CHAPTER III

RESULTS

Establishment of a rat model with renal and hepatic injuries induced by cadmium exposure

Change in body weight of rats after cadmium treatment

At the start of the experiment, the mean body weight average ± standard error of the cadmium treated rats (294.00 ± 9.16 g) was similar to the control rats (279.33 ± 9.34 g). The body weight of the control rats gradually increased during the experiment. Although the average weight of cadmium treated rats (306.67 ± 33.83 g) was not significantly different from that of the control rats (402.00 ± 12.86 g) at the end of the experiment, they appear quite different in Figure 10. The mass of every rat is reported in Table 2 and the daily change in mean weight for each treatment is shown in Figure 10.

Appearance and behavior of rats after cadmium treatment

Three days after the start of cadmium exposure, treated rats began to show abnormalities in appearance muscle necrosis at the injection area and facial swelling (Figure 11B). By day five, nasal bleeding and hunched back were observed (Figure 11D). Hair loss and scarring (Figure 11D and 11E) occurred in the last week of cadmium treatment. Moreover, cadmium treated rats ate and drank less than the control rats.

Table 2 Body weight (grams) of control rats treated with normal saline and rats dosed with $CdCl_2$ (1.2 mg/kg/day) by subcutaneous injection for 20 doses during 26 days

No. of Cd Doses	Control rats (NSS treated)			Body weight Mean ± SE (g)	$CdCl_2$ treated rats			Body weight Mean ± SE (g)
	Rat1	Rat2	Rat3		Rat4	Rat5	Rat6	
1	282	294	262	279 ± 9	300	276	306	294 ± 9
2	290	306	272	289 ± 10	306	280	310	299 ± 9
3	300	310	282	297 ± 8	300	274	310	295 ± 11
4	300	314	284	299 ± 9	300	268	312	293 ± 13
5	318	314	286	306 ± 10	308	270	320	299 ± 15
6	324	332	308	321 ± 7	316	232	292	280 ± 75
7	334	340	318	331 ± 7	324	230	298	284 ± 28
8	332	250	320	334 ± 9	330	232	300	287 ± 29
9	340	358	326	341 ± 9	330	232	302	288 ± 29
10	350	360	332	347 ± 8	330	240	302	291 ± 27
11	358	378	348	361 ± 9	304	252	288	281 ± 15
12	364	382	348	365 ± 10	310	258	292	287 ± 15
13	386	394	354	372 ± 12	310	260	304	291 ± 16
14	368	404	358	377 ± 14	324	250	324	299 ± 25
15	372	410	366	383 ± 14	338	230	338	302 ± 36
16	390	420	380	397 ± 12	336	236	312	295 ± 30
17	384	422	382	396 ± 13	330	218	324	291 ± 36
18	392	424	382	399 ± 13	346	224	326	299 ± 38
19	396	430	384	403 ± 14	348	230	348	309 ± 39
20	398	426	382	402 ± 13	350	240	330	307 ± 34

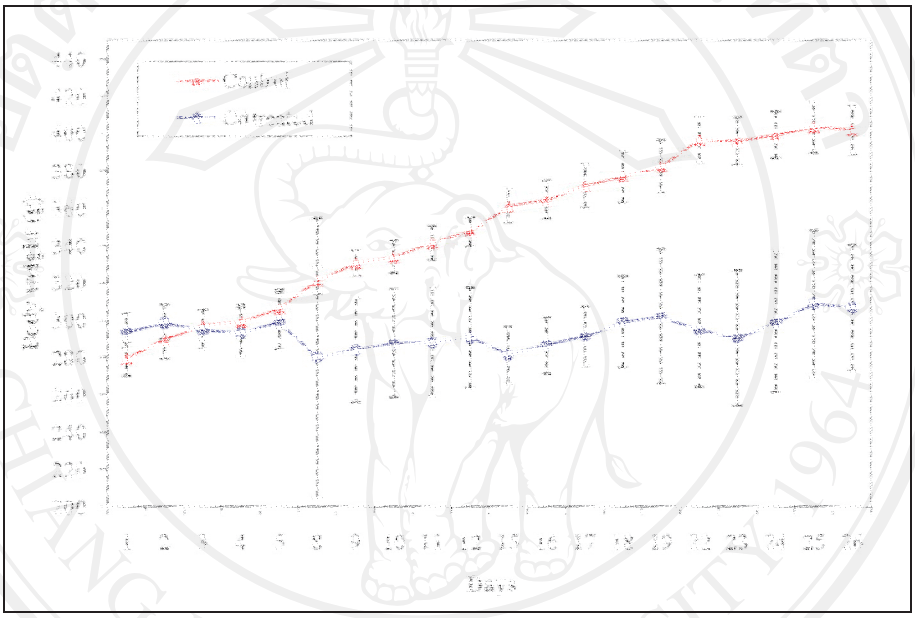

Figure 10 Change in body weight of cadmium treated and control rats during the 26 day experimental period. Each point represents mean of three rats and the error bars represent the standard error (SE).

