

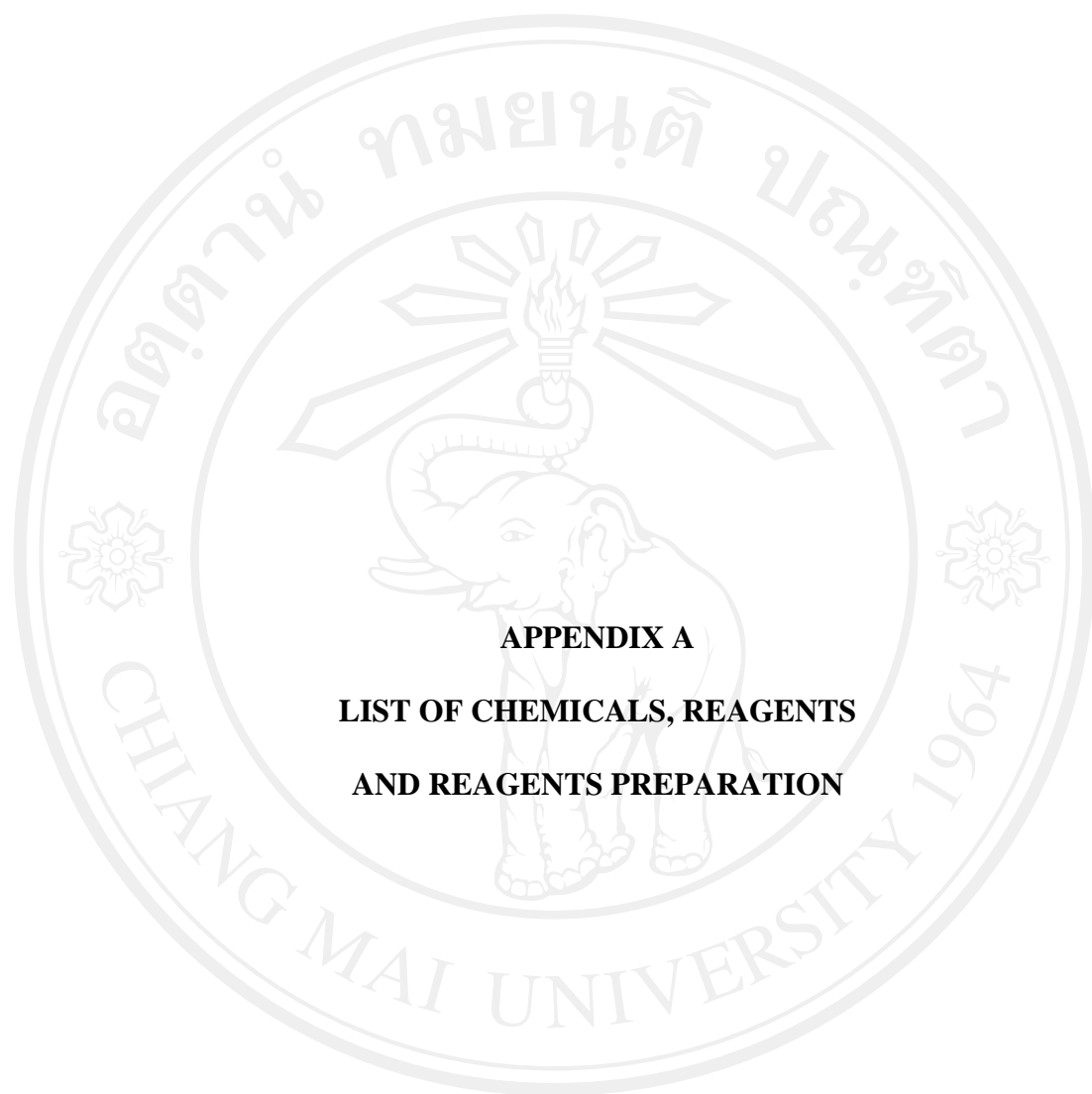


APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved



APPENDIX A

**LIST OF CHEMICALS, REAGENTS
AND REAGENTS PREPARATION**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University
All rights reserved

1. List of chemicals and reagents

Chemical and Reagents	Sources
Cadmium chloride	RANKEM, India
Cadmium atomic spectroscopy standard 1000 mg/L	Sigma-Aldrich, Germany
95% Ethyl alcohol	Commercial grade, Thailand
Hexane	Commercial grade, Thailand
Methanol	Commercial grade, Thailand
Nitric acid	MERCK, Germany
Propanol	MERCK, Germany
Silica gel 60 for CC (70-230 mesh)	MERCK, Germany
Sodium Chloride	RCI Labscan Limited, Thailand
Thiopental sodium	ABBOTT, Italy
TritonX-100	Fisher Science, UK

2. List of reagents preparation

1.0 mg/kg Cadmium chloride (CdCl_2)

- $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ 0.6227 g

adjusted volume to 150 mL with 0.9% NaCl

5% Monobasic ammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$)

- $\text{NH}_4\text{H}_2\text{PO}_4$ 2.5 g

adjusted volume to 100 mL with ultrapure water

5% Nitric acid (HNO_3)

- Stock HNO_3 (65% v/v) 7.7 mL

adjusted volume to 100 mL with ultrapure water

0.1% Nitric acid (HNO_3)

- Stock HNO_3 (65% v/v) 1.5 mL

adjusted volume to 1,000 mL with ultrapure water

Rinse solution

- Propanol 100 mL

- TritonX-100 100 μL

adjusted volume to 1,000 mL with ultrapure water

10 ppb Cd standard

- Stock Cd standard (1,000 $\mu\text{g/L}$) in 0.1% HNO_3 100 μL
adjusted volume to 10 mL with 0.1% HNO_3
- Prepared Cd standard above 100 μL
adjusted volume to 10 mL with 0.1% HNO_3

1 ppb Cd standard

- Cd standard (10 $\mu\text{g/L}$) in 0.1% HNO_3 1,000 μL
adjusted volume to 10 mL with 0.1% HNO_3



APPENDIX B

LIST OF RAW DATA

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

1. List of tables of raw data in the study of effect of *T. laurifolia* Lindl. leaf crude extract on cadmium induced hepatorenal toxicities

