CHAPTER V

DISCUSSIONS AND CONCLUSIONS

Administration of single oral dose ethanol (5 g/kg) to rats significantly increased levels of ALT and AST indicating the damage of plasma membrane and /or necrosis of hepatocytes. However, there were no changes in STg, HTg, GSH, MDA, TNF-α and IL-1β probably due to insufficient time of ethanol exposure and insufficient amount of toxic metabolites formed to induce membrane peroxidation, metabolic disturbances and activation of kupffer cell released cytokines. Administration of PA extract at a dose of 75 mg/kg 24 hours before ethanol (5 g/kg) showed the best hepotoprotective result by bringing the levels of ALT and AST induced by ethanol back to normal similar to SL. This study confirmed the hepatoprotective activity of PA extract against toxic dose of ethanol similar to provious report against paracetamol (วันดี อุดมอักษร, 2543; Wongnava *et al.*, 2000) and CCl_4 (Venkatesan *et al.*,2003; Harish and Shivanandappa, 2006).

Administration of ethanol (4 g/kg/day) for 21 days resulted in significantly increased levels of ALT and AST, the most important and specific serum marker for liver injury (Hodgson and Smart, 2001). These findings indicate changes in the permeability of membrane which due to necrosis and/or deterioration in integrity of membrane.

Administration of PA extract (75 mg/kg/day) for 7 days after ethanol significantly decreased levels of ALT and AST and brought back to normal level. This effect suggested that the PA extract stabilize membranes and prevent enzyme leakage from hepatocytes similar to the previous report of silymarin, a known hepatoprotective agent and picroliv, the active constituent isolated from Picrorhiza kurroa (Sarawat *et al.*, 1999). Syamasundar (1985) reported that lignans such as phyllanthin and hypophyllanthin in *P. amarus* extract could inhibit ALT and AST release against CCl_4 and GalN induced cytotoxicity in primary cultured rat hepatocytes. Furthermore, *P. amarus* contains the phenolic compounds such as flavonoids and tannins acting as antioxidant, may be by preventing lipid membrane peroxidation and resulting in inhibition of ALT and AST leakage. These chemical constituents of PA may possess combined action of stabilizing membranes and

preventing enzyme leakage which may responsible for hepatoprotective mechanism of PA against liver injury from ethanol.

Fatty liver is the accumulation of fat especially triglyceride in the liver induced by over ethanol consumption. Elevation of liver fat may be by increasing of triglyceride and fatty acid synthesis, decreasing of fatty acid oxidation, increasing in rate of fatty acid delivery to liver and mobilization of lipid from peripheral depots, decreasing lipoprotein (VLDL) releasing to blood (Zimmerman, 1999). Moreover, elevation of triglyceride in serum after ethanol consumption may be due to decreased activity of lipoprotein lipase which involved in the uptake of triglyceride riched lipoprotein (VLDL) by extra hepatic tissue, increased in hepatic triglyceride and VLDL production (Lamp, Wood and Fallon, 1979; Mahendran and Shyamala Devi, 2001).

In this study, administration of ethanol for 21 days to rats significantly increased HTg level corresponded with fat vacuoles found in hepatocytes confirmed by positive staining of Oil red O. Administration of PA extract (75 mg/kg/day) for 7 days after ethanol showed significant decrease in HTg level and fat vacuoles. These results indicated that PA extract could decrease hepatic triglyceride, may be by interfering with the above mechanisms of fatty liver.

Lipid peroxidation caused by oxidative stress is the results of enhanced generation of free radicals and a depletion of the antioxidants in the defense system. The increase in hepatic malondialdehyde (MDA) level suggests the enhanced lipid peroxidation leading to liver injury. In this study, administration of ethanol for 21 days to rats significantly increased MDA level which can bring back to normal level by administration of PA extract (75 mg/kg/day) for 7 days after ethanol. This observation demonstrates the antiperoxidative and antioxidant effects of PA extract. In this respect, Venkatesan *et al.* (2003), Harish *et al.* (2005), Raphael, Sabu and Kuttan (2002) and Kumar and Kuttan (2004) also reported that PA extract had inhibitory effect on lipid peroxidation.

These actions of PA may be the actions of several active constituents especially the phenolic compound which reported to have the character of quenching oxygen-devided free radicals by donating a hydrogen atom or an electron to free radicals. Kumaran and Karunakaran (2006) reported that the content of phenolic compounds in *P.amarus* correlated with its antioxidant effect. There are

many types of phenolic compounds in this herb as follows: lignans such as phyllanthin and hypophyllanthin, flavonoids such as quercetin and astragalin, and ellagitannins such as amarinic acid, amarin and phyllanthisiin D (Kumar and Kuttan, 2004; Raphael and Kuttan, 2003). Furthermore, PA is reported to contain catechin and epigallocatechin (Kumar and Kuttan, 2004) the well known antioxidant like those constituents in green and black tea (*Camellia sinensis* O.Kuntze) which Ostrowska *et al.* (2004), Luczaj and Skrzydlewska (2004) reported their antioxidant action and inhibition of lipid peroxidation in rats received chronic ethanol consumption. Therefore, the combined action of these constituents makes PA a good antioxidant and an effective inhibitor of lipid peroxidation.