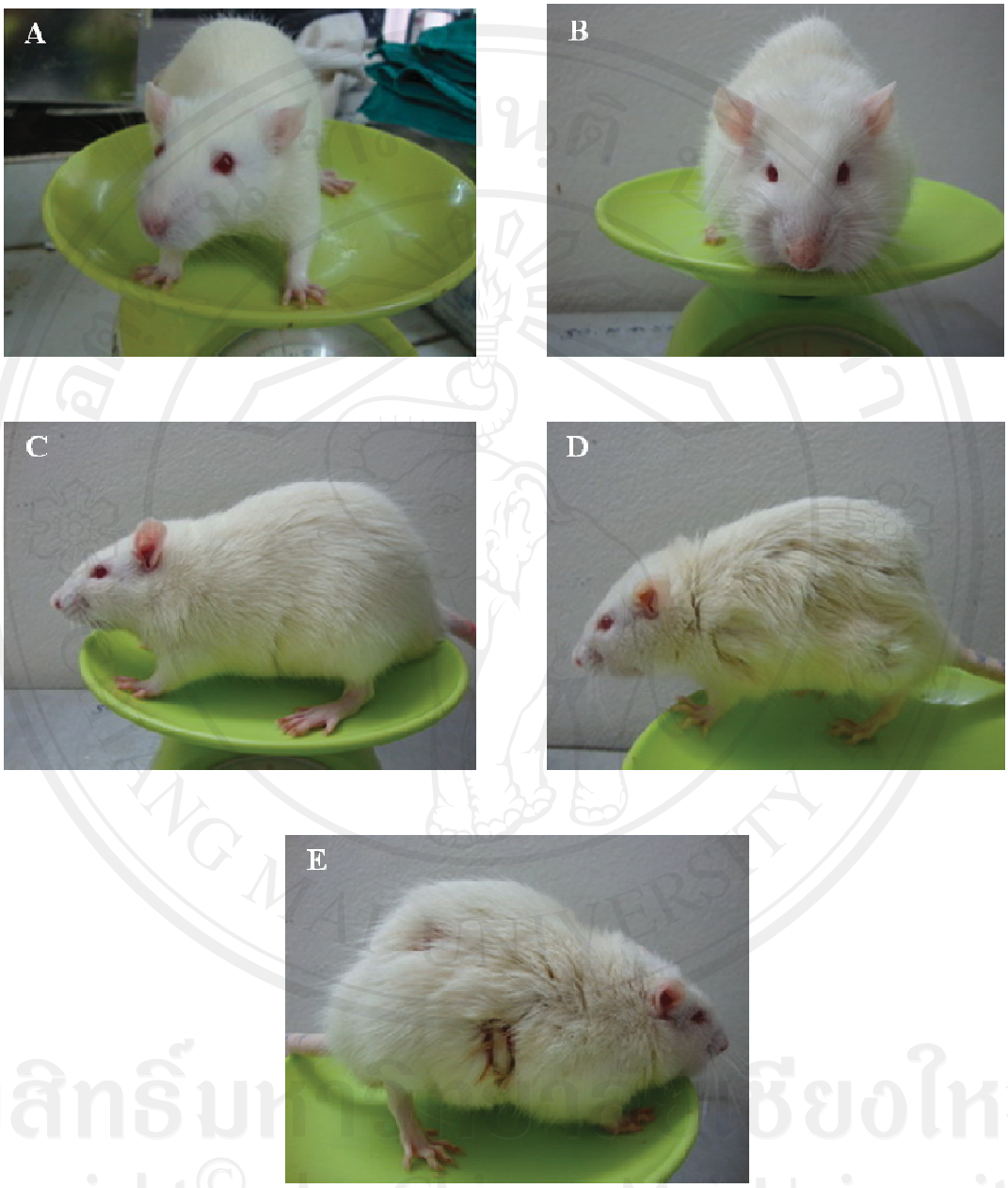

Figure 11 Abnormalities in the appearance of cadmium treated rats were swollen face (B), hunched back and hair loss (D) and skin necrosis (E). Animals in the control group treated with normal saline are shown for comparison (A and C).

The degree of abnormality in appearance and behavior increased progressively over time. Treated rats had passive head tap and were much more sensitive to touch the control rats. They also showed statue position, aggression and fear of contact.

Urinary and blood cadmium concentrations

The concentrations of urinary cadmium in control rats (56.00 ± 31.34 μg/gCr) and cadmium treated rats (202.33 ± 73.13 μg/gCr) were not different at day 0. However, the concentration of urinary cadmium at day 20 was significantly higher in cadmium treated rats (220,792.33 ± 1,714.00 μg/gCr) than for the control rats (4.67 ± 1.67 μg/gCr) (Table 3).

Cadmium treatment resulted in a significant increase in blood cadmium level (5,114.33 ± 1,081 μg/L) to approximately 5,000 times the concentration in the blood of the control rats (1.67 ± 0.33 μg/L) (Table 3).

Weight of kidney and liver after cadmium treatment

The weight of kidneys and livers from control rats (left kidney: 1.16 ± 0.09 g, right kidney: 1.28 ± 0.04 g, liver: 11.75 ± 0.25 g) were not statistically different to those from cadmium treated rats (left kidney: 1.30 ± 0.11 g, right kidney: 1.34 ± 0.13g, liver: 18.96 ± 1.91 g), though these organs from cadmium treated rats seemed enlarged compared to those from the control rats (Table 4).

