CHAPTER 4

DISCUSSION AND CONCLUSION

^{1}H NMR spectra of the PG fraction of *T. laurifolia* leaf extract had chemical shifts that were specified as phenolic and glycoside compounds and they were expected to be the important constituents of the leaves which demonstrated in this study as protective agents on Cd toxicities. The finding of phenolic compounds in plant extract was similarly to the previous studies of Purnima and Gupta 1978; Thongsaard and Marsden, 2002; Oonsivilai *et al.*, 2007; Oonsivilai *et al.*, 2008. In addition, the finding of glycoside were similarly to the finding in the studies of Kanchanapoom *et al.*, 2002 and Morkmek *et al.*, 2010.

Adding TL leaf extract in rat's drinking water before Cd administration help prevent abnormal appearances, behaviors and organ damage caused by Cd. This finding reassured that TL leaf extract did not affect rat's behaviors and internal organs. Wisitpongpan *et al.* (2003) and Chivapat *et al.* (2009) also reported that TL leaf extract had no toxic effects in rats when giving high dose of the extract up to 2,000 mg/kg/day to the rats.

The result of this study show that crude extract of the TL leaves has more protective effects on Cd toxicities than the PG fraction of TL leaf extract. This was presumably due to more kinds of chemical constituents in the crude extract of the *T. laurifolia* leaves than in the isolated-PG fraction. Perhaps the effect of TL leaf extract that help prevent Cd toxicity needs more than one type of chemicals in

a synergistic manner. The result of this study is similar to the study of Oonsivilai *et al.* (2008) who reported that the water extract of TL leaf showed higher antioxidant activity than the ethanol extract. It is possible that the protective effect of TL leaf extract on Cd toxicity might be due to some other mechanisms such as reduction of free radicals formation etc. The exact mechanism needs to be investigated in more detail.

The results also demonstrated that giving Cd to rats for 20 days (sub-acute Cd exposure) decreased rats body weight significantly ($p<0.05$) and consumption of TL leaf extract via drinking water before exposed to Cd was clearly shown to reduce weight loss caused by Cd. Water intake of the rats treated with Cd alone decreased significantly ($p<0.05$) compared to the control rats. This result was similar to the study of Brzóska *et al.* (2003) who reported that rats exposed to Cd alone depressed the rat's consumption of drinking fluid. Interestingly, in the last ten days of our experiment the rats treated with Cd alone drank water more than the water they drank in the previous ten days and we don't know of the reason. This finding was similarly to the study of Jihen *et al.* (2008) who reported that trend of water consumption of rats treated with Cd did not steady decrease. In contrast, the rats pretreated with TL leaf extract drank more water than the rats treated with Cd alone. The results confirm that TL leaf extract can protect weight loss induced by Cd.

Under normal condition, circulating Cd which bound to low molecular weight endogenous substances such as metallothionein, cysteine or glutathione is filtered through the glomerulus and efficiently taken up by the epithelial cells of the proximal tubule. Only small amount of the compounds will be excreted in the urine. During

Cd exposure the presence of Cd in the urine resulted from the normal turnover and shedding of epithelial cells, mainly reflect the level of Cd exposure. However, over a long time exposure, the concentration of Cd in the epithelial cells increases to the point that more cells begin to die and slough off into the urine. It is at this point that the urinary excretion of Cd increases markedly. Therefore, urinary Cd can be used as a good biomarker for chronic exposure to Cd. Our finding showed that pretreatment with TL crude extract or the PG fraction of the leaf extract did not reduce urinary Cd concentration.

Cd concentration in whole blood mainly reflects the exposure during recent weeks or months (WHO, 1992). The present study showed high blood Cd concentration in Cd treated rats significantly ($p<0.05$) compared to the control without Cd treatment. Pretreatment with TL crude extract or the PG fraction of the leaf extract also did not reduce the blood Cd concentration.

Histopathology of the kidney in rats treated with Cd showed proximal tubular necrosis and glomerular dilation similar to several studies of Augley *et al.*, 1984; Brzóska *et al.*, 2003; Jihen *et al.*, 2008; Renugadevi and Prabu, 2009, 2010a. The liver showed necrosis with pyknotic nuclei and dilation of sinusoid which was also shown in the previous studies of Jihen *et al.*, 2008; Tarasub *et al.*, 2008; Karadeniz *et al.*, 2009; Renugadevi and Prabu, 2010b and El-Sokkary *et al.*, 2010.

Cd induced nephrotoxicity and hepatotoxicity has been reported to be the cause of a releasing of Cd ions from cadmium-metallothionein complex. After absorption, Cd transported in blood and bound to albumin before taken up by the liver where Cd ions are released from albumin and induce synthesis of metallothionein.

When the synthesis of metallothionein becomes insufficient for binding Cd ions in the liver, it will lead to cause hepatocyte injury. A small quantity of Cd-metallothionein complex is released into blood circulation from hepatocytes and efficiently transported through the glomerular membrane before taken up by renal tubular cells where it is degraded by lysosomal enzymes. The Cd ions then be released and induced the synthesis of metallothionein, which bound and retained Cd in the kidney for a very long time. Part of the Cd-metallothionein complex will be degraded in tubular lumen before reabsorption. Hence, Cd ions release to tubular lumen resulted to renal tubular damage (Brzóska *et al.*, 2003; Klaassen *et al.*, 2009).

Rats pretreated with *T. laurifolia* leaf crude extract clearly did not show histopathological changes of both kidney and liver but the rats pretreated with the PG fraction of TL leaf extract still showed kidney damage but the number of the rats that had kidney damage were shown to have less severity than the kidney of rats treated with Cd alone. However, there was no histological changes in the liver indicated that the PG fraction of TL leaf extract could protect liver damage caused by Cd but could not protect kidney.

In conclusion, this study demonstrated drinking TL leaf extract could reduce the systemic toxicities of Cd including structural damage of the kidney and liver of the rats. However, the extract could not reduce the accumulation of Cd in blood and urine of the rats.