In recent year, it has become increasing evident that bacteria in the intestines play a key role in ethanol induced liver injury. Ethanol consumption caused elevation of endotoxin (lipopolysaccharide, LPS), an integral part of the cell wall of Gram-negative bacteria, pass through the intestinal wall into the portal blood and enter to the liver. These endotoxins stimulated the kupffer cell to produce pro-inflammatory cytokines such as TNF-α and IL-1β, both cytokines are considered to be important mediators in inflammation and cell death, resulting in liver injury (Jarvellainen *et al.*, 1999; Boelsterli, 2003). In this study, administration of ethanol for 21 days to rats showed significantly increased TNF-α and IL-1β levels, giving PA extract (75 mg/kg/day) for 7 days after ethanol significantly decreased TNF-α level. Kiemer *et al.* (2003) reported that hexane and ethanol-aqueous extract of *P. amarus* attenuated the LPS induced secretion of TNF-α in Raw264.7 macrophages and in human whole blood, and reduced TNF-α production in LPS treated mice. Therefore, PA extract may protect against liver injury by inhibition of pro-inflammatory cytokines production and may be due to its antioxidant effect by inhibition of free radicals stimulated the transcription of pro-inflammatory cytokines.

Histopathological observations after rats received ethanol for 21 days found that centrilobular area was the predominant site of liver injury due to the greater amounts of cytochrome P450 (CYP2E1) responsible for ethanol oxidation together with the lower level of GSH and receiving low oxygen and nutrient concentrations making this area the sensitive site to injury induced by ethanol (Klaassen, 2001). Hepatocyte swelling caused by the binding of acetaldehyde to tubulin of

microtubules blocking the secretion of proteins as well as ethanol increased lipid, water and electrolytes in cell, resulting in hepatocytes to enlarge or balloon (Lieber, 2004). Increasing area of sinusoidal spaces caused by the effect of acetaldehyde in vasodilation (Eriksson, 2001). Loss of glycogen was showed by irregular of intracytoplasmic pink-red glycogen accumulation of the PAS staining. ATP in cell was depleted and used energy came from glycogen instead of ATP. Furthermore, It also found the proliferation of bile duct at the portal tract which was an adaptive change that increased the ability of the liver to excrete cytotoxic materials into the bile. (Ballantyne, Marrs and Syversen, 2000). These changes induced by ethanol consumption for 21 days and administration of PA extract (75 mg/kg/day) for 7 days after ethanol could reduce the pathological changes and greatly reverted the morphological pattern of the liver to normal. The chemical constituents of PA may responsible for the reduction of oxidative stress of hepatocyte and mitochondria, inhibition of acetaldehyde binding and inflammation.

Moreover, administration of PA extract showed the enhanced regeneration of liver with the large number of cell division (mitotic figure) similar to silymarin treatment. Flora *et al.* (1998) reported that silymarin could enhance protein synthesis by silybin, a active constituent of silymarin, stimulated the activity of ribosomal RNA polymerase, resulting in regeneration and production of hepatocytes. PA extract may acts like silymalin in enhancement liver regeneration.

In rats administered with ethanol for 21 days and then self recovery for 7 days showed returning of clinical chemistry parameters back to normal level, but histopathological observation still found the morphological changes and reversing of the cell to normal was slower than rats received PA extract and SL.

Administration of PA extract alone for 7 days caused no changes in all clinical chemistry parameters and histopathological examination. These results indicated that PA extract by itself caused no toxic effect to rats.

In conclusions, administration of aqueous extract from *Phyllanthus amarus* at a dose of 75 mg/kg before or after ethanol consumption showed a significant hepatoprotective effect in ethanol treated rats and *P. amarus* by itself caused no toxic effect assessed by clinical chemistry parameters together with histopathological examination. The possible mechanism of *P. amarus* for hepatoprotective effect may be due to its stabilizing membranes and preventing enzyme leakage from cells, antioxidation, inhibition of fatty liver, inhibition of TNF-α production and enhancement of liver regeneration. Thus, it is possible that *P. amarus* may be a choice of alternative prevention and treatment in alcoholic liver disease.

Further studies should be performed to confirm the effect of PA extract on enzymatic antioxidant system such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and non-enzymatic antioxidant system such as ascorbic acid, α-tocopherol besides reduced glutathione (GSH). Effect of PA on the inhibitory mechanism of fatty liver and pro-inflammatory cytokines as well as the active constituents which responsible for hepatoprotective effect against ethanol induced liver injury should be studies in detail. In addition, the long-term exposure to PA related to its effectiveness and toxicity should also be investigated.