Table 13 Body weight (g) of the rats treated with CdCl₂ 1.0 mg/kg BW (group 1)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SEM
1	228	242	212	228	164	224	216 ± 11
2	240	228	218	238	202	224	225 ± 6
3	238	238	218	238	220	232	231 ± 4
4	246	246	228	254	232	234	240 ± 4
5	244	250	230	250	230	236	240 ± 4
6	246	250	232	242	240	240	242 ± 4
7	244	250	238	262	242	238	246 ± 4
8	248	262	240	262	260	248	253 ± 4
9	256	256	238	268	256	250	254 ± 4
10	250	264	242	262	262	252	255 ± 4
11	256	260	246	262	264	254	257 ± 3
12	262	272	254	286	270	264	268 ± 4
13	266	274	256	284	274	262	269 ± 4
14	266	272	258	292	280	262	272 ± 5
15	272	278	258	288	284	268	275 ± 4
16	268	278	262	302	288	268	278 ± 6
17	280	280	270	316	298	276	287 ± 7
18	274	282	274	308	300	280	286 ± 6
19	280	282	272	312	296	274	286 ± 6
20	282	286	276	320	300	278	290 ± 7

Table 13 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean \pm SEM
21	264	266	256	298	282	258	271 \pm 7
22	278	278	278	312	296	274	286 \pm 6
23	284	282	278	314	282	278	286 \pm 6
24	298	276	262	322	300	276	289 \pm 9
25	298	272	260	328	304	268	288 \pm 11
26	302	268	248	340	300	258	286 \pm 14
27	304	270	240	352	302	252	287 \pm 17
28	310	272	226	344	294	246	282 \pm 18
29	310	278	228	362	298	242	286 \pm 20
30	314	268	238	356	302	242	287 \pm 19
31	316	272	238	368	296	244	289 \pm 20
32	320	270	228	370	290	240	286 \pm 22
33	320	258	210	342	282	230	274 \pm 21
34	314	262	206	328	272	230	269 \pm 19
35	320	276	202	310	264	238	268 \pm 18
36	316	288	216	308	260	240	271 \pm 16
37	316	274	226	Died	258	242	263 \pm 15
38	308	274	228	-	262	244	263 \pm 14
39	312	294	238	-	254	246	269 \pm 14
40	312	300	226	-	246	248	266 \pm 17

SEM = standard error of mean

Table 14 Body weight (g) of the rats administrated with 0.1 mg/mL *T. laurifolia* leaf extract in drinking water before and during Cd treatment (group 2)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean \pm SEM
1	230	226	228	218	228	246	229 \pm 4
2	238	236	232	224	232	252	236 \pm 4
3	240	240	238	230	242	264	242 \pm 5
4	242	244	240	234	238	264	244 \pm 4
5	242	242	240	236	246	266	245 \pm 4
6	250	248	248	240	248	264	250 \pm 3
7	252	250	250	242	250	276	253 \pm 5
8	250	258	254	252	258	280	259 \pm 4
9	254	258	258	248	258	282	260 \pm 5
10	258	262	258	256	264	286	264 \pm 5
11	270	270	266	254	266	296	270 \pm 6
12	268	272	270	260	266	296	272 \pm 5
13	264	272	270	254	272	290	270 \pm 5
14	270	274	274	262	270	294	274 \pm 4
15	272	272	272	262	272	298	275 \pm 5
16	280	284	282	266	278	312	284 \pm 6
17	282	282	280	270	276	312	284 \pm 6
18	276	282	284	266	276	310	282 \pm 6
19	288	288	290	270	274	314	287 \pm 6
20	286	296	296	278	282	318	293 \pm 6

Table 14 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean \pm SEM
21	262	268	268	248	254	294	266 \pm 7
22	284	286	288	264	276	312	285 \pm 6
23	290	300	298	272	282	322	294 \pm 7
24	294	302	296	278	284	322	296 \pm 6
25	308	318	300	280	288	324	303 \pm 7
26	308	312	304	278	288	320	302 \pm 6
27	312	324	292	280	298	318	304 \pm 7
28	316	320	310	282	298	314	307 \pm 6
29	320	328	310	284	310	320	312 \pm 6
30	328	330	318	280	306	330	315 \pm 8
31	328	324	310	282	312	330	314 \pm 7
32	324	314	306	264	292	328	305 \pm 10
33	316	302	312	280	296	340	308 \pm 8
34	310	300	310	282	300	348	308 \pm 9
35	304	294	304	290	306	350	308 \pm 9
36	294	288	312	294	298	344	305 \pm 8
37	284	284	318	300	306	340	305 \pm 9
38	274	302	322	Died	304	330	306 \pm 10
39	270	294	322	-	302	338	305 \pm 12
40	290	280	310	-	296	340	303 \pm 10

Copyright © by Chiang Mai University

All rights reserved

Table 15 Water consumption (mL) of the rat treated with CdCl₂ (group 1)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SEM
1	24	5	20	29	60	23	27 ± 8
2	26	24	25	35	45	31	31 ± 3
3	38	27	35	62	46	25	39 ± 6
4	46	26	33	87	57	31	47 ± 9
5	63	38	39	100	86	38	61 ± 11
6	34	19	37	97	61	29	46 ± 12
7	35	26	45	97	81	28	52 ± 12
8	46	43	46	96	76	26	56 ± 11
9	53	35	42	97	79	29	56 ± 11
10	47	29	47	99	64	26	52 ± 11
11	31	24	28	98	46	25	42 ± 12
12	36	35	36	80	61	31	47 ± 8
13	36	48	34	97	69	26	52 ± 11
14	39	39	34	98	65	28	51 ± 11
15	29	35	29	98	43	26	43 ± 11
16	35	28	40	69	45	27	41 ± 6
17	40	37	41	99	73	28	53 ± 11
18	54	40	40	97	66	35	55 ± 10
19	35	32	33	70	50	27	41 ± 7
20	55	58	40	85	115	36	65 ± 13

Table 15 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean \pm SEM
21	50	43	46	69	55	38	50 \pm 5
22	73	25	27	79	24	25	42 \pm 11
23	71	22	11	91	23	20	40 \pm 14
24	68	22	17	96	24	23	42 \pm 14
25	70	26	13	98	10	22	40 \pm 15
26	47	17	8	98	39	14	37 \pm 14
27	74	26	10	99	7	13	38 \pm 16
28	52	25	14	100	14	14	37 \pm 14
29	57	8	31	99	23	15	39 \pm 14
30	35	17	28	76	8	22	31 \pm 10
31	25	16	2	50	6	15	19 \pm 7
32	17	9	1	8	102	23	27 \pm 16
33	19	22	31	1	3	58	22 \pm 9
34	22	27	18	2	6	32	18 \pm 5
35	19	26	24	10	7	30	19 \pm 4
36	23	11	24	3	10	26	16 \pm 4
37	18	9	20	Died	13	21	16 \pm 2
38	29	51	29	-	6	27	28 \pm 7
39	18	25	3	-	4	18	14 \pm 4
40	45	24	6	-	14	13	20 \pm 7