Histopathology of kidney and liver

The structure of the glomeruli and proximal convoluted tubules in the control rat kidneys were normal (Figure 12A). The kidney tissues in the control group had

normal cuboidal shaped cells with defined epithelial cell linings. The kidneys of the cadmium treated rats had proximal renal tubular damage (Figure 12B and 12C) and protein casts were present in the collecting tubules (Figure 12B). The cuboidal shaped cells were disrupted and the epithelial cell linings were undefined. The intracellular spaces and lumen increased in size (Figure 12C). Some glomeruli showed increased cellularity and diminished capsule space.

The hepatic architecture in the control rats was unremarkable (Figure 13A). Whereas the livers of rats exposed to cadmium (Figure 13B and 13C) showed a range of degenerative changes including swollen hepatocytes with pale cytoplasm and condensed nuclear chromatins with pyknosis, vacuolated hepatocytes with enlarged nuclei, single cell necrosis, marked by contracted cells detached from the others and densely hyperchromatic nuclear chromatins. Sinusoidal widening was also observed in all animals that received cadmium treatment.

Note: The rat model study was awarded the "Best Poster" at 2nd National Conference in Toxicology on December 17-18, 2009. The abstract is included as Appendix C. [*Morkmek N, Lertpresertsuke N, Ruangyuttikarn W. Establishment of a cadmium induced renal tubular damaged rats model for investigating the effect of Thunbergia laurifolia Lindl. leaves extract on cadmium renal toxicity. Thai J Toxicology 2009; 24(2): 181*]

Table 3 Cadmium in urine (day 0 and day 20) and blood (day20) of control rats injected subcutaneously (1.2 ml saline/kg/day) and treated rats (1.2 mg Cd/kg/day) with 20 doses over 26 days

Cd concentrations	Day	Control rats				$CdCl_2$ treated rats			
		Rat 1	Rat 2	Rat 3	Mean ± SE	Rat 4	Rat 5	Rat 6	Mean ± SE
Urinary Cd	0	117	13	38	56.00 ± 31.34	100	163	344	202.33 ± 73.13
(µg/gCr)	26	3	3	8	4.67 ± 1.67	138,506	384,219	139,652	220,792.33 ± 1,714.00*
Blood Cd (µg/L)	26	2	1	2	1.67 ± 0.33	2,951	6,151	6,241	5,114.33 ± 1,081.98

* $p < 0.05$

Table 4 Weight of organs from control and cadmium treated rats after exposure

Organs	Control rats (g)				$CdCl_2$ treated rats (g)			
	Rat 1	Rat 2	Rat 3	Mean ± SE	Rat 4	Rat 5	Rat 6	Mean ± SE
Left kidney	0.986	1.205	1.302	1.16 ± 0.09	1.515	1.257	1.138	1.30 ± 0.11
Right kidney	1.285	1.347	1.213	1.28 ± 0.04	1.594	1.296	1.140	1.34 ± 0.13
Liver	11.342	12.196	11.713	11.75 ± 0.25	22.375	15.771	18.738	18.96 ± 1.91

