CHAPTER 3

RESULTS

3.1 Isolation of *T. laurifolia* Lindl. leaf extract

Fine powder residues of 18 fractions of the *T. laurifolia* leaf extract were obtained from column chromatography (Figure 5) and each fraction was examined on TLC plate (Figure 6). Dried weight, solubility and solvent used in the TLC system for isolating each fraction of the extract were shown in Table 1

3.2 NMR spectra of the *T. laurifolia* Lindl. leaf extract

The criteria for choosing an interested fraction was considered on the quantity of dried weight, solubility and amount of phenolic and glycoside compounds contained in the fraction after identifying and characterizing the chemical constituents of the fractions by ^{1}H NMR. The NMR spectra of fraction 1 to 7 had no significant amount of phenolic and glycoside compounds as shown in Figure 7 (the NMR spectra of fraction 2 was not performed). Figure 8 showed the NMR spectra of the fractions 13 and 14 which were suit to be our interested fractions due to they had an appropriated amount of the highly soluble compounds of phenolic and glycoside with the chemical shift ^{1}H-NMR (ppm, DMSO-d_6) at δ 1.80 (4H, s), δ 2.50 (1H, s), δ 3.15 (2H, s), δ 3.25 (2H, s), δ 3.42 (6H, s), δ 3.62 (2H, d, J = 14.9 Hz), δ 4.85 (1H, d, J = 11.4 Hz) as glycoside compound and δ 5.18 (1H, d, J = 3.3 Hz), δ 6.15 (1H, d, J = 15.9 Hz), δ 6.47 (1H, d, J = 8.9 Hz), δ 6.60 (1H, d, J = 8.0 Hz), δ 6.67 (1H, d, J = 15.8

Hz) as phenolic compound. Thus, the three fractions (13^{th}, 14^{th} and 15^{th} fractions) were pooled to be our selected fraction or PG fraction and evaporated to dryness under nitrogen gas before keeping in the refrigerator at temperature of 0-4^{o}C for animal experiments.

3.3 Animal experiment 1: Effect of *T. laurifolia* Lindl. leaf crude extract on cadmium induced hepatorenal toxicities

Physical appearance and behavior of rats

Physical appearances were found abnormally in both groups of rats after receiving $CdCl_2$ solution subcutaneously (Figure 9). However, the severity and number of rats shown in Table 2 demonstrated that rats pretreated with crude extract of *T. laurifolia* leaf in drinking water had delayed appearance signs and symptoms of Cd toxicity. Moreover, two rats in Cd treated group died during the experiment but all rats that were pretreated with the crude extract survived.

After treatment with $CdCl_2$ solution, all rats were aggressive, consumed less food and water. They all scared of contact and put their face at the corner's cage most of the time, but rats pretreated with *T. laurifolia* leaf crude extract were shown to have abnormal behavior less than the rats treated with $CdCl_2$ alone.

Figure 5 Column chromatography (50 mm diameter, 50 cm height) packed with silica gel **(A)**, loading with crude extract of the *T. laurifolia* leaves dissolved in 95% ethanol **(B)** and the fractions (500 mL) were eluted by different ratio of ethyl acetate and methanol **(C)**. The fractionated process took about six hours per one experiment.

(H = hexane, E = ethyl acetate, M = methanol)

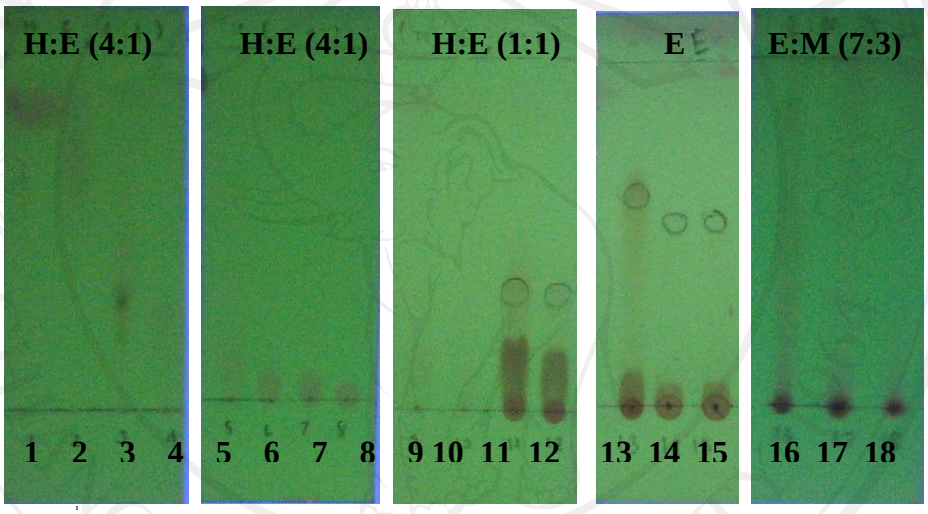

Figure 6 Thin layer chromatogram of the 18 fractions of *T. laurifolia* leaf extract

Table 1 Dried weight, solubility and ratio of solvents used in the TLC system for isolating each fraction of the *T. laurifolia* leaf extract

Fraction No.	Dried weight (g)	Solubility in	Solvent ratio
1	0.145	Hexane	H : E = 4:1
2	0.227	Hexane	
3	0.035	Hexane	
4	0.125	Hexane	
5	0.146	Hexane	
6	0.132	Hexane	
7	0.138	Hexane	
8	0.149	Hexane	
9	0.151	Ethyl acetate	H : E = 1:1
10	0.119	Ethyl acetate	
11	0.211	Ethyl acetate	
12	0.180	Ethyl acetate	
13	0.330	Water	E
14	0.215	Water	
15	0.210	Water	
16	0.111	Methanol	E: M = 7:3
17	0.219	Methanol	
18	0.169	Methanol	

(H = hexane, E = ethyl acetate, M = methanol)

