not only serum liver enzymes were used to investigate liver inflammation, but cytokines expressions involved in inflammation were also evaluated. ApoE knockout mice fed with high cholesterol diet exhibited an increase in liver inflammation. These mice also demonstrated the over-expression of pro-inflammatory cytokines, IL-1β and MCP-1, as well as no alteration of anti-inflammatory cytokine, IL-10 (Yin et al., 2009). Presently, drug of choice for treatment of hypercholesterolemia is statins. Unfortunately, besides rhabdomyolysis, elevated liver enzymes and liver failure have been found in patients treating with statins (Russo, Scobey, and Bonkovsky, 2009). Consistent with a previous study in animals, Cheerana Yomchot (2007) found that

rabbits fed with high cholesterol diet combined with simvastatin exhibited higher levels of ALT as compared to rabbits with high cholesterol diet control. In this study, the expressions of pro- and anti-inflammatory cytokines in the livers of the same rabbits studied by Cheerana Yomchot (2007) were investigated. The results showed that simvastatin significantly increased levels of pro-inflammatory cytokines including MCP-1 and TNF-α but it did not alter the expression of IL-1β and the anti-inflammatory cytokines, IL-10 and TGF-β. In contrast, treatment with 400 mg/kg/day of *C. comosa* did not alter liver enzyme in rabbits fed with high cholesterol diet (Cheerana Yomchot, 2007). Even though this result was not consistent to a study of Songpol Chivapat et al. (2003) who found elevated ALT level in male rats treated with 800 mg/kg/day of *C. comosa* extract for 90 consecutive days. In this study, advantageous effect of *C. comosa* on the liver was shown by the result showing that *C. comosa* did not increase the expression of pro-inflammatory cytokines, IL-1β, MCP-1, and TNF-α, but increased the anti-inflammatory cytokine, IL-10, instead. The findings that simvastatin increased serum hepatic ALT in the study of Cheerana Yomchot (2007) and increased liver TNF-α in this study could be more or less associated. Generally, hepatotoxic exposure leads to Kupffer cell activation resulting in release of cytokines such as IL-1β, TNF-α, etc (Lacour et al., 2005). These cytokines cause further release of reactive oxygen species from leukocytes (Shetty, Lalor, and Adams, 2008) resulting in liver cell damage then hepatic enzyme release (Garg and Aggarwal, 2002).

In conclusion, this study demonstrated that long term treatment with *C. comosa* rhizome decreased pro-inflammatory cytokines including IL-1β, MCP-1 and TNF-α in aorta of rabbits fed with high-cholesterol diet but did not affect anti-inflammation cytokines (IL-10, TGF-β) in the aorta. In the liver, *C. comosa* increased IL-10 without an alteration of pro- and anti-inflammatory cytokines including IL-1β, MCP-1, and TNF-α. In contrast, simvastatin increased the expression of pro-inflammatory cytokine, IL-1β but did not alter MCP-1 and TNF-α as well as anti-inflammatory cytokines in aorta of rabbits fed with high-cholesterol diet. Whereas in the liver, simvastatin increased pro-inflammatory cytokines including MCP-1 and TNF-α without an advantageous effect on anti-inflammatory cytokines (IL-10, TGF-β). Further study to explore cytokine synthesis to confirm the alteration of gene expression is suggested.