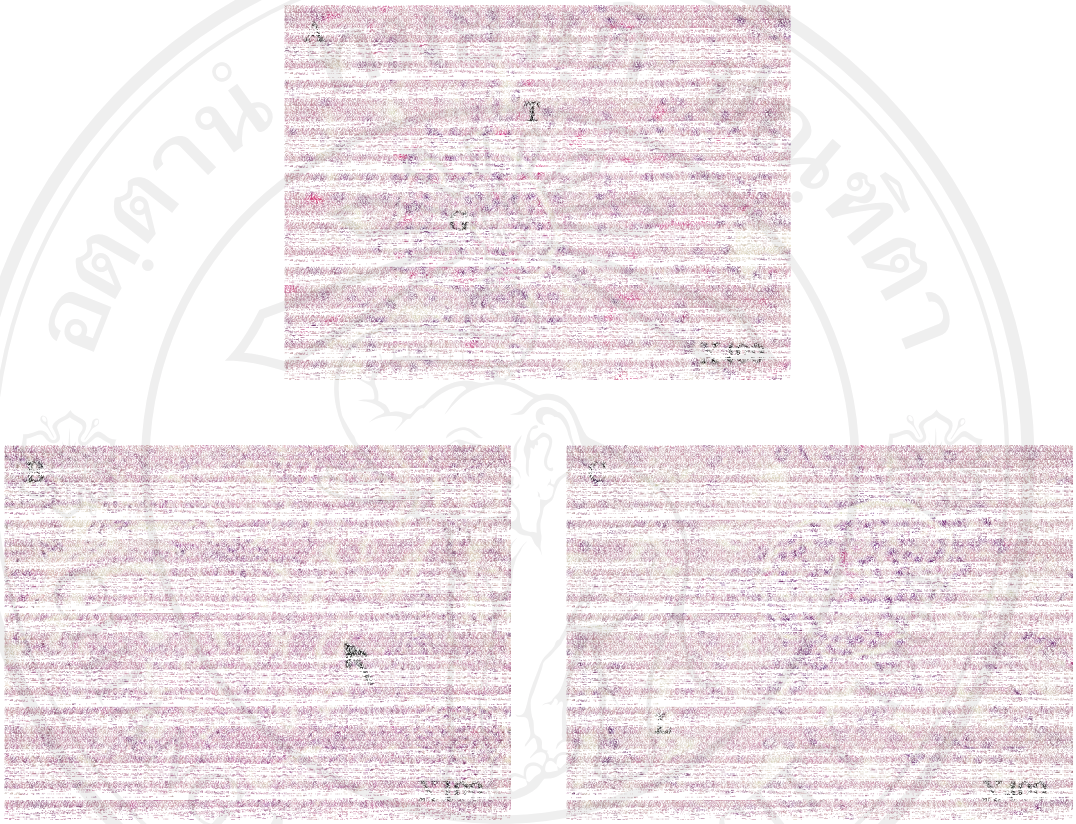

Figure 12 Histopathology of a normal (control) rat kidney (A) showing normal structures in the glomerulus (G) and the proximal convoluted tubules (T). The histopathology of a rat kidney after treatment with cadmium (B and C) shows proximal tubular damage with protein casts (arrow) and dilation of lumen (L).

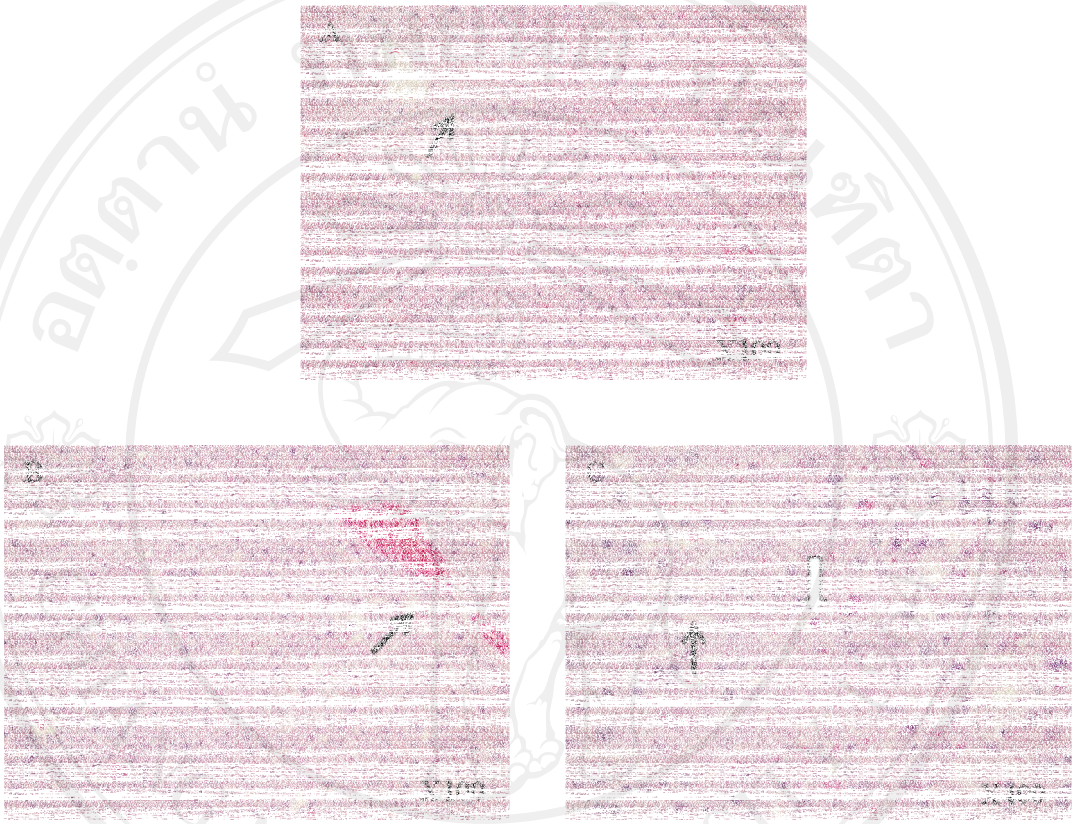

Figure 13 The histopathology of a section of liver from a control rat (A) showing normal hepatic architecture. The liver from a rat exposed to cadmium (B and C) shows degenerative hepatocytes with sinusoidal widening (black arrow) and single cell necrosis (white arrow, C).

Characterization of the constituents of *T. laurifolia* leaves

The NMR spectra of the aqueous extract of *T. laurifolia* leaves collected in the rainy season (July 2009) and the cool season (October 2009) were very similar (Figure 14A and 14B). The NMR study showed the leaf extract contained benzyl, hexyl and hexenyl glucosides.