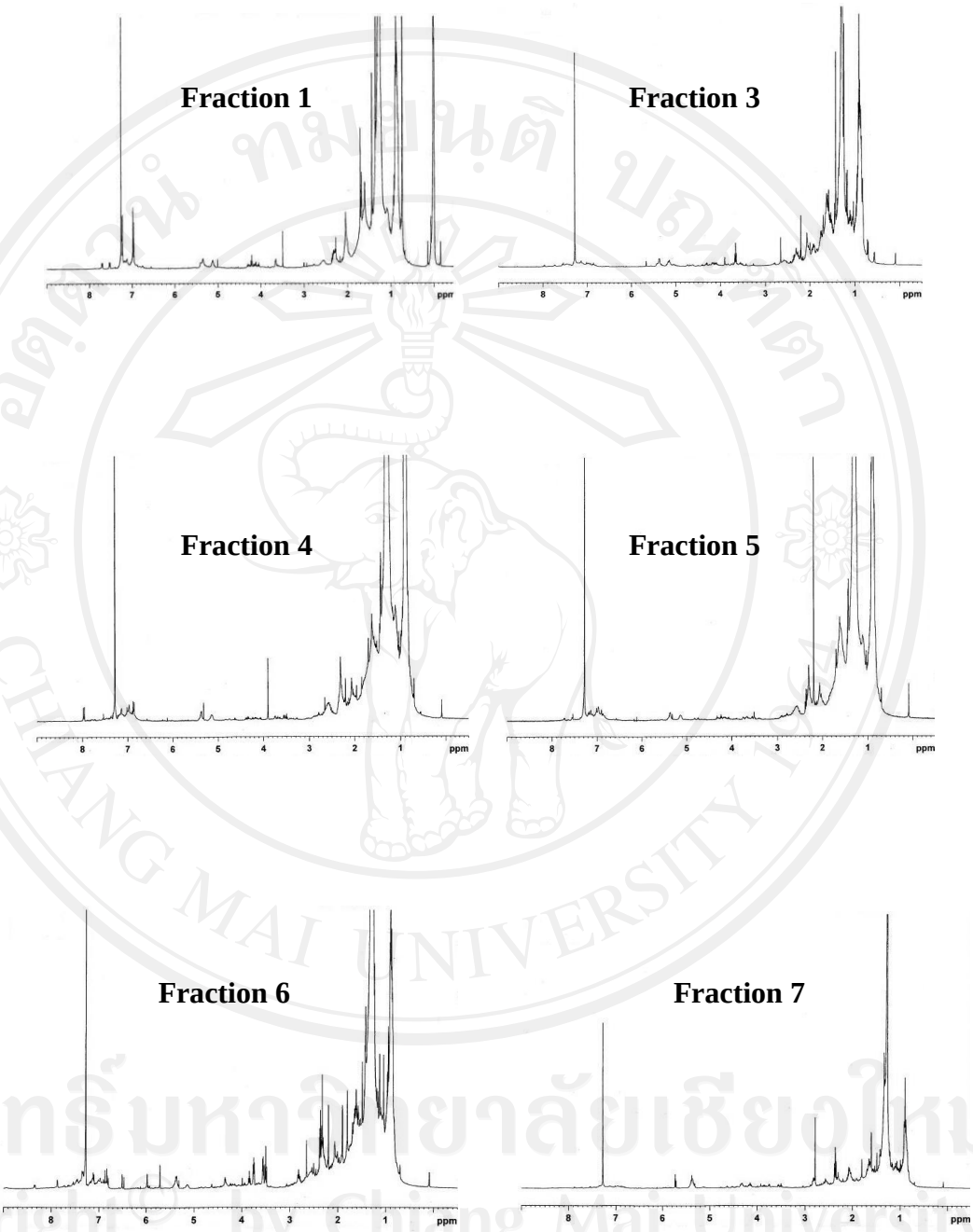

Figure 7 NMR spectra of the 1st and the 3rd – 7 th fraction of *T. laurifolia* leaf extract showing no phenolic and glycoside compounds

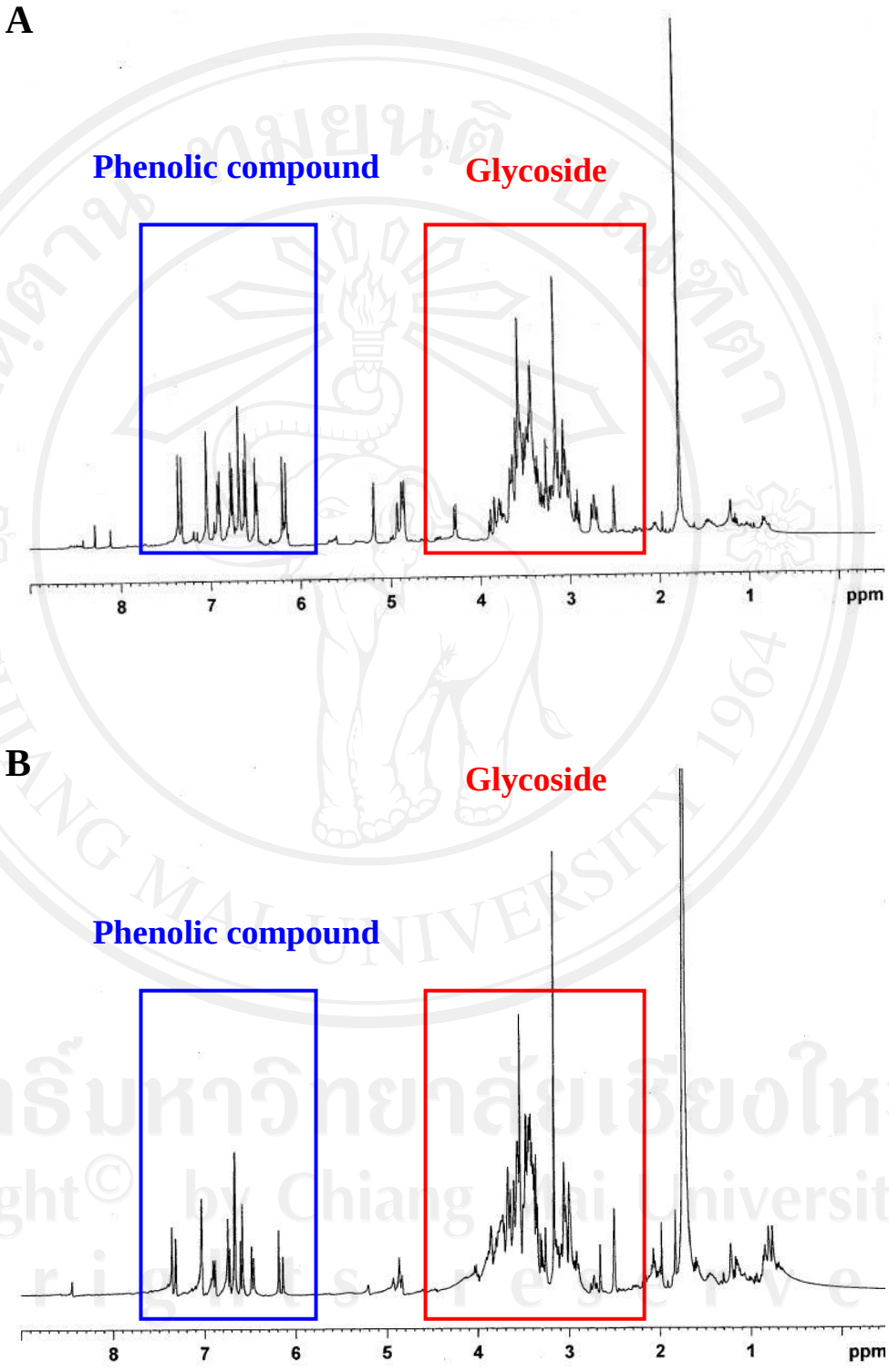

Figure 8 NMR spectra of the 13th fraction **(A)** and the 14th fraction **(B)** of *T. laurifolia* leaf extract showing contents of phenolic and glycoside compounds