Table 16 Water consumption (mL) of the rats pretreated with *T. laurifolia* leaf extract (group 2)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean \pm SEM
1	29	35	31	36	27	35	32 \pm 2
2	26	36	36	45	34	29	34 \pm 3
3	33	33	47	63	34	28	40 \pm 5
4	42	40	63	81	60	29	53 \pm 8
5	32	37	30	53	31	28	35 \pm 4
6	37	39	38	67	34	31	41 \pm 5
7	100	41	79	99	56	45	70 \pm 11
8	76	42	62	90	63	35	61 \pm 9
9	50	49	60	75	55	37	54 \pm 5
10	32	33	36	61	28	29	37 \pm 5
11	43	38	68	80	58	29	53 \pm 8
12	45	43	91	98	65	48	65 \pm 10
13	48	50	87	100	65	31	64 \pm 11
14	35	24	46	72	47	30	42 \pm 7
15	43	31	48	67	46	40	46 \pm 5
16	48	25	66	97	46	37	53 \pm 11
17	44	29	77	92	47	35	54 \pm 10
18	45	28	47	61	25	31	40 \pm 6
19	48	23	59	74	38	37	47 \pm 8
20	63	43	63	52	60	46	55 \pm 4

Table 16 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean \pm SEM
21	45	54	54	84	47	42	54 \pm 6
22	35	50	38	85	33	32	46 \pm 9
23	33	41	37	85	28	22	41 \pm 9
24	53	48	66	93	52	35	58 \pm 8
25	92	51	72	92	52	23	64 \pm 11
26	97	92	92	96	89	38	84 \pm 9
27	46	43	55	47	64	21	46 \pm 6
28	62	46	39	46	55	31	47 \pm 5
29	47	20	58	16	49	26	36 \pm 7
30	29	12	33	23	31	24	25 \pm 3
31	16	3	18	92	94	17	40 \pm 17
32	14	8	23	32	28	27	22 \pm 4
33	9	10	16	33	27	23	20 \pm 4
34	8	8	15	43	31	24	22 \pm 6
35	15	6	25	35	98	16	33 \pm 14
36	10	12	28	26	58	18	25 \pm 7
37	5	35	28	10	56	21	26 \pm 8
38	9	8	15	Died	52	29	23 \pm 8
39	32	6	8	-	40	22	22 \pm 7
40	10	55	9	-	45	32	30 \pm 9

Table 17 Urinary and blood Cd concentrations of rats in group 1 (CdCl₂)

Cd concentrations in	Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SEM
Urine (µg/g Cr)	0	106.42	12.93	14.12	16.83	31.11	3.94	30.89 ± 15.53
	20	38.91	5.05	13.13	16.00	45.33	20.80	23.20 ± 6.38
	40	162,208.33	20,729.41	103,765.90	Died	54,262.07	56,490.07	79,491.16 ± 24,545.76
Blood (µg/L)	40	6,045.30	3,679.20	Died	Died	6,502.80	5,372.10	5,399.85 ± 618.77

Table 18 Urinary and blood Cd concentration of group 2 (TL + CdCl₂)

Cd concentrations in	Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SEM
Urine (µg/g Cr)	0	82.11	5.65	25.00	45.26	42.36	8.97	34.90 ± 11.58
	20	19.12	3.52	14.62	3.70	41.83	39.91	20.45 ± 6.92
	40	114,032.59	69,400.00	133,352.94	Died	16,566.37	24,041.18	71,478.62 ± 23,355.14
Blood (µg/L)	40	5,387.70	5,481.00	5,229.00	Died	5,864.40	3,483.60	5,089.14 ± 533.59

TL = *T. laurifolia* leaf crude extract

Table 19 Organ's weight (g) of rats in group 1 (CdCl₂)

Organs	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SEM
Right kidney	1.02	1.06	1.05	1.12	0.91	0.83	1.00 ± 0.04
Left kidney	0.97	0.95	0.93	1.07	0.83	0.76	0.92 ± 0.05
Liver	12.88	13.57	9.83	16.00	9.68	9.04	11.83 ± 1.12

Table 20 Organ's weight (g) of rats in group 2 (TL + CdCl₂)

Organs	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SEM
Right kidney	0.76	0.89	0.99	1.30	0.91	1.18	1.00 ± 0.08
Left kidney	0.85	0.96	0.88	0.98	0.91	1.21	0.96 ± 0.05
Liver	11.94	11.26	11.44	13.05	12.61	15.30	12.60 ± 0.61

TL = *T. laurifolia* leaf crude extract

2. List of tables of raw data of the study of effect of the selected fraction (PG fraction) of *T. laurifolia* Lindl. leaf extract on cadmium induced hepatorenal toxicities

Table 21 Body weight (g) of the control rats (group 1)

Day	Rat 1	Rat 2	Rat 3	Mean ± SEM	Day	Rat 1	Rat 2	Rat 3	Mean ± SEM
1	248	214	176	213 ± 21	21	280	304	274	286 ± 9
2	226	206	156	196 ± 21	22	296	324	282	301 ± 12
3	242	234	184	220 ± 18	23	298	330	292	307 ± 12
4	248	244	196	229 ± 17	24	300	334	292	309 ± 13
5	258	250	198	235 ± 19	25	298	330	294	307 ± 11
6	258	258	210	242 ± 16	26	302	338	300	313 ± 12
7	272	270	218	253 ± 18	27	306	332	296	311 ± 11
8	272	270	228	257 ± 14	28	310	340	300	317 ± 12
9	270	276	234	260 ± 13	29	304	334	304	314 ± 10
10	278	286	244	269 ± 13	30	304	346	306	319 ± 14
11	282	290	250	274 ± 12	31	314	340	304	319 ± 11
12	274	290	250	271 ± 12	32	302	342	298	314 ± 14
13	282	306	262	283 ± 13	33	308	334	300	314 ± 10
14	282	306	260	283 ± 13	34	306	342	304	317 ± 12
15	286	312	268	289 ± 13	35	318	354	318	330 ± 12
16	288	314	272	291 ± 12	36	314	346	310	323 ± 11
17	290	312	276	293 ± 10	37	316	350	314	327 ± 12
18	294	320	286	300 ± 10	38	316	352	318	329 ± 12
19	300	332	288	307 ± 13	39	312	350	320	327 ± 12
20	300	328	290	306 ± 11	40	312	354	318	328 ± 13

