CHAPTER IV

DISCUSSION AND CONCLUSION

Cadmium exposure results in accumulation of cadmium predominately in the kidney and liver and increases concentrations in the blood and urine (Jarup *et al*., 1998). The cadmium concentration in urine is influenced primarily by the body burden and is proportional to the cadmium concentration in the kidney. Thus, it is useful for estimating long-term cadmium exposure. The concentration of cadmium in blood reflects recent exposure and blood cadmium levels the body burden during acute exposure (Jarup, 2002). The results of this study showed blood cadmium levels were significantly higher cadmium treated rats and urinary cadmium concentrations were also higher than the controls.

Cadmium exposure lowers the body, kidney and liver weights of rats (Brzoska *et al*., 2003; Haouem *et al*., 2007; Suru, 2008). Since homeostasis of essential metals is tightly regulated, the consequence of cadmium intoxication depends heavily on nutritional status and due to persistence of cadmium in humans affects the cellular metabolism and growth (Samir *et al*., 2000; Hispard *et al*., 2007). In this study, cadmium treated rats ate and drank less compared to the control rats. Moreover, cadmium treated rats appeared and behaved abnormally after treatment which might cause cadmium induced toxicity and lowered appetite in rats. In animal toxicity

studies, organ toxicity can be shown by loss of organ weight after exposure (Anderson *et al*., 1999). In this study, there was no significant difference in the weight of body, kidneys and livers between control and cadmium treated rats which were in accord with the study of Chwelatiuk, *et al* (2006). These might result from a few numbers of experimental rats. But the organs of the cadmium treated rats appeared to be larger than the control rats.

Cadmium induced necrosis in the proximal tubular cells of the kidney and in hepatocytes which was similar to finding reported by Aoyagi *et al*., 2003; Brzoska *et al*., 2003 and Esrefoglu *et al*., 2007. Cadmium induced nephrotoxicity and hepatotoxicity is thought to be mediated through the cadmium-metallothionein (CdMT) complex which is synthesized in the liver, released into circulation and taken up by renal proximal tubule cells. When synthesis of MT is insufficient to bind cadmium ions in the liver, free cadmium causes hepatocytes damage and the CdMT complex is released into bloodstream. The complex in the plasma is then filtered through the glomeruli in the kidney and taken up by the proximal tubular cells (Dudley *et al*., 1985; Sudo *et al*., 1996).

Studies conducted on mammals have revealed kidney injury associated with the release of the CdMT complex into the peripheral circulation after liver saturation. On its way through the kidneys, this complex mainly causes injury in the cortical region, reaching the proximal tubules and causing a gradual loss of the organ's function (Dorian and Klaassen, 1995). This study showed that cadmium treated rats had high cadmium concentrations in their blood which affected the hepatocytes and the renal cortex especially the proximal tubular cells. One histopathological change that was a

characteristic of the livers of many cadmium exposed rats was vacuolation or fatty change which was an excessive accumulation of fat in the cytoplasm. The large vacuole in the cell forced the nuclei to the periphery of the hepatocyte and this was usually accompanied by nuclear atrophy (Bogiswariy, 2008).

This study is the first study of detoxification of cadmium in rats by *T. laurifolia* leaf extract. Lyophilized leaf extract administered to rats at 500 mg/kg BW for 28 days did not affect rat behavior (Wisitpongpan *et al*., 2003). A lower dosage of 125 mg/kg BW that was used in this study had no abnormal effect on the animals.

During the period of cadmium exposure, the treated rats showed abnormal appearance and behavior such as aggression and fear of touch. This caused difficulties in feeding leaf extract to the cadmium treated rats. Other routes for administering leaf extract should be considered in future experiments.

The high death rate of treated rats during cadmium exposure indicated the cadmium dose was too high or these experimental rats were more sensitive to cadmium than the rats used in the previous experiments to establish the rat model for renal and hepatic injuries induced by cadmium. The antagonistic and prophylactic effects of *T. laulifolia* for cadmium toxicity could not be defined at the cadmium dose used in this study because all rats died before the planned date to end the experiment. However, rats in the prophylactic study which were pre-treated or co-treated with plant extract had less severe abnormalities in appearance and behavior than the positive control rats treated with cadmium only.

The histopathology results showed that pre-treatment with *T .laurifolia* was more effective than co-treatment in preventing cell damage induced by cadmium. The leaf

extract might interact with cadmium in the body but the mechanism for the interaction at the on cellular level was not investigated. *T. laurifolia* leaf extract administered by gavage did reduce the level of kidney and liver damage observed by histopathology but did not prevent mortality at high cadmium exposure. Therefore, future studies should investigate lower cadmium doses and alternative methods to administer *T. laurifolia* such as diluted in drinking water.

The compounds found in *T .laurifolia* leaves in this study were similar to those previously reported by Kanchanapoom *et al* (2002) namely two iridoid glycosides (8-*epi*-grandiforic and 3'-*O*-β-glucopyranosyl-stibericoside), benzyl β-glucopyranoside, benzyl β-2-*O*-β-glucopyranosyl, glucopyranoside, grandifloric acid, *E*-2-hexynyl β-glucopyranoside, hexanol β-glucopyranoside, 6-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosylapigenin.

In conclusion, a model of renal and hepatic injuries in rats after exposure to cadmium subcutaneous injection was established and used this model to investigate the antagonistic and prophylactic properties of an aqueous extract of the leaves of *T. laurifolia*. This study found that *T. laurifolia* leaf extract treatment provided some protection against cadmium toxicity when administered prior to cadmium exposure. However, the extract did not reduce the concentration of cadmium in the blood and urine of treated rats. Methods used to administer the leaf extract and the cadmium dosage could be modified to further investigate the detoxifying capacities of *T. laurifolia*.