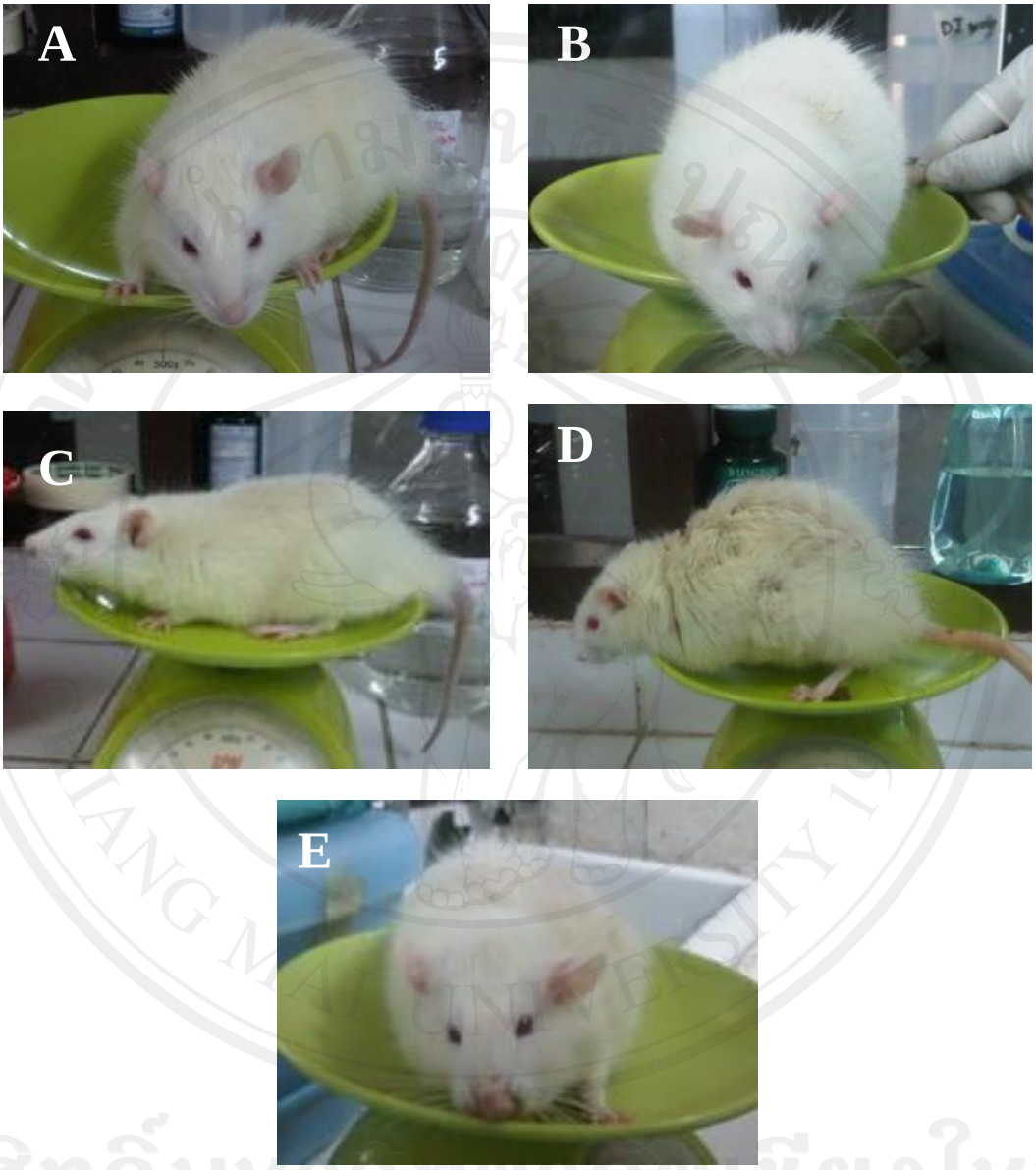

Figure 9 Physical appearances of the experimented rats with swollen face **(B)**, hunched posture and scar at the injecting site **(D)** and bleeding nose **(E)** were seen after $CdCl_2$ solution (1.0 mg/kg) administration compared to the rat treated with NSS **(A)** or *T. laurifolia* leaf extract **(C)** before $CdCl_2$ administration.

Table 2 Number of rats in group 1 (treated with $CdCl_2$ alone) and group 2 (treated with *T. laurifolia* leaf crude extract before and during $CdCl_2$ administration) that showed abnormal appearance of Cd toxicity

Appearance	**Group 1 ($CdCl_2$)**	**Group 2 (TL + $CdCl_2$)**
1. Bleeding nose	Day 3 (1/6)	Day 5 (4/6)
2. Face swelling	Day 10 (6/6)	Day 11 (6/6)
3. Falling hair	Day 7 (6/6)	Day 11 (6/6)
4. Hunched posture	Day 6 (6/6)	Day 10 (6/6)

Values represent mean of the day that found abnormal appearance of rats and values in parenthesis represent number of rats that were observed having abnormal appearance per the number of experimented rats in each group.

TL = *T. laurifolia* leaf crude extract

Rat's body weight

Body weight of rats in both group during day 1 to day 20, without (group 1) and with (group 2) pretreatment with *T. laurifolia* leaf crude extract in drinking water were significantly different ($p<0.05$) from the body weight of rats in day 21 to day 40 as shown in Figure 10. This result indicated that crude extract of *T. laurifolia* leaves help reduce weight loss due to cadmium toxicity.

Water consumption

During days 1-20, both groups of experimented rats consumed similar volume of water per day but after Cd treatment commenced on day 20, rats in both groups consumed less water. In contrast, the group provided with *T. laurifolia* leaf crude extract consumed significantly more water than rats without pretreatment with the extract ($p<0.05$, Figures 11).

Urinary cadmium concentration

Rats in both groups had extremely high urinary Cd level, therefore, the *T. laurifolia* leaf crude extract had no effect on the urinary Cd concentration. Table 3 Shows the amount of Cd quantified by a GFAAS showing non-significant differences of the Cd levels but significantly different from the Cd levels in urine of rats before Cd treatment.

Blood cadmium concentration

Blood Cd concentrations in both groups of rats after 20 days exposure to Cd, were shown in Table 4 indicated that *T. laurifolia* leaf crude extract also had no effect on the concentrations of Cd in blood.

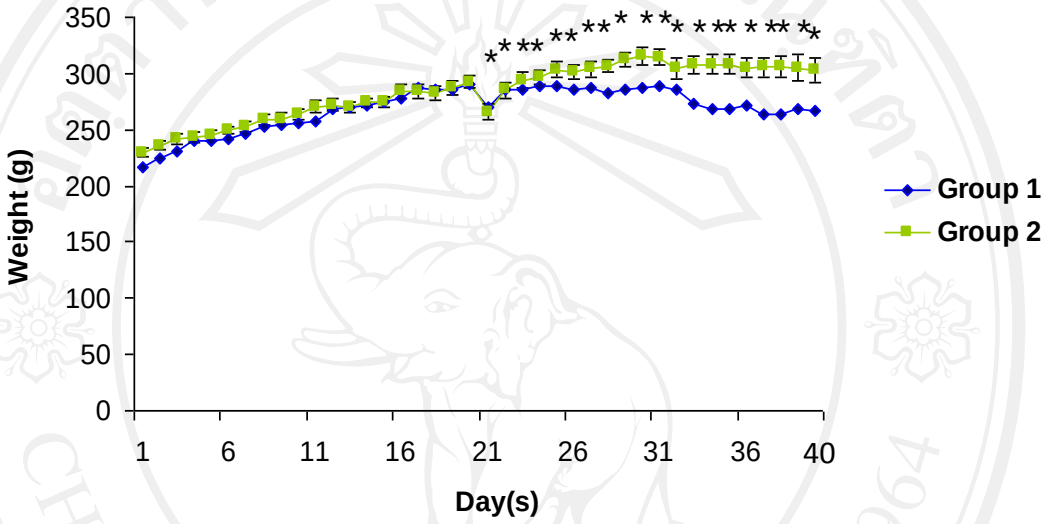

Figure 10 Rats exposed to Cd (group 1) after day 20 suffered weight loss but the loss was much more limited in rats which consumed drinking water containing *T. laurifolia* leaf crude extract (group 2). All values are mean ± standard error of mean (SEM) of six rats. An * indicates statistically significant difference among the average body weight of rats in two groups (p<0.05).

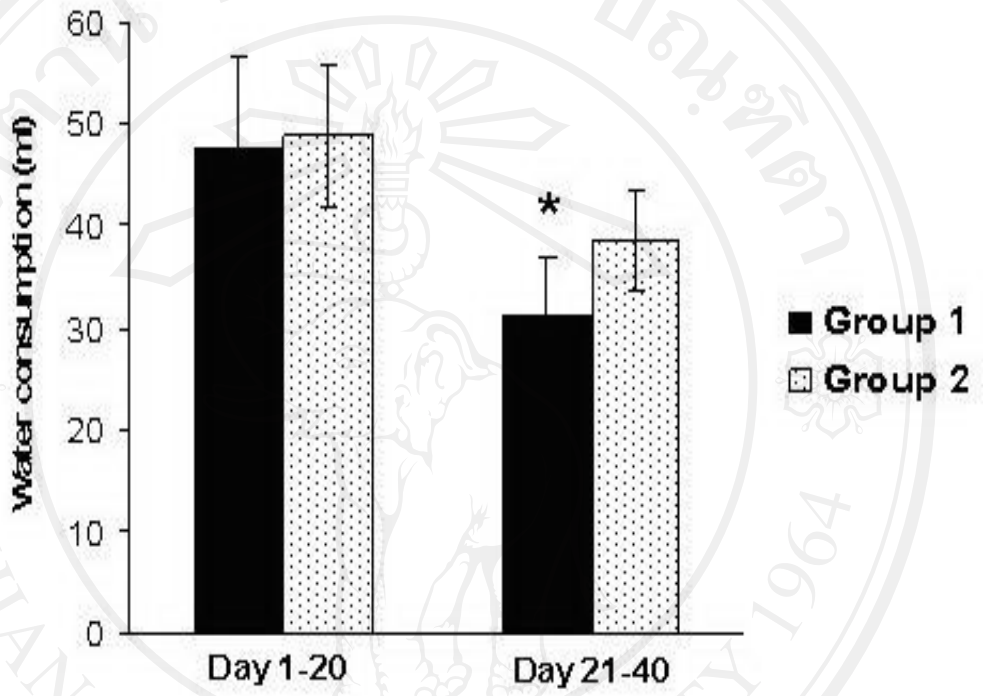

Figure 11 Water consumption of male Wistar rats in Cd treated (group 1) and pretreated with *T. laurifolia* leaf crude extract in drinking water (group 2) before (Day 1-20) and after Cd treatment (Day 21-40). All values are mean ± SEM of six rats. An * indicates statistically significant difference among the two groups (p<0.05).