Table 22 Body weight (g) of the rats treated with 1.0 mg/kg BW CdCl₂ (group 2)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
1	290	270	298	286	252	279 ± 8
2	266	252	268	256	232	255 ± 6
3	282	272	288	292	260	279 ± 6
4	296	284	292	286	268	285 ± 5
5	294	274	292	280	266	281 ± 5
6	304	292	304	296	284	296 ± 4
7	300	280	310	284	278	290 ± 6
8	302	300	318	312	284	303 ± 6
9	304	308	326	320	290	310 ± 6
10	308	308	324	318	282	308 ± 7
11	306	318	330	340	294	318 ± 8
12	316	328	326	338	300	322 ± 6
13	310	326	332	338	300	321 ± 7
14	310	330	336	342	308	325 ± 7
15	316	332	340	342	306	327 ± 7
16	318	336	348	356	306	333 ± 9
17	320	346	344	356	312	336 ± 8
18	324	342	342	352	308	334 ± 8
19	322	342	352	354	318	338 ± 7
20	322	350	352	362	322	342 ± 8

Table 22 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean \pm SEM
21	302	336	334	346	304	324 \pm 9
22	308	338	344	346	304	328 \pm 9
23	300	328	342	340	304	323 \pm 9
24	300	312	350	334	304	320 \pm 10
25	286	302	350	320	312	314 \pm 11
26	280	288	352	312	316	310 \pm 13
27	274	280	352	302	326	307 \pm 15
28	266	272	350	292	320	300 \pm 16
29	264	266	352	280	324	297 \pm 17
30	242	252	350	262	320	285 \pm 21
31	242	258	342	254	298	279 \pm 18
32	240	Died	326	258	302	282 \pm 20
33	254	-	334	Died	302	297 \pm 23
34	240	-	314	-	296	283 \pm 22
35	250	-	312	-	302	288 \pm 19
36	250	-	324	-	306	293 \pm 22
37	248	-	324	-	294	289 \pm 22
38	270	-	338	-	304	304 \pm 20
39	280	-	332	-	302	305 \pm 15
40	296	-	326	-	304	309 \pm 9

Table 23 Body weight (g) of the rats treated with the PG fraction of *T. laurifolia* leaf extract

Day	Rat 1	Rat 2	Rat 3	Mean ± SEM	Day	Rat 1	Rat 2	Rat 3	Mean ± SEM
1	264	214	236	238 ± 14	21	300	248	280	276 ± 15
2	238	198	218	218 ± 12	22	320	250	300	290 ± 21
3	264	212	240	239 ± 15	23	322	250	300	291 ± 21
4	266	212	246	241 ± 16	24	320	250	302	291 ± 21
5	268	222	250	247 ± 13	25	326	258	308	297 ± 20
6	278	228	246	251 ± 15	26	328	258	302	296 ± 20
7	286	240	248	258 ± 14	27	332	274	294	300 ± 17
8	282	240	252	258 ± 13	28	334	276	294	301 ± 17
9	290	250	256	265 ± 12	29	332	280	296	303 ± 15
10	296	250	260	269 ± 14	30	332	282	290	301 ± 16
11	296	256	264	272 ± 12	31	336	282	306	308 ± 16
12	300	252	264	272 ± 14	32	342	288	318	316 ± 16
13	310	260	280	283 ± 15	33	336	290	314	313 ± 13
14	312	266	280	286 ± 14	34	336	290	322	316 ± 14
15	310	270	280	287 ± 12	35	338	298	318	318 ± 12
16	312	270	294	292 ± 12	36	340	290	330	320 ± 15
17	310	272	296	293 ± 11	37	342	296	326	321 ± 13
18	300	276	298	291 ± 8	38	332	292	324	316 ± 12
19	322	264	302	296 ± 17	39	338	296	324	319 ± 12
20	314	258	304	292 ± 17	40	342	294	332	323 ± 15

Table 24 Body weight (g) of the rats pretreated with the PG fraction of *T. laurifolia* leaf before and during treated with CdCl₂ (group 4)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
1	264	258	218	250	290	256 ± 12
2	242	240	194	234	266	235 ± 12
3	262	260	204	256	298	256 ± 15
4	256	262	228	262	302	262 ± 12
5	254	270	236	270	288	264 ± 9
6	250	268	236	268	280	260 ± 8
7	248	276	242	268	276	262 ± 7
8	248	280	244	282	282	267 ± 9
9	242	282	256	282	288	270 ± 9
10	246	288	252	290	282	272 ± 9
11	246	288	256	300	286	275 ± 10
12	244	302	270	302	278	279 ± 11
13	246	300	268	306	274	279 ± 11
14	274	308	256	290	310	288 ± 10
15	288	314	282	310	322	303 ± 8
16	290	310	284	316	320	304 ± 7
17	284	316	292	314	326	306 ± 8
18	280	320	296	312	328	307 ± 9
19	290	320	292	310	314	305 ± 6
20	294	320	300	314	338	313 ± 8

Table 24 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean \pm SEM
21	274	300	280	292	312	292 \pm 7
22	288	306	292	318	326	306 \pm 7
23	280	300	296	316	332	305 \pm 9
24	278	300	300	320	342	308 \pm 11
25	292	306	308	324	352	316 \pm 10
26	296	306	312	320	358	318 \pm 11
27	292	304	312	316	360	317 \pm 12
28	304	306	312	314	358	319 \pm 10
29	308	308	320	330	340	321 \pm 6
30	310	310	310	330	348	322 \pm 8
31	320	318	300	328	340	321 \pm 7
32	320	324	290	330	332	319 \pm 8
33	312	330	290	318	328	316 \pm 7
34	314	320	296	316	322	314 \pm 5
35	322	314	294	314	314	312 \pm 5
36	304	308	290	300	320	304 \pm 5
37	304	318	290	310	330	310 \pm 7
38	308	318	284	306	336	310 \pm 8
39	314	322	288	316	344	317 \pm 9
40	306	324	290	312	340	314 \pm 8

Table 25 Water consumption (mL) of the control rats (group 1)