Detoxification experiments

Antagonistic effect of *T. laurifolia* on cadmium toxicities

The cadmium treated rats showed abnormal appearance including bleeding nose, hunched back, hair loss (Figure 15) and abnormal behavior including passive head tap response, sensitivity to touch, statue position, black tail and aggression. These responses appeared more severe than those of the cadmium treated rats in the earlier experiment to establish the rat model. The cadmium treated rats ate and drank less than the controls and all the treated rats died before the end of the cadmium treatment (26 days). Therefore, it was impossible to quantify the cadmium concentration of urine and blood of live treated rats. The kidneys and livers were collected for histopathological examination.

The kidneys of three experimental rats showed marked tubular necrosis with sloughing of tubular epithelial cells (Figure 16B) and swollen glomeruli compared to control rats (Figure 16A). There was no histopathological change in the liver (Figure 17).

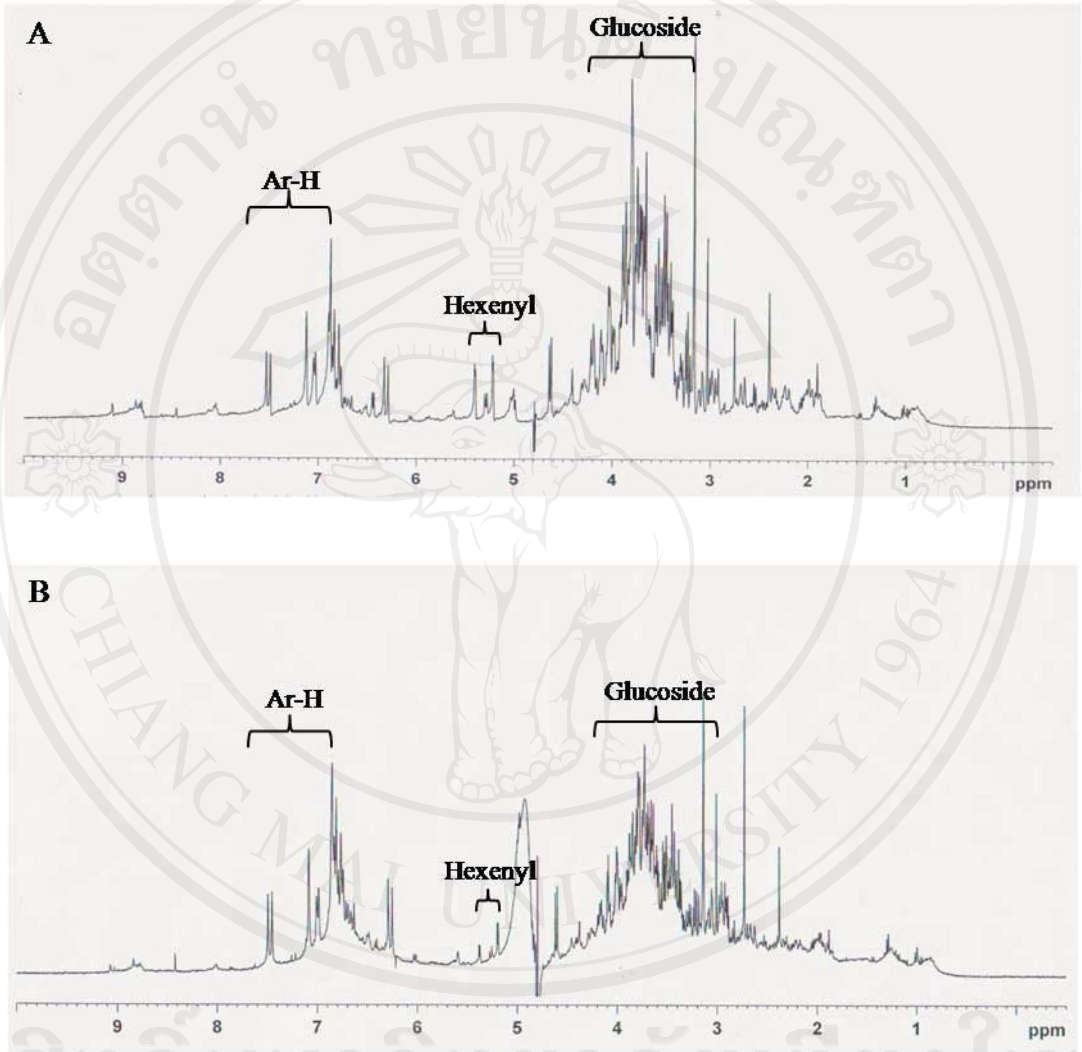

Figure 14 NMR spectra of the aqueous extract of *Thunbergia laurifolia* leaves collected in July (A) and October (B) 2009 showing similar constituents.