Table 3 Urinary Cd concentrations in rats exposed to Cd without (group 1) and with (group 2) pretreatment with *T. laurifolia* leaf crude extract

Rats	**U-Cd (µg/g Cr)**		
	Day 0	**Day 20**	**Day 40**
Group 1	30.9 ± 15.5	23.2 ± 6.4	79,491.2 ± 24,545.8
Group 2	34.9 ± 11.6	20.4 ± 6.9	71,478.6 ± 23,355.1

Values represent mean ± SEM of six rats but day 40 represents four rats in group 1 and five rats in group 2 because 1-2 rats died before urine collection.

Table 4 Comparison of blood Cd concentrations of rats treated with Cd only (group 1) and the concentrations of blood Cd in rats pretreated with *T. laurifolia* leaf crude extract in drinking water (group 2)

Rats	**Blood Cd (µg/L)**
Group 1 ($CdCl_2$ treatment only)	5,399.9 ± 618.8
Group 2 (pretreatment with TL)	5,089.1 ± 533.6

Values represent mean ± SEM of four rats in group 1 and five rats in group 2 because 1-2 rats died before blood collection.

TL = *T. laurifolia* leaf crude extract

Kidney and liver's weight of rat

Table 5 demonstrated that the kidney and liver's weight of Cd treated rats and *T. laurifolia* pretreated rats were not significantly difference. However, the *T. laurifolia* pretreated rats appeared to have larger liver's weight than the liver's weight of rats treated with Cd only.

Histopathological examination

Light microscopic examination of the rat's kidneys indicated *T. laurifolia* leaf crude extract could protect kidney from damage caused by Cd. The kidney cortex of rats exposed to Cd without *T. laurifolia* leaf crude extract (Figure 12B) showed abnormalities including glomeruli widening, cloudy swelling of tubules, lumen widening, irregular shaped epithelial cells**,** blurred structure of tubular epithelium, abnormal defined nuclei and pale cytoplasm. In contrast, the histology of glomeruli in rat's kidneys exposed to Cd and *T. laurifolia* leaf crude extract pretreatment (Figure 12C) was not different from glomeruli of the normal rats in Figure 12A (the result shown in Figure 12A was from our previous study with normal rat without any treatment). These plates clearly demonstrated that the kidney tubule and glomeruli structure were preserved in rats exposed to both *T. laurifolia* leaf crude extract and Cd.

The liver of Cd treated group (Figure 13B and 13C) showed focal necrosis, vacuolated cytoplasm, pyknotic nucleus, chromatin condensation and sinusoidal widening and more severe degree of damage found in died rats compared to rats pretreated with *T. laurifolia* leaf crude extract (Figure 13D). The liver histopathology of the rat pretreated with TL leaf extract was similar to the liver of the control rat (no Cd treatment) as shown in Figure 13A.

Table 6 and 7 showed the histopathological grading of the rats kidney and liver damages due to Cd and protective effect of *T. laurifolia* leaf crude extract on Cd toxicities, respectively.

The results from animal experiment 1 show that *T. laurifolia* leaf crude extract effectively protected kidney and liver damages caused by Cd. This work was presented as a poster at the 3rd National Conference in Toxicology on November 25-26, 2010 and also has been published in Thai Journal of Toxicology (Chattaviriya *et al*., 2010).

The raw data of the results is shown in appendix B. A reprint of the published paper is shown in appendix C.

Table 5 Kidney and liver's weight of the experimented rats treated with $CdCl_2$ (group 1) and rats pretreated with *T. laurifolia* leaf crude extract before $CdCl_2$ administration

Organs		Group 1 ($CdCl_2$)	Group 2 (TL + $CdCl_2$)
Kidney (g)	**Right**	1.00 ± 0.04	1.00 ± 0.08
	Left	0.92 ± 0.05	0.96 ± 0.05
Liver (g)		11.83 ± 1.12	12.60 ± 0.61

Values represent mean ± SEM of six rats, TL = *T. laurifolia* leaf crude extract

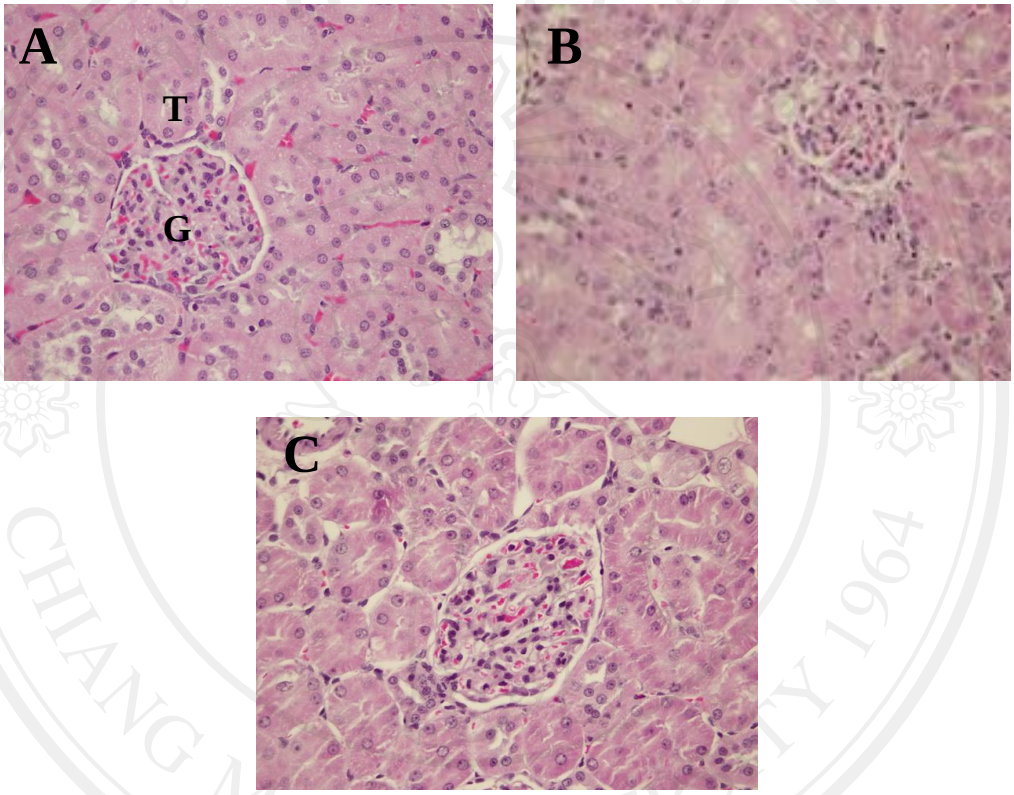

Figure 12 Histopathology (H&E, x400) of tubule (T) and glomeruli (G) in the kidney cortex of a normal, untreated rat **(A);** rat exposed to cadmium chloride at 1.0 mg/kg for 20 days **(B);** and rat exposed to both *T. laurifolia* leaf crude extract and $CdCl_2$ **(C)**.