Day	Rat 1	Rat 2	Rat 3	Mean ± SEM	Day	Rat 1	Rat 2	Rat 3	Mean ± SEM
1	33	40	41	38 ± 3	21	40	41	43	41 ± 1
2	31	39	44	38 ± 4	22	17	33	26	25 ± 5
3	28	31	48	36 ± 6	23	28	29	44	34 ± 5
4	27	28	50	35 ± 8	24	27	28	43	33 ± 5
5	25	30	40	32 ± 4	25	31	31	40	34 ± 3
6	28	25	42	32 ± 5	26	28	24	30	27 ± 2
7	26	31	39	32 ± 4	27	30	33	39	34 ± 3
8	27	28	42	32 ± 5	28	29	29	41	33 ± 4
9	28	34	41	34 ± 4	29	30	26	37	31 ± 3
10	28	32	35	32 ± 2	30	26	31	35	31 ± 3
11	29	35	33	32 ± 2	31	25	24	42	30 ± 6
12	27	38	43	36 ± 5	32	30	32	41	34 ± 3
13	30	35	34	33 ± 2	33	24	28	44	32 ± 6
14	31	29	40	33 ± 3	34	24	26	41	30 ± 5
15	30	30	41	34 ± 4	35	27	41	29	32 ± 4
16	27	27	44	33 ± 6	36	29	29	40	33 ± 4
17	30	29	42	34 ± 4	37	28	28	39	32 ± 4
18	34	29	38	34 ± 3	38	23	26	37	29 ± 4
19	29	25	40	31 ± 4	39	26	32	41	33 ± 4
20	30	29	43	34 ± 5	40	38	22	32	31 ± 5

Table 26 Water consumption (mL) of the rats treated with 1.0 mg/kg BW CdCl₂
(group 2)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
1	17	46	66	46	90	53 ± 12
2	32	48	39	49	51	44 ± 4
3	26	42	35	40	40	37 ± 3
4	22	43	33	44	48	38 ± 5
5	25	48	41	55	44	43 ± 5
6	23	34	33	41	39	34 ± 3
7	21	47	36	46	30	36 ± 5
8	33	38	31	44	34	36 ± 2
9	23	35	29	41	31	32 ± 3
10	24	36	32	37	30	32 ± 2
11	24	35	26	33	31	30 ± 2
12	22	23	33	28	32	28 ± 2
13	31	29	29	30	32	30 ± 1
14	28	28	34	33	30	31 ± 1
15	21	24	32	26	28	26 ± 2
16	26	29	31	33	28	29 ± 1
17	21	26	33	27	30	27 ± 2
18	26	23	35	27	35	29 ± 2
19	20	27	39	27	31	29 ± 3
20	8	46	36	36	61	37 ± 9

Table 26 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean \pm SEM
21	17	22	35	26	33	27 \pm 3
22	10	14	25	30	24	21 \pm 4
23	11	9	31	19	20	18 \pm 4
24	4	8	26	10	28	15 \pm 5
25	5	7	30	18	26	17 \pm 5
26	7	6	31	9	26	16 \pm 5
27	2	4	22	8	22	12 \pm 4
28	4	3	19	7	21	11 \pm 4
29	6	8	24	8	16	12 \pm 3
30	3	5	19	7	6	8 \pm 3
31	23	11	26	9	30	20 \pm 4
32	31	Died	33	3	19	22 \pm 6
33	17	-	20	Died	15	17 \pm 1
34	33	-	17	-	18	23 \pm 4
35	59	-	39	-	25	41 \pm 8
36	47	-	22	-	18	29 \pm 7
37	42	-	42	-	22	35 \pm 5
38	38	-	29	-	22	30 \pm 4
39	45	-	32	-	24	34 \pm 5
40	16	-	9	-	16	14 \pm 2

Table 27 Water consumption (mL) of the rats treated with PG fraction of *T. laurifolia* leaf extract (group 3)

Day	Rat 1	Rat 2	Rat 3	Mean ± SEM	Day	Rat 1	Rat 2	Rat 3	Mean ± SEM
1	31	23	27	27 ± 2	21	43	16	46	35 ± 10
2	39	29	39	36 ± 3	22	28	17	38	28 ± 6
3	13	16	28	19 ± 5	23	22	15	41	26 ± 8
4	20	18	25	21 ± 2	24	28	18	34	27 ± 5
5	21	29	18	23 ± 3	25	21	20	27	23 ± 2
6	22	31	17	23 ± 4	26	26	44	12	27 ± 9
7	19	25	20	21 ± 2	27	23	29	14	22 ± 4
8	22	30	14	22 ± 5	28	23	28	16	22 ± 3
9	26	33	23	27 ± 3	29	24	28	15	22 ± 4
10	21	27	20	23 ± 2	30	21	25	15	20 ± 3
11	22	30	16	23 ± 4	31	27	29	63	40 ± 12
12	25	25	22	24 ± 1	32	17	23	42	27 ± 8
13	20	27	23	23 ± 2	33	23	28	42	31 ± 6
14	23	28	25	25 ± 1	34	21	25	39	28 ± 5
15	24	26	32	27 ± 2	35	18	23	35	25 ± 5
16	18	23	31	24 ± 4	36	21	26	36	28 ± 4
17	10	28	28	22 ± 6	37	16	25	42	28 ± 8
18	39	11	34	28 ± 9	38	23	24	41	29 ± 6
19	23	16	33	24 ± 5	39	23	21	44	29 ± 7
20	38	39	45	41 ± 2	40	36	36	33	35 ± 1

Table 28 Water consumption (mL) of the rats pretreated with the PG fraction of *T. laurifolia* leaf extract before and during treated with CdCl₂ (group 4)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
1	37	25	39	39	55	39 ± 5
2	31	31	12	31	43	30 ± 5
3	6	33	48	6	33	25 ± 8
4	9	27	34	43	8	24 ± 7
5	5	24	31	29	9	20 ± 5
6	9	29	30	34	10	22 ± 5
7	14	33	39	34	25	29 ± 4
8	6	31	40	30	13	24 ± 6
9	8	30	39	28	11	23 ± 6
10	8	42	51	38	9	30 ± 9
11	10	34	49	32	30	31 ± 6
12	9	32	55	40	16	30 ± 8
13	55	24	5	6	34	25 ± 9
14	32	26	56	47	41	40 ± 5
15	27	20	34	44	38	33 ± 4
16	13	38	35	28	45	32 ± 5
17	8	36	34	24	46	30 ± 6
18	20	32	33	13	46	29 ± 6
19	24	33	32	35	53	35 ± 5
20	40	28	39	42	29	36 ± 3