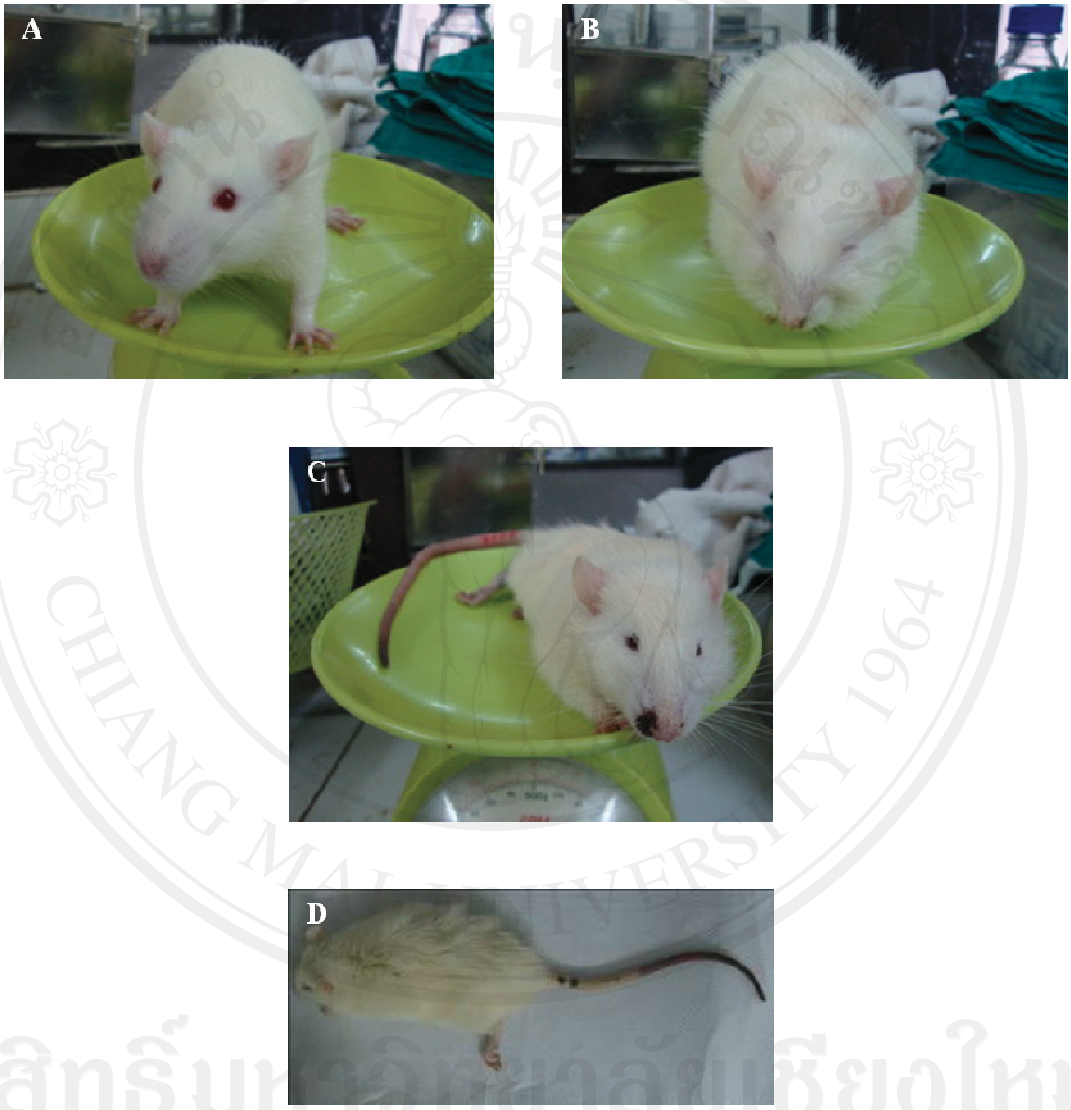

Figure 15 The appearance of control rats (A) compared to rats after cadmium treatment showing abnormalities such as swollen face (B), bleeding nose (C) and black tail (D).

Figure 16 The histopathology of the kidney of a control rat (A) shows the normal structure of the glomerulus (G) and the proximal convoluted tubules (T). The kidney tissue from a rat treated with cadmium (B) shows proximal tubular necrosis, sloughing of tubular epithelial cells and a swollen glomerulus.

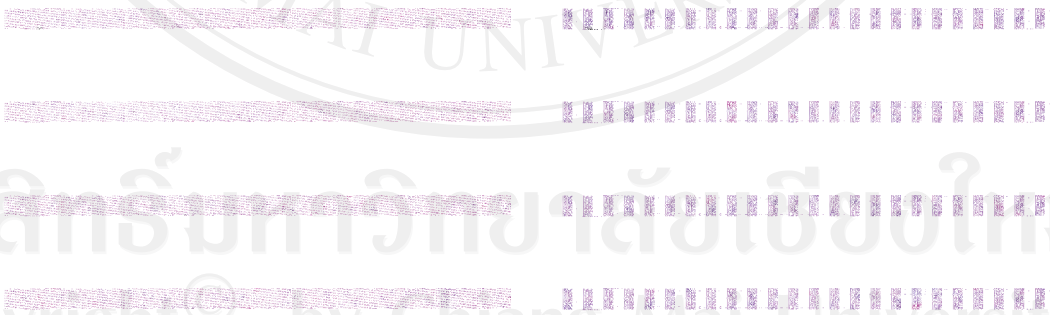

Figure 17 Section of the liver of a normal rat (A) and cadmium treated rat (B), showing similar histopathology.