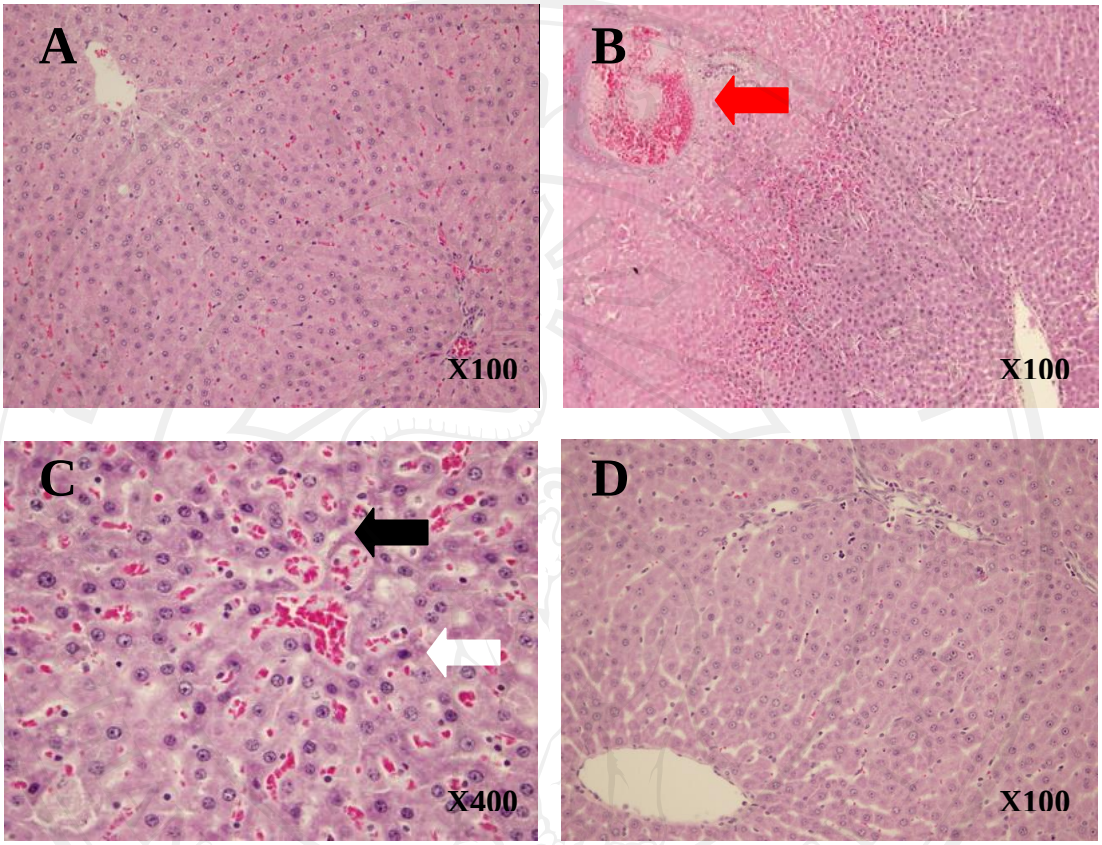

Figure 13 Comparison of histopathology of the liver of rat exposed to $CdCl_2$ (1.0 mg/kg BW) showing focal necrosis (red arrow), vacuolated cytoplasm, pyknotic nucleus and chromatin condensation (white arrow) and sinusoidal widening (black arrow) in **B and C** compared to histology of control rat **(A)** with normal liver. Pretreatment with *T. laurifolia* leaf crude extract before and during Cd exposure also did not show histopathology changes **(D)**.

Table 6 Histopathological changes (grading as 0, +1 to +3 damages) of the rat's kidney after Cd induced toxicity with and without pretreatment of the TL leaf extract

Rats	Kidney											
	Glomerular swelling				Proximal tubular degeneration				Interstitial inflammation			
	+3	+2	+1	0	+3	+2	+1	0	+3	+2	+1	0
Group 1 ($CdCl_2$) (n=6)	6/6	-	-	-	5/6	1/6	-	-	5/6	1/6	-	-
Group 2 (TL + $CdCl_2$) (n=6)	-	-	4/6	2/6	-	-	-	6/6	-	-	-	6/6

Values represent number of rats that observed histopathology. +3 = profound degree or diffuse > 50 % of tissue; +2 = moderate degree or scattered < 50 % of tissue; +1 = mild degree or randomly observed < 10 % of tissue; 0 = absence of the change. TL = *T. laurifolia* leaf crude extract

Table 7 Histopathological changes (grading) of the rat's liver after treatments

Rats	Liver											
	Hepatocytic necrosis				Hepatocytic pyknotic nuclei				Dilation of sinusoid			
	+3	+2	+1	0	+3	+2	+1	0	+3	+2	+1	0
Group 1 ($CdCl_2$) (n=6)	6/6	-	-	-	4/6	2/6	-	-	2/6	1/6	2/6	1/6
Group 2 (TL + $CdCl_2$) (n=6)	-	-	-	6/6	-	-	-	6/6	-	-	1/6	5/6

Values represent the number of rats that observed histopathology. +3 = profound degree or diffuse > 50 % of tissue;

+2 = moderate degree or scattered < 50 % of tissue; +1 = mild degree or randomly observed < 10 % of tissue;

0 = absence of the change. TL = *T. laurifolia* leaf crude extract

3.4 Animal experiment 2: Effect of the PG fraction of *T. laurifolia* Lindl. leaf extract on cadmium induced hepatorenal toxicities

Physical appearance and behavior of rats

Physical appearance and behavior of rats after treated with Cd alone were shown abnormally similar to those rats in the experiment 1 with the crude extract. This experiment also found two rats in the Cd treated group died on day 32 and day 33. Pretreatment with the PG fraction of *T. laurifolia* leaf extract in drinking water also showed abnormal appearances and behaviors but the severity of abnormalities were observed less than the rats treated with Cd alone. Moreover, the day that was found rats with abnormal appearance and behavior was later day than the day that rats treated with $CdCl_2$ alone were observed (Table 8).

Rats treated with the PG fraction of *T. laurifolia* leaf extract and the control rats (n=3) treated with only normal saline solution subcutaneously did not show any abnormal physical appearance and behavior.

Rat's body weight

Means of the rat's body weight were shown in Figure 14 and 15. The body weight of rat's treated with normal saline and the PG fraction of *T. laurifolia* leaf extract (F.TL) alone for 40 days gradually increased during the 40 days experiment. In contrast to the Cd treated rats that showed significantly decreased body weight (p<0.05) in day 21-40. An administration of F.TL before and during $CdCl_2$ injection for 20 days help decrease the body weight of rats due to Cd toxicity in day 21-40 but the decrease was not significantly different compared to the control rats (p>0.05). There were two rats treated with $CdCl_2$ died on day 32.

Water consumption

Water consumption of the experimented rats was shown in Figure 16. During days 1-20, every rats consumed similar volume of water per day. After day 20 (day 21-40) rats in group 2 (Cd treated alone) consumed significantly ($p<0.05$) less water than control rats. However, the rats that were provided with the PG fraction of *T. laurifolia* leaf extract (F.TL) before and during $CdCl_2$ treatment consumed significantly ($p<0.05$) more water than rats treated with $CdCl_2$ alone.

Urinary and blood cadmium concentrations

All rats that were treated with $CdCl_2$ had high urinary Cd concentrations, even though they were pretreated with the PG fraction of *T. laurifolia* leaf extract (F.TL) when compared to the control rats treated with normal saline or treated with only F.TL (Table 9).

Kidney and liver's weight of rats

Weight of the kidneys and livers of rats in this experiment was not significantly different among the control rats (group 1), Cd treated rats (group 2) and rats pretreated with the PG fraction of *T. laurifolia* leaf extract (group 4). However, the data appeared to show that rats in group 2 and 4 with $CdCl_2$ treatment had larger organs than the control rats (Table 10).

Table 8 The day that abnormal appearances of rats caused by cadmium toxicity were seen in rats treated with $CdCl_2$ alone and in rats treated with the PG fraction of *T. laurifolia* leaf extract before $CdCl_2$ administration

Abnormal appearances	**No. Rats ($CdCl_2$)**	**No. Rats (F.TL + $CdCl_2$)**
1. Bleeding nose	Day 5 (3/5)	Day 10 (1/5)
2. Face swelling	Day 5 (3/5)	Day 7 (3/5)
3. Falling hair	Day 6 (3/5)	Day 7 (3/5)
4. Hunched posture	Day 4 (5/5)	Day 5 (5/5)

Values represent mean of the day that found abnormal appearance of rats and values in parenthesis represent number of rats that were found abnormal appearance per the number of experimented rats in each group.