Table 28 (continued)

Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean \pm SEM
21	28	21	36	42	50	35 \pm 5
22	6	25	25	18	36	22 \pm 5
23	12	31	29	26	28	25 \pm 3
24	42	22	28	28	35	31 \pm 3
25	20	18	26	17	32	23 \pm 3
26	26	20	27	15	39	25 \pm 4
27	31	20	22	15	25	23 \pm 3
28	26	19	25	37	13	24 \pm 4
29	28	23	13	30	18	22 \pm 3
30	29	24	10	31	15	22 \pm 4
31	29	27	7	34	7	21 \pm 6
32	23	27	13	26	6	19 \pm 4
33	26	15	21	21	21	21 \pm 2
34	23	9	15	18	10	15 \pm 3
35	16	20	24	12	17	18 \pm 2
36	27	20	18	23	55	29 \pm 7
37	28	20	18	26	32	25 \pm 3
38	27	17	15	25	35	24 \pm 4
39	24	21	21	21	39	25 \pm 3
40	19	36	27	17	28	25 \pm 3

Table 29 Urinary and blood concentrations of the control rats (group 1)

Cd concentrations	Day	Rat 1	Rat 2	Rat 3	Mean ± SEM
Urinary Cd (µg/g Cr)	0	4.88	13.35	30.16	16.13 ± 7.44
	20	0.58	1.12	0.31	0.67 ± 0.24
	40	2.03	0.48	0.77	1.09 ± 0.48
Blood Cd (µg/L)	40	2.21	1.91	2.19	2.10 ± 0.10

Table 30 Urinary and blood concentration of Cd exposure rats (group 2)

Cd concentrations	Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
Urinary Cd (µg/g Cr)	0	1.46	3.89	1.41	3.35	7.29	3.48 ± 1.07
	20	0.40	0.84	2.00	1.72	2.72	1.54 ± 0.41
	40	122,899.63	Died	52,616.08	Died	104,053.37	93,189.69 ± 21,028.54
Blood Cd (µg/L)	40	2,238.90	Died	3,286.80	Died	3,160.20	2,895.30 ± 330.62

Table 31 Urinary and blood concentrations of rats treated with the PG fraction of *T. laurifolia* leaf extract for 40 days (group 3)

Cd concentrations	Day	Rat 1	Rat 2	Rat 3	Mean ± SEM
Urinary Cd (µg/g Cr)	0	2.97	5.63	2.68	3.76 ± 0.94
	20	1.82	1.62	9.32	4.25 ± 2.54
	40	0.32	1.80	0.39	0.84 ± 0.48
Blood Cd (µg/L)	40	1.99	2.45	2.69	2.37 ± 0.21

Table 32 Urinary and blood Cd concentrations of rats pretreated with the PG fraction of *T. laurifolia* leaf extract before and during treated with CdCl₂ (group 4)

Cd concentrations	Day	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
Urinary Cd (µg/g Cr)	0	1.02	2.92	7.58	4.11	2.63	3.65 ± 1.10
	20	2.37	1.87	0.56	2.86	1.24	1.78 ± 0.41
	40	74,867.50	51,790.12	82,238.68	85,000.00	231,965.81	105,172.42 ± 32,175.01
Blood Cd (µg/L)	40	4,305.00	2,814.90	3,527.40	2,575.80	3,049.50	3,254.52 ± 305.56

Copyright © by Chiang Mai University
All rights reserved

Table 33 Organ's weight (g) of the control rats (group 1)

Organs	Rat 1	Rat 2	Rat 3	Mean ± SEM
Right kidney	0.92	1.35	1.11	1.13 ± 0.13
Left kidney	0.98	1.33	1.21	1.17 ± 0.10
Liver	9.73	10.83	9.64	10.07 ± 0.39

Table 34 Organ's weight (g) of the Cd treated rats (group 2)

Organs	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
Right kidney	1.36	1.22	1.46	1.15	1.44	1.33 ± 0.06
Left kidney	1.38	1.25	1.32	1.06	1.48	1.30 ± 0.07
Liver	15.51	10.03	14.94	9.19	14.90	12.92 ± 1.36

Table 35 Organ's weight (g) of rats treated with the PG fraction of *T. laurifolia* leaf extract (group 3)

Organs	Rat 1	Rat 2	Rat 3	Mean ± SEM
Right kidney	1.30	1.18	0.96	1.14 ± 0.10
Left kidney	0.97	1.10	0.98	1.02 ± 0.04
Liver	10.14	10.20	10.85	10.40 ± 0.23

Table 36 Organ's weight (g) of rats pretreated with the PG fraction of *T. laurifolia* leaf extract before and during treated with CdCl₂ (group 4)

Organs	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± SEM
Right kidney	1.55	1.40	1.24	1.35	1.34	1.37 ± 0.05
Left kidney	1.44	1.37	1.24	1.30	1.51	1.37 ± 0.05
Liver	14.78	14.26	14.34	14.46	16.43	14.85 ± 0.40



APPENDIX C

PUBLISHED ABSTRACT AND ARTICLE

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

การดื่มน้ำสกัดใบรางจืดช่วยป้องกันการเกิดพิษของแคดเมียมต่อไตหนูขาว

พลอยไพลิน ฉัตตะวิริยะ¹, ัญญฐิตา หมอกเมฆ¹, นิรัชร์ เลิศประเสริฐสุข¹ และ วีระวรรณ เรืองยุทธิการณ์^{2*}

¹ ภาควิชานิติเวชศาสตร์, คณะแพทยศาสตร์, มหาวิทยาลัยเชียงใหม่, จังหวัดเชียงใหม่, ประเทศไทย

² ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์, มหาวิทยาลัยเชียงใหม่, จังหวัดเชียงใหม่, ประเทศไทย