Prophylactic effect of *T. laurifolia* on cadmium toxicities

In this experiment, *T. laurifolia* leaf extract was fed to rats before cadmium treatment. Again, all the treated rats died before the end of the 26 day dose treatment. Thus, urine and blood samples could not be collected from survivors. But kidneys and livers were collected for histopathological examination.

Before the cadmium treated animals died, all showed abnormalities in appearance and behavior which were similar to those exhibited by the rats in the antagonistic effect experiment. However, the rats fed *T. laurifolia* leaf extract either before or during the cadmium dosing showed less severe abnormal effects than the rats with cadmium treatment only and no feeding of *T. laurifolia* leaf extract.

Histopathological examination found differences in the kidneys of the animals in the three groups in the experiment. The untreated control group (Figure 18A) had normal glomerulus and tubule structures in their kidneys. The positive control group (cadmium treatment only) showed kidney damage such as tubular necrosis and atrophy of the glomeruli and the cadmium treatment clearly affected the brush border and nucleus of the proximal tubule (Figure 18B). In contrast, the kidneys of cadmium exposed animals which were fed leaf extract before cadmium treatment appeared undamaged especially the proximal convoluted tubules (Figure 18C). Finally, kidneys from animals co-treated with *T. laurifolia* extract and cadmium showed various histopathological changes. One rat showed normal histology, a second showed renal tubular degeneration (Figure 18D) and the other four rats showed tubular necrosis which was less severe than in rats treated with cadmium without *T. laurifolia*.

The livers of the positive control rats showed severe vacuolar necrosis, fatty infiltration and fibrosis (Figure 19B) compared to normal rat's liver (Figure 19A). Pre-treatment with the leaf extract (Figure 19C) reduced the number of lesions compared to those observed in the positive control rats. Two of the six rats pretreated with leaf extract had normal liver histology (Figure 19D) and whilst the others did show necrosis, it was not as severe as in rats treated with cadmium alone.

Note: The rat model study and the constituents of the leaf extract were presented (oral presentation) at the 3rd National Conference in Toxicology on November 25-26, 2010 and published in Thai Journal of Toxicology. The published paper is shown in Appendix C. [*Morkmek N, Chattaviriya P, Lertpresertsuke N, Chuncharunee S, Ruangyuttikarn W. Detoxification of cadmium induced renal and hepatic injuries in rats by Thunbergia laurifolia Lindl. leaf extract. Thai J Toxicology 2010; 25(2): 115-23*]

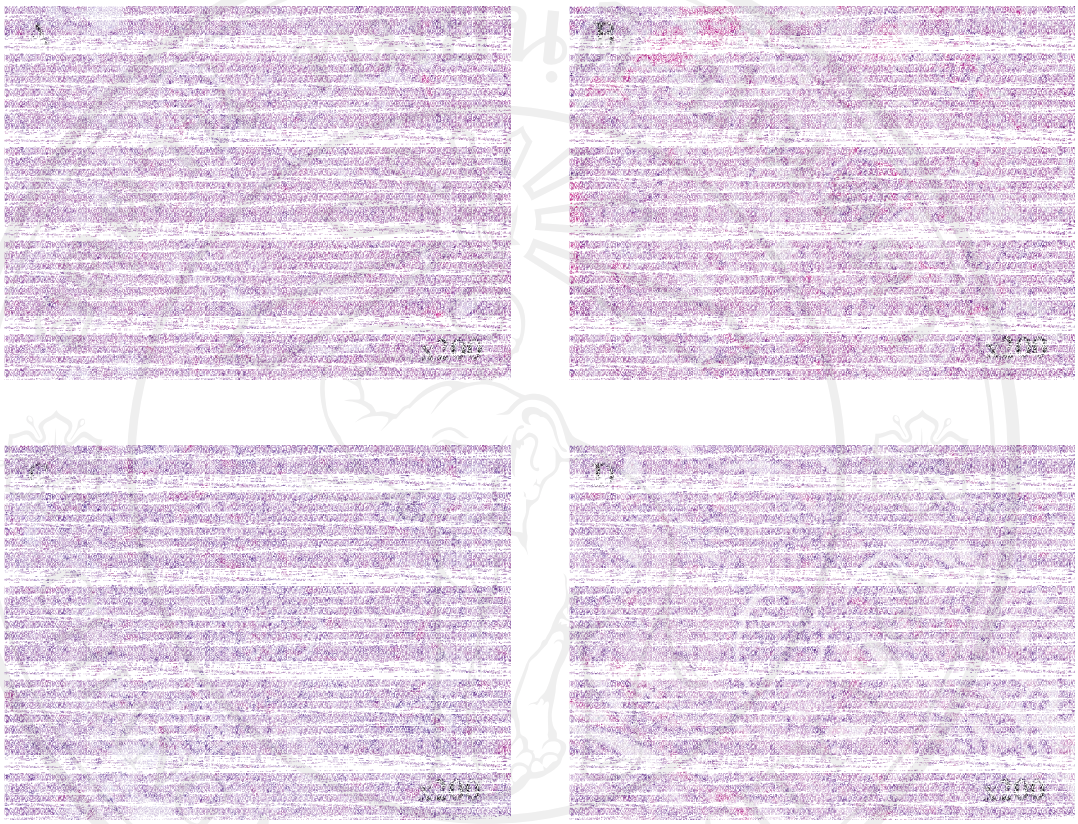

Figure 18 Histopathology of the kidney from a normal rat showing the structure of glomerulus and proximal convoluted tubules (A). A kidney from a rat treated with cadmium only (no leaf extract) showing proximal tubular necrosis (B). A kidney from a rat pre-treated with *T. laurifolia* followed by $CdCl_2$ injection showing degenerative changes (C). A kidney from a rat co-treated with *T .laurifolia* and $CdCl_2$ showing necrosis and degeneration (D).