F.TL = The PG fraction of *T. laurifolia* leaf extract

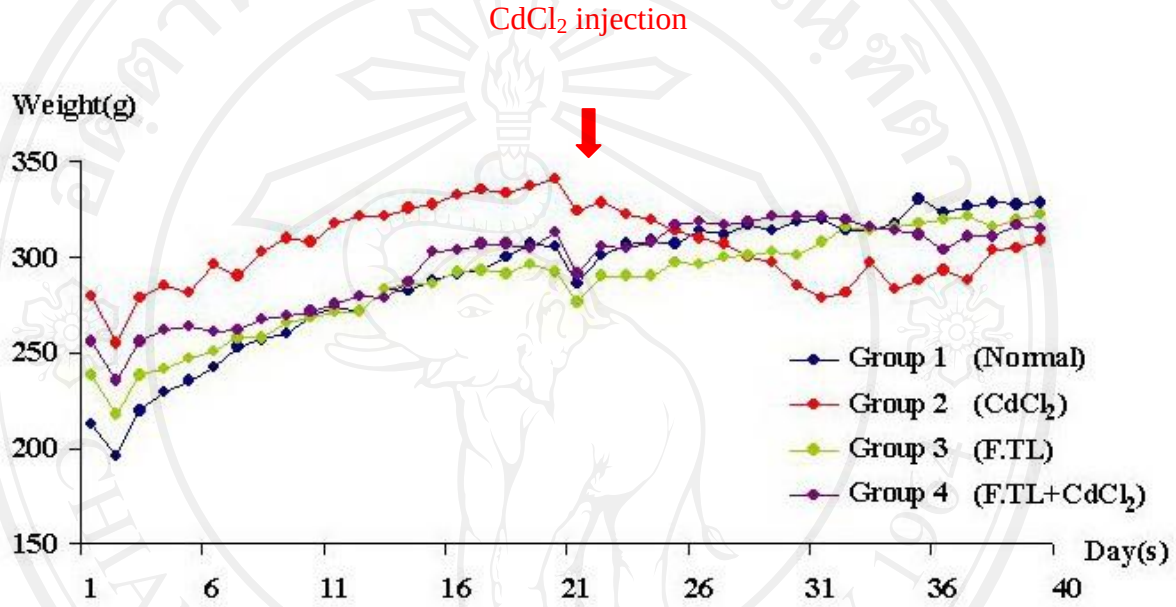

Figure 14 Body weight of rats that were subcutaneously injected with normal saline (group 1), 1.0 mg/kg BW $CdCl_2$ (group 2), feeding with the PG fraction of *T. laurifolia* leaf extract (F.TL) in drinking water (group 3) and feeding with F.TL for 20 days followed by $CdCl_2$ injection on day 21-40 (group 4). Each point represents mean of five rats (group 2 and 4) and three rats in group 1 and 3.

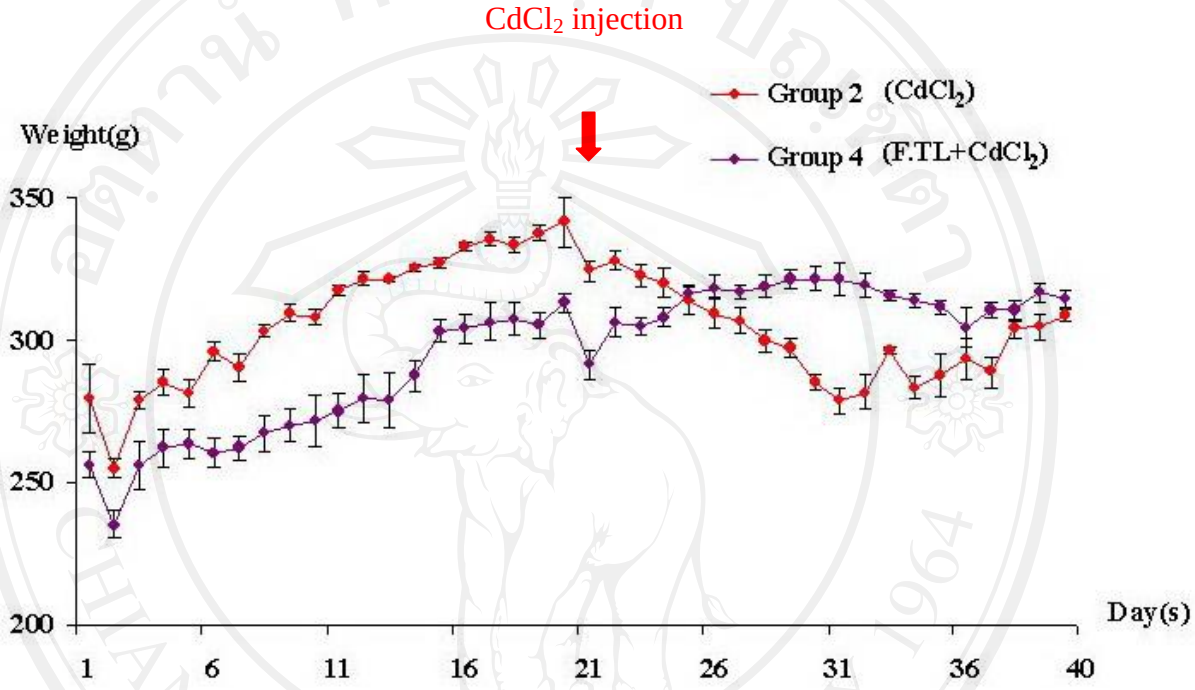

Figure 15 Comparison of the average body weight of rats (n=5) treated with $CdCl_2$ 1.0 mg/kg after day 20 (red arrow) and the average body weight of rats (n=5) pretreatment with the PG fraction of *T. laurifolia* leaf extract (F.TL) before and during $CdCl_2$ administration. Each point represents mean ± SEM of five rats.

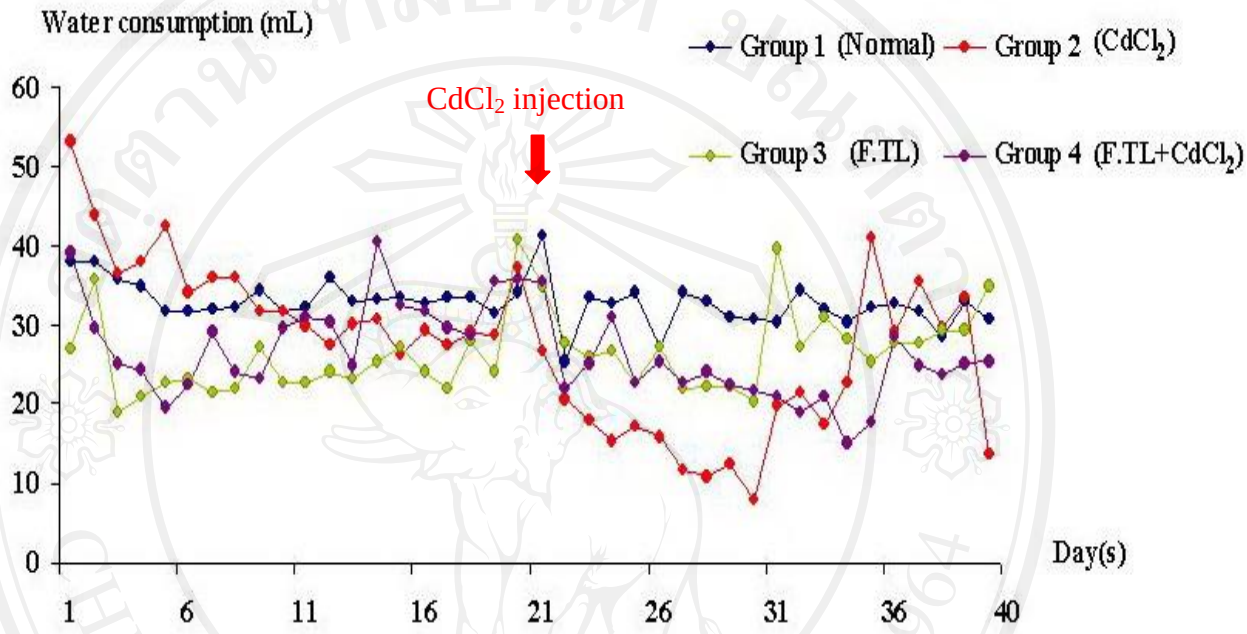

Figure 16 Mean volume of water consumption of the experimented rats in 40 days. Group 1 was control rats treated with normal saline, group 2 was treated with $CdCl_2$ 1.0 mg/kg BW after day 20 (red arrow), group 3 was treated with the PG fraction of *T. laurifolia* leaf extract (F.TL) for 40 days and group 4 was treated with F.TL and $CdCl_2$. Each point represents mean volume of water consumption of five rats except day 32-40 of rats in group 2 represent values of three rats.