บทคัดย่อ

รางจืด หรือชื่อทางวิทยาศาสตร์คือ *Thunbergia laurifolia* Lindl. เป็นสมุนไพรไทยที่มีสรรพคุณในการถอนพิษต่างๆ ปัจจุบันพบว่าการตกค้างแคดเมียมในสิ่งแวดล้อมเป็นปัญหาสำคัญของประเทศ โดยพบมากบริเวณอำเภอแม่สอด จังหวัดตาก ประชากรที่อาศัยอยู่ในบริเวณนี้เป็นกลุ่มที่มีความเสี่ยงสูงต่อการได้รับแคดเมียมแบบเรื้อรังอันอาจก่อให้เกิดความผิดปกติต่อไตและกระดูกได้ ดังนั้นเพื่อศึกษาว่าสารสกัดใบรางจืดสามารถป้องกันการเกิดพิษของแคดเมียมต่อไต ได้หรือไม่ งานวิจัยนี้ได้ใช้หนูขาวเพศผู้ 12 ตัว แบ่งเป็นสองกลุ่ม ๆ ละ 6 ตัว โดยกลุ่มแรกเป็นกลุ่มควบคุมให้น้ำกลั่นเป็นน้ำดื่ม 20 วันก่อนการให้สารละลายแคดเมียมคลอไรด์ความเข้มข้น 1.0 มิลลิกรัมต่อกิโลกรัม โดยการฉีดเข้าใต้ผิวหนังเป็นเวลา 20 วัน กลุ่มที่สองให้สารสกัดใบรางจืด 0.1 มิลลิกรัมต่อมิลลิลิตรในน้ำดื่มก่อนการให้สารละลายแคดเมียมคลอไรด์ 20 วัน และให้ต่อเนื่องไปพร้อมกับการได้รับสารละลายแคดเมียมคลอไรด์ เช่นเดียวกับหนูกลุ่มที่หนึ่ง ผลการศึกษาพบว่าหนูในกลุ่มที่ได้รับสารสกัดใบรางจืดมีน้ำหนักตัวสูงกว่าหนูกลุ่มควบคุม มที่ได้รับสารละลายแคดเมียมคลอไรด์เพียงอย่างเดียวอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) อย่างไรก็ตามแม้ว่าสารสกัดใบรางจืดไม่สามารถลดปริมาณแคดเมียมที่สะสมในเลือดและปัสสาวะของหนูขาวที่ได้รับแคดเมียมได้ แต่สามารถช่วยป้องกันความผิดปกติที่ตรวจพบทางจุลพยาธิวิทยาที่ไตของหนูขาวได้ ผลการวิจัยครั้งนี้แสดงให้เห็นว่าสารสกัดใบรางจืดสามารถป้องกันการเกิดพิษของแคดเมียมต่อไตหนูขาวได้ และยังคงพิษที่ระบบอื่นๆของร่างกาย ผลการศึกษานี้จึงอาจเป็นประโยชน์สำหรับการนำไปประยุกต์ใช้ลดความเป็นพิษของแคดเมียมในกลุ่มประชากรที่อาศัยอยู่ในบริเวณที่มีความเสี่ยงจากการได้รับแคดเมียมปนเปื้อนในอาหารและน้ำดื่ม

คำสำคัญ: แคดเมียม, รางจืด, พิษต่อไต, หนูขาว

*Corresponding author: รศ. ดร. วีระวรรณ เรืองยุทธิการณ์

สาขาวิชาพิษวิทยา ภาควิชานิติเวชศาสตร์

คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ 50200

Email: werawan52@gmail.com

Drinking *Thunbergia laurifolia* Lindl. Leaf Extract Helps Prevent Renal Toxicity Induced by Cadmium in Rats

Chattaviriya P¹, Morkmek N¹, Lertprasertsuke N², Ruangyuttikarn W^{1*}

¹Division of Toxicology, Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Thailand.

²Department of Pathology, Faculty of Medicine, Chiang Mai University, Thailand

ABSTRACT

Thunbergia laurifolia Lindl. (TL) or “Rang Jerd” is a Thai herbal medicine used as an antidote for several poisonous agents. Cadmium (Cd) is an environmental pollutant in Mae Sot district, Tak province, Thailand. Chronic exposure to Cd causes renal and bone dysfunction in exposed human populations. In order to investigate whether TL leaf extract could prevent Cd induced renal toxicity, two groups of male Wistar rats, six rats each were injected with cadmium chloride solution (CdCl₂) at the concentration of 1.0 mg/kg BW for 20 days. Group 1 was serving as control and fed distilled water for 20 days before Cd administration while group 2 was administered TL leaf extract at 0.1 mg/ml in drinking water for 20 days before and during injection of CdCl₂ at the same concentration as in group 1. The body weight of rats pretreated with TL leaf extract before Cd exposure in group 2 was significantly ($p < 0.05$) greater than that of rats given with Cd alone. However, TL leaf extract did not reduce the levels of Cd in blood and urine of the Cd exposed rats. The rats in group 2 did not show histopathological changes in the kidney that were observed in the control group which given Cd alone. Therefore, this study demonstrated that TL leaf extract can protect against Cd induced structural damage in rat kidney and also reduce other systemic toxicity. TL leaf extract may be useful for reducing Cd toxicity in human populations exposed to Cd in food and drinking water.

Keywords: cadmium, *Thunbergia laurifolia* Lindl., renal toxicity, rats

*Corresponding author: Assoc. Prof. Dr. Werawan Ruangyuttikarn,

Department of Forensic Medicine, Faculty of Medicine,

Chiang Mai University, Chiang Mai 50200, Thailand

Email: werawan52@gmail.com

INTRODUCTION

Thunbergia laurifolia Lindl. (TL) is a herbal medicine used as an antidote for several poisonous agents in Thai traditional medicine. The Thai name for this plant is "Rang Jerd" and its English name is "Babbler's Bill".¹ It is a shrub with small oblong or ovate leaves and bluish-purple flowers. It can be divided into three types designated by flower color: white, yellow, or purple. Purple varieties are believed to possess compounds that deliver health benefits particularly from materials of the stem, root and leaves.² It is commonly consumed as herbal tea. Various parts of the plant have been used for various medicine purposes e.g. aqueous extracts of fresh leaves, dried leaves, dried root and bark were used as antidote for insecticide³⁻⁵, ethyl alcohol, arsenic and strychnine poisoning.⁶ The dried root was also used as an anti-inflammatory⁷ and antipyretic agent.⁸ The aqueous extract from leaves is reported to be harmless to rats with no behavioral effects.⁹ Moreover, the crude extract is reported to have no cytotoxicity and high antioxidant activity.¹⁰