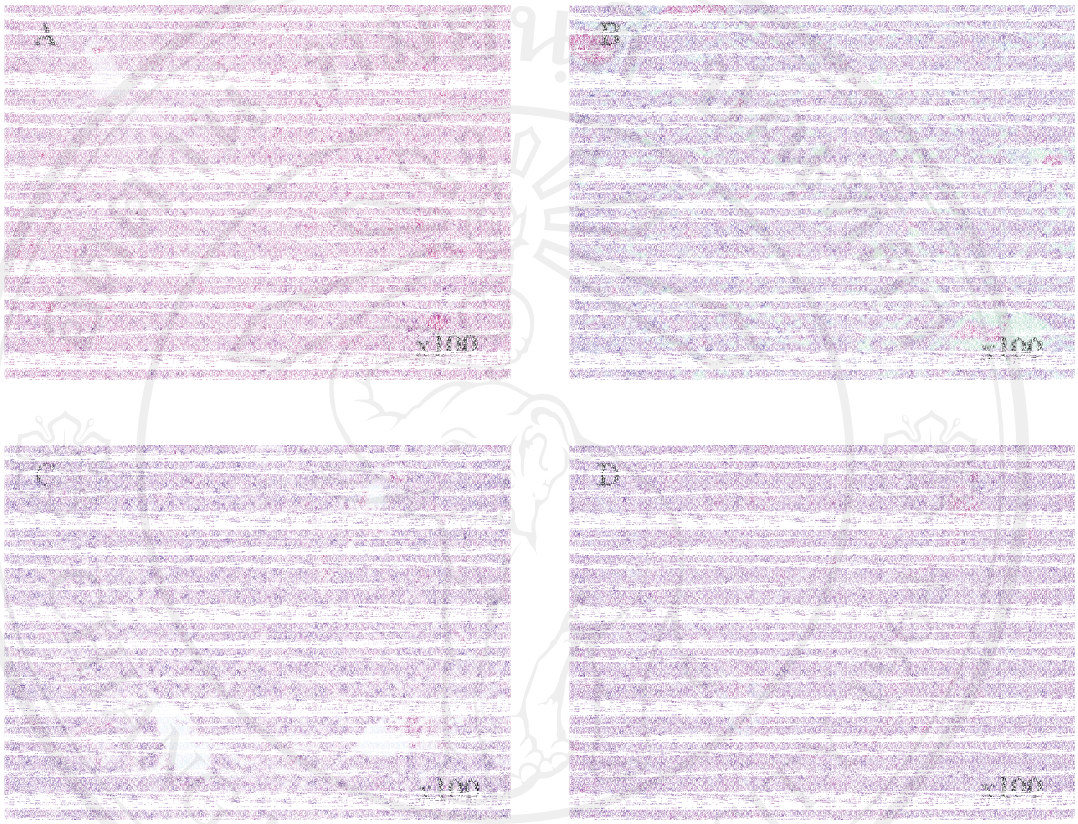

Figure 19 Histopathology of the liver taken from a normal rat showing glomerulus and proximal convoluted tubules structures (A). Liver from a rat exposed to cadmium showing vacuolar necrosis, fatty infiltration and fibrosis (B). Liver from a rat pre-treated by oral administration of *T. laurifolia* then followed by $CdCl_2$ injection showing degenerative changes (C) whereas rats co-treated with *T. laurifolia* leaf extract (orally) and $CdCl_2$ (by injection) showed normal histology (D) and necrosis.

Conclusion

In conclusion, a rat model of cadmium toxicities was established whereby subcutaneously injection of $CdCl_2$ resulted in renal and hepatic injuries. This model was used to study the antagonistic and prophylactic effects of *T. laurifolia* leaf extract. Unfortunately, all cadmium treated rats died before the end of the experiment. Although the *T. laulifolia* leaf extract treatment did not prevent mortality in cadmium treated rats, the changes in appearance and behavior due to cadmium toxicities were less severe in the rats fed leaf extract than in those which only received $CdCl_2$. The histopathology of kidney and liver tissues showed that rats fed *T. laulifolia* leaf extract sustained less cadmium induced tissues damage than the unfed rats.

The ^{1}H NMR study of *T. laurifolia* leaf extract showed that the extract contained benzyl, hexyl and hexenyl glucosides. These constituents might be responsible for the suppression of kidney and liver tissue damage.