Table 9 Urinary and blood Cd concentrations of untreated rats (group 1) and rats treated with $CdCl_2$ (group 2), the PG fraction of *T. laurifolia* leaf extract (F.TL) for 40 days in drinking water (group 3) and pretreated with F.TL before and during $CdCl_2$ exposure (group 4)

	Urinary Cd (µg/g Cr)			Blood Cd (µg/L)
Rats	**Day 0**	**Day 20**	**Day 40**	**Day 40**
Group 1 (control)	16.13 ± 7.44	0.67 ± 0.24	1.09 ± 0.48	2.10 ± 0.10
Group 2 ($CdCl_2$)	3.48 ± 1.07	1.54 ± 0.41	93,189.69 ± 21,028.54*	2,895.30 ± 330.62*
Group 3 (F.TL)	3.76 ± 0.94	4.25 ± 2.54	0.84 ± 0.48	2.37 ± 0.21
Group 4 (F.TL + $CdCl_2$)	3.65 ± 1.10	1.78 ± 0.41	105,172.42 ± 32,175.01*	3,254.52 ± 305.56 *

Values represent mean ± SEM of three rats (group 1 and group 3) and five rats (group 2 and group 4) but in day 40 the values represent mean ± SEM of three rats in group 2 because two rats died. An * indicates statistically significant difference compared to the control rats (p<0.05)

Table 10 Kidney and liver's weight (g) of untreated rats (group 1) and treated with $CdCl_2$ (group 2), the PG fraction of *T. laurifolia* leaf extract (F.TL) for 40 days in drinking water (group 3) and pretreated with F.TL before and during $CdCl_2$ treatment (group 4)

Organs		**Group 1 (normal)**	**Group 2 ($CdCl_2$)**	**Group 3 (F.TL)**	**Group 4 (F.TL + $CdCl_2$)**
Kidney	**Right**	1.13 ± 0.13	1.33 ± 0.06	1.14 ± 0.10	1.37 ± 0.05
	Left	1.17 ± 0.10	1.30 ± 0.07	1.02 ± 0.04	1.37 ± 0.05
Liver		10.07 ± 0.39	12.92 ± 1.36	10.40 ± 0.23	14.85 ± 0.40

Values represent mean ± SEM of three rats (group 1 and group 3) and five rats (group 2 and group 4)

Histopathological examination

Histopathology of the kidneys in Cd treated rats (Figure 17B and 18B) showed the same structural damage of the kidney (proximal tubular necrosis and/or degeneration, glomerular widening, lumen widening etc.) found in rats of the animal experiment 1 compared to the control rats (Figure 17A and 18A). Pretreatment with the PG fraction of *T. laurifolia* leaf extract (F.TL) before and during Cd administration (Figure 17D and 18D) could not help decrease the damages but the severity of the damages in the kidneys of rats pretreated with F.TL and Cd was seen less than those found in rats treated with Cd alone (Table 11) whereas rats fed with F.TL for 40 days did not showed any histopathological changes in the kidney (Figure 17C and 18C). Therefore, F.TL had no toxic effect on rat's kidney.

Histopathology of the rat's liver before and after treatment with F.TL and $CdCl_2$ were shown in Figure 19 and Table 12. The results showed that F.TL could protect liver damages from Cd toxicity while the extract itself did not affect the liver.

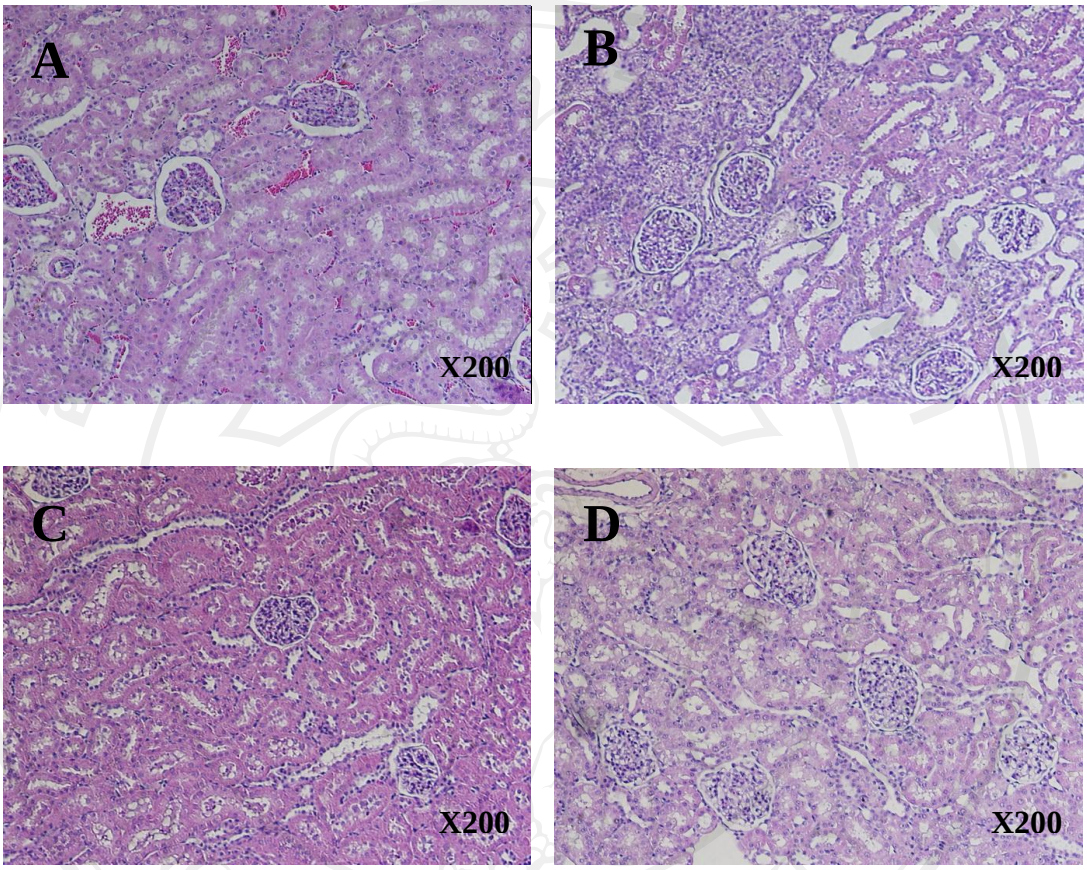

Figure 17 Histopathology of normal kidney **(A)** compared to structural damages of the kidney of rats treated with $CdCl_2$ showing proximal tubular necrosis and glomerular widening **(B)** and normal kidney of rats pretreated with the PG fraction of *T. laurifolia* leaf extract (F.TL) for 40 days **(C)**. Slightly proximal tubular necrosis and glomerular widening were seen in the liver of rats pretreatment with F.TL before and during $CdCl_2$ treatment **(D)**.