Cadmium (Cd) is a naturally occurring minor element, one of the metallic components in the earth's crust and oceans, and present everywhere in our environment. It is an important industrial and environmental pollutant that can affect multiple organ systems and has a biological half-life of about 30 years in humans.¹¹ Human Cd exposure is mostly from food and water as well as cigarette smoke and contaminated air. Human and animal studies have shown that pulmonary absorption is higher than gastrointestinal absorption; approximately 50% of inhaled Cd is absorbed in the blood circulation, but gastrointestinal absorption of Cd is reported to be only 3-8% of the ingested load.¹² The absorption of Cd in humans depends on physiological status (age, dietary intake, iron storage, gender and smoking habits). High ingestion of Cd causes acute gastroenteritis.¹³ Long-term occupational exposure to Cd causes severe chronic effects,

predominantly in the lung and kidney. Cd mainly accumulates in the kidney, where it causes generalized dysfunction of the proximal tubules, characterized by polyuria and increase in urinary excretion of low-molecular-weight proteins, electrolytes, amino acids and glucose¹⁴ as well as histopathological changes including proximal tubular cell degeneration, interstitial inflammation and fibrosis, glomerular swelling, atrophic and pyknotic nuclei, vacuoles, apoptosis and necrosis.^{15,16}

Recently in Thailand, environmental pollution of Cd has been discovered in Mae Sot district, Tak province.¹⁷ Cd, presumably released from zinc mining in Mae Sot area, has contaminated water and soil and has entered the human food chain by uptake into rice grown in the district. A large-scale health impact survey in the district¹⁸ reported that residents had high level of Cd exposure with 9.2% of subjects having urinary Cd between 5-10 $\mu\text{g/gCr}$ and 2.5% with urinary Cd >10 $\mu\text{g/gCr}$, compared to the maximum recommended level of the urinary Cd of 2 $\mu\text{g/gCr}$.¹⁹

Other studies of the health of Mae Sot residents have found high levels of renal dysfunction, particularly in farmers who ate their own, locally grown rice, each day²⁰ and accelerated bone resorption due to impaired calcium reabsorption in the renal tubules.²¹

However, there are no specific treatments for minimizing Cd toxicity in this exposed population. There is also no report of the use of TL leaf extract to treat Cd induced renal toxicity. Therefore, our study tested the hypothesis that TL leaf extract supplied in drinking water to rats, may reduce renal toxicity induced by high exposure to Cd.

MATERIALS AND METHODS

Obtaining of Thunbergia laurifolia Lindl. leaves

Fresh, mature leaves of *Thunbergia laurifolia* Lindl. were collected from Ob Khan National Park, Hangdong district, Chiang Mai province, Thailand and identified at the Queen Sirikit Botanic Garden, Mae Rim district, Chiang Mai province.²² The leaves were washed with tap water, dried and ground to powder, then stored in amber glass bottles at room temperature before extraction.

Extraction of TL leaves

T. laurifolia leaf powder was soaked in boiled distilled water (1:10 w/v) for 1 hr then filtered through three layers of gauze followed by Whatman No.4 filter paper. The filtrate was lyophilized and stored in a desiccator at 4°C. The extract was redissolved in distilled water to desired concentrations just prior to use.

TL leaf extract dosage preparation

The dosage of the TL leaf extract for rats was calculated to approximate a human dosage from drinking 3 cups of TL tea per day. This was estimated as 0.1 mg/ml of TL leaf extract supplied in distilled water 120 ml, which was the rat daily drinking water ration.

Animal treatment

Twelve adult male Wistar rats (200-250 g) were used in this study. The study protocol was approved by the Animal Ethics Committee, Faculty of Medicine, Chiang Mai University. The rats were acclimatized under controlled experimental conditions of room temperature of 25±2°C with 12 hr light and 12 hr dark cycle and humidity of 50±10% for one week before experiments. They had free access to drinking water and standard rodent pellets throughout the experiment.

The rats were divided into two groups of six. The positive control group (group 1)

were provided distilled water without TL leaf extract for 20 days, then treated with daily subcutaneously injection of CdCl₂ solution (1.0 mg/kg) in isotonic saline for 20 more days. The treatment group (group 2) was provided TL leaf extract (0.1mg/ml) in drinking water for 20 days prior to the commencement of the CdCl₂ treatment (1.0 mg/kg) for 20 more days and the TL leaf extract supply in drinking water was continued throughout the experiment.

The body weight and water consumption of each rat was measured daily. Twenty four hour urine samples were collected using metabolic cages from each rat on three occasions; Day 0 or 1; Day 20; and Day 40. At the end of experiment (Day 40), all rats were anesthetized with sodium phenobarbital and blood was taken via cardiac puncture. The kidneys were removed, washed with normal saline, weighed and kept in neutral-buffered formalin solution for histopathological examination.

Quantification of urinary creatinine and cadmium

The creatinine level in rat urine was measured using Jaffe reaction²³ with spectrophotometer at 500 nm. Urinary Cd concentrations were measured by graphite furnace atomic absorption spectrometer (GFAAS) with Zeeman-GFAAS background correction (Varian SpectraA800Z). The Cd standard curve was established using standard Cd solution. The standard solution was mixed with a modifier and diluted to 1, 3 and 5 µg/l. The modifier was used as a blank. The urine sample was mixed with modifier before analysis and put into an autosampler under the previous described of the GFAAS standardized condition.²⁴

Quantification of blood cadmium

Blood Cd concentrations were also measured by GFAAS with Zeeman-GFAAS background correction but the sample

preparation was different from the process prepared for urinary Cd measurement. Five hundred microliters of whole blood was mixed with 1 ml of 5% nitric acid in the micro-test tube, then vigorously mixed for 30 seconds and held at room temperature for 1 hr. The micro-test tube was centrifuged at 12,000 rpm for 5 min at 20°C, before removing the supernatant to a new micro-test tube and centrifuged again at 12,000 rpm for 5 min. The supernatant was injected onto the GFAAS with the developed temperature program.²¹

Histopathological examination

The kidneys were perfused, taken out and washed with normal saline for removing excessive blood. They were dissected and fixed in 10% neutral-buffered formalin. Representative sections were selected for histopathological processing. The tissues were embedded in paraffin blocks and cut as five micron sections, stained with hematoxylin-eosin and examined under light microscope.²⁵

Statistical analysis

Data were expressed as mean \pm standard error of mean (SEM) and compared between groups using Student's t-test. Differences at $p < 0.05$ were considered significant.

RESULTS

Rat body weight

Body weight of Cd treated rats in both groups of the experiments, without (group 1) and with (group 2) pretreatment with TL leaf extract in drinking water as TL tea, were significantly different ($p < 0.05$) from day 21 to day 40 as shown in Figure 1. The results showed that pretreatment with TL leaf extract in drinking water can