CHAPTER V

DISCUSSION AND CONCLUSION

Histopathology of NASH is similar to that of alcoholic induced hepatitis with the presence of macrovesicular steatosis, hepatocyte ballooning, necroinflammation, Mallory bodies, and fibrosis (Chalasani et al., 2004). To study the pathogenesis or therapeutic methods of NASH, there are many models which have been used such as genetic model (obese rats), methionine and choline deficient diets, high fat liquid diet, and 100% fat deit. In this study, 100% fat diet was chosen to induce NASH in Sprague-Dawley rats as this procedure took short-time, had no technical difficulty and provided the same pattern pathological change as in human.

Effect of 100% Fat Diet for 6 Weeks in Rats

By feeding rats with 100% fat diet, the hepatic lesions of NASH were apparent within 6 weeks. Histopathological examination showed macrovesicular steatosis, hepatocyte ballooning, Mallory bodies, and mild to moderate inflammation. One hundred percent fat diet caused mobilizing of free fatty acid (FFA) from adipose tissue and transporting into hepatocytes. In this condition, the liver failed to synthesize apolipoprotein that used for packaging and exporting of fat from the liver, therefore triglycerides (TG) accumulated in liver (Brody, 1999). β-oxidation of FFA in hepatocytes produced reactive oxygen species (ROS) which activated lipid peroxidation (Benzie, 1996). ROS and lipid peroxidation caused direct damage to hepatocytes by disrupting membranes, protein, and DNA (De Knegt, 2001 and Day, 2002). The damage and lipid peroxidation products induced an inflammatory response.

In 100% fat diet-fed rats, body weight decreased significantly ($p<0.05$) as compared with normal control group. While serum cholesterol significantly increased,

serum TG level was unchanged with 100% fat diet. Feeding 100% fat diet for 6 weeks caused a loss of body weight that may be due to a metabolic imbalance of carbohydrate, protein, and fat. Moreover, 100% fat diet contained highly saturated fat which may increase blood cholesterol concentration by 15 to 25% (Guyton, 2000). This result was from an increase of fat deposition in the liver which then provides the increased quantities of acetyl CoA in the liver cell for production of cholesterol (Guyton, 2000). The increased cholesterol was found in this experiment and had been observed in another study that used 10% lard oil and 2% cholesterol supplement adding into the standard diet (Fan et al., 2003).

Serum aspartate aminotransferases (AST) and alanine aminotransferases (ALT) are useful screening tests for detecting liver injury (Kaplowitz, 1992). They are found in hepatocytes and can not diffuse out of the cells in normal situation. When the hepatocyte is injured, plasma membrane can be disrupted and the leakage through extracellular fluid of the enzyme occurs where they can be detected at abnormal levels in the serum (Robbins, 1974). Several experiments found that AST and ALT increased in NASH rats (Weltman et al., 1996; Leclercq et al., 2000; Fan et al., 2003; Kirsch et al., 2003 and George et al., 2003). In contrast, AST and ALT decreased significatly with 6 weeks of 100% fat diet in this study. The decreased serum transaminases may be due to poor nutrition or hepatocyte death. Rats fed 100% with fat diet derived main energy from fat, when there were low in vitamin and mineral contents. The decreased AST and ALT levels were probably due to nutritional deficiency of pyridoxal phosphate which is a cofactor for both AST and ALT to catalyze the transfer of the α amino group from aspartate or alanine to α-ketoglutarate with made the release of pyruvate, oxaloacetate, and glutamate (Kaplowitz, 1992). In addition, oxidative stress condition may be a cause of hepatocyte death, therefore, aminotrasferases can not be produced.

FFA causes oxidative stress that has the potential to induce NASH (Sligte et al., 2004). FFA in the body is increased and this is associated with state of starvation (Sligte et al., 2004). Stored FFA can be mobilized from adipose tissue through lipolysis (Sligte et al., 2004). FFA metabolism increases the production of ROS which activated lipid peroxidation. The results are the disruption of membrane and the production of reactive metabolites such as MDA (Benzie, 1996). This study found high hepatic MDA in 100% fat- diet fed rats that agreed with the other experimental NASH rats (Leclercq et al., 2000; Fan et al., 2003; Kirsch et al., 2003 and George et al., 2003). Also, an increasing in total glutathione in whole blood with 100% fat diet feeding could be explained by compensatory protection mechanism against oxidative stress.

Effect of N-acetylcysteine on Body Weight, Serum Biochemical Parameters, Oxidative Stress Markers, and Liver Histopathology in Rats with Nonalcoholic Steatohepatitis

After induced NASH with 100% fat diet for 6 weeks and treated with diet control or diet plus NAC, the body weight in rats with NASH increased nearly to the level of normal control group by 10 weeks. High dose of NAC caused nausea, vomiting, and gastrointestinal disturbance (Kelly, 1998). Therefore, in NASH+diet+NAC_{500} group, the body weight was lower when compared with NASH+diet group. This may be caused by poor appendage side effects of high dose of NAC.

In the diet treatment alone and diet plus NAC groups, total glutathione, serum AST, ALT, cholesterol, TG, and hepatic MDA returned to normal levels as in the control group. In addition, the pathological changes of liver in these groups were improved. These results emphasized how crucial the nutritional composition of the diet is. Good proportion of nutrients (i.e., carbohydrate, lipid, and protein) is essential for

growth and maintenance. These nutrients supply energy, promote growth, repair body tissues, and regulate body processes (Guthrie, 1986). It is, therefore, concluded that resumed normal diet can attenuate oxidative stress, improve biochemical parameters, and liver histopathology in rats with NASH. However, addition of NAC with diet is not better than control diet alone in this study.

Some limitations of our study deserve further discussion. First, the model in this study represented malnutrition condition, but general patients with NASH were obese. However, both conditions provided the same pathological changes. Second, a previous study has showed that 100% fat induced NASH in rats after 6 weeks and 12 weeks (Thong-Ngam et al., 2005). This study chose a 6-week duration because extreme weight loss found at 12 weeks may cause death of the animals. Third, in Cayman's GSH assy kit, sample preparation for measurement of GSSG (oxidized form) is generally difficult. Therefore, this study measured only total glutathione. Finally, there have been no studies of correlation between erythrocyte glutathione (total glutathione, GSH, and GSSG) and hepatic glutathione in rats with NASH. This study found high total glutathione in whole blood (main source from erythrocytes) of rats with NASH. However, it was not able to infer hepatic glutathione.

Further studies should be conducted to explore the hypothesis that the decreased AST and ALT levels were probably due to deficiency of pyridoxal phosphate in rats with NASH. More studies should also examine both reduced and oxidized forms in erythrocytes and hepatic tissue.

In conclusion, within 6 weeks 100% fat diet induced macrovesicular steatosis, hepatocyte ballooning, and inflammation in rats similar to histopathology of NASH. Treatment of NASH with diet or diet plus NAC could attenuate oxidative stress as well as improve biochemical parameters and liver histopathology. However, the result of

addition of NAC is not better than diet treatment alone. Further studies of NAC in NASH from other causes are necessary.