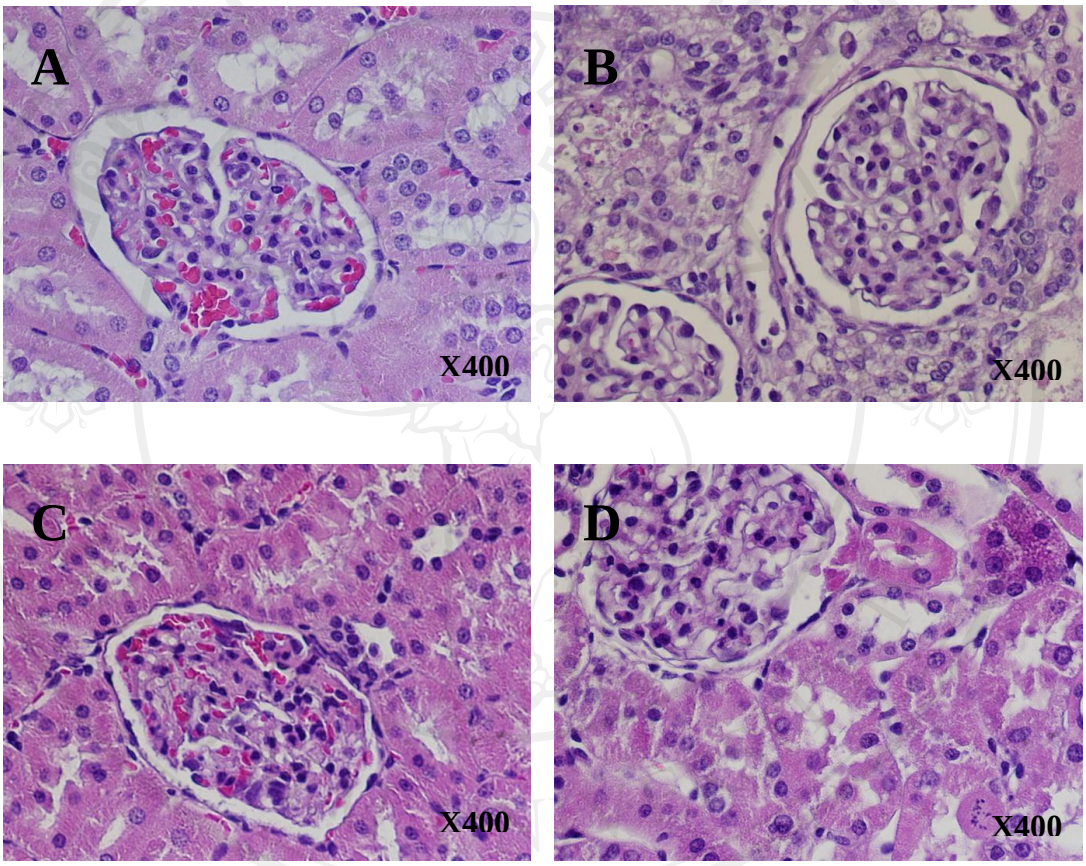

Figure 18 Showing histopathology of the rats kidney as in Figure 18 with 2 times expansion (X400)

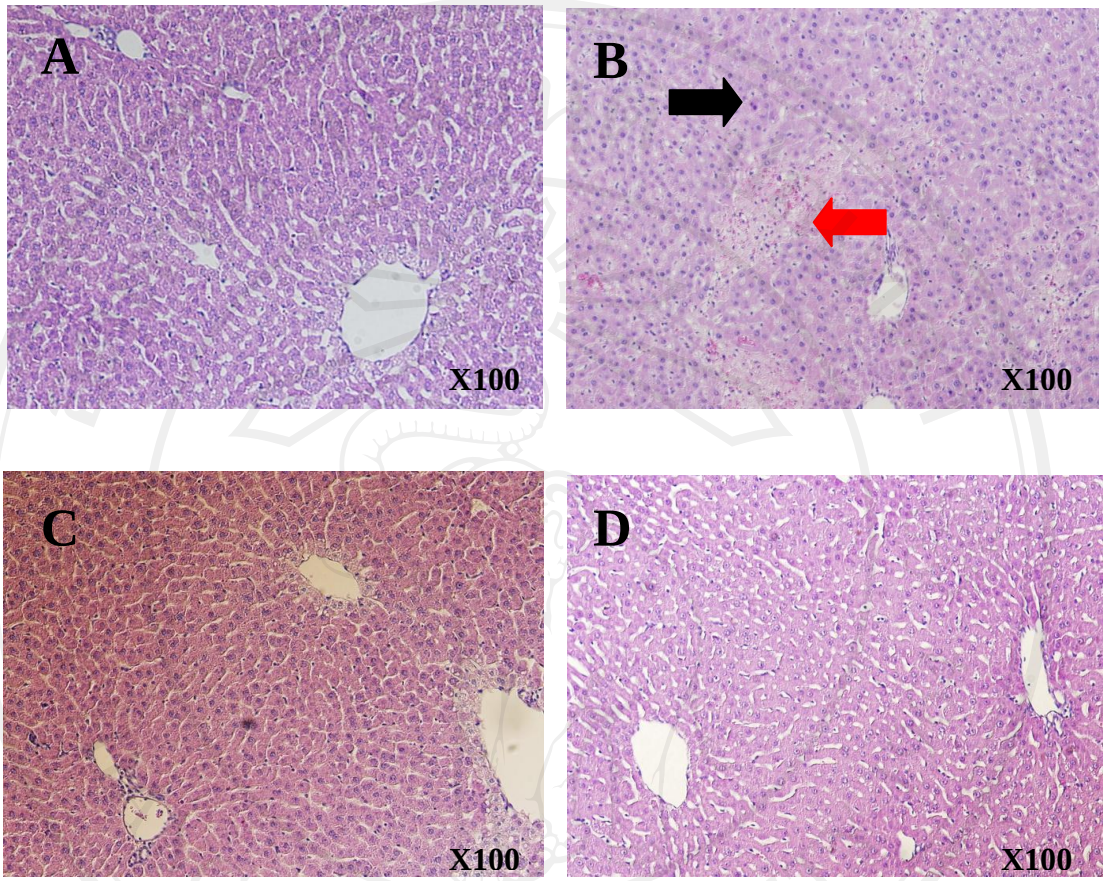

Figure 19 Histopathology of rats normal liver **(A)** compared to the damaged liver of rats treated with $CdCl_2$ **(B)** with focal necrosis (red arrow) and pyknotic nucleus (black arrow), and normal liver of rats pretreated with the PG fraction of *T. laurifolia* leaf extract (F.TL) in drinking water for 40 days **(C)** and 20 days before administration of $CdCl_2$ **(D)**.

Table 11 Histopathology grading of kidney damage in animal experiment 2

Rats	Kidney											
	Glomerular swelling				Proximal tubular degeneration				Interstitial inflammation			
	+3	+2	+1	0	+3	+2	+1	0	+3	+2	+1	0
Group 1 (Normal) (n=3)	-	-	-	3/3	-	-	-	3/3	-	-	-	3/3
Group 2 ($CdCl_2$) (n=5)	4/5	-	1/5	-	3/5	1/5	1/5	-	4/5	-	1/5	-
Group 3 (F.TL) (n=3)	-	-	-	3/3	-	-	-	3/3	-	-	3/3	-
Group 4 (F.TL + $CdCl_2$) (n=5)	2/5	3/5	-	-	2/5	2/5	1/5	-	1/5	1/5	3/5	-

Values represent the number of rats that had kidney damage; +3 = profound degree or diffuse > 50 % of tissue;

+2 = moderate degree or scattered < 50 % of tissue; +1 = mild degree or randomly observed < 10 % of tissue;

0 = absence of the change in the rats. F.TL = The PG fraction of *T. laurifolia* leaf extract

Table 12 Histopathology grading of liver damage in animal experiment 2

Rats	Liver											
	Hepatocytic necrosis				Hepatocytic pyknotic nuclei				Dilation of sinusoid			
	+3	+2	+1	0	+3	+2	+1	0	+3	+2	+1	0
Group 1 (Normal) (n=3)	-	-	-	3/3	-	-	-	3/3	-	-	-	3/3
Group 2 ($CdCl_2$) (n=5)	5/5	-	-	-	4/5	1/5	-	-	3/5	-	-	2/5
Group 3 (F.TL) (n=3)	-	-	-	3/3	-	-	-	3/3	-	-	-	3/3
Group 4 (F.TL + $CdCl_2$) (n=5)	-	-	-	3/3	-	-	-	5/5	-	-	-	5/5

Values represent the number of rats that had liver damage; +3 = profound degree or diffuse > 50 % of tissue;

+2 = moderate degree or scattered < 50 % of tissue; +1 = mild degree or randomly observed < 10 % of tissue;

0 = absence of the change in the rats. F.TL = The PG fraction of *T. laurifolia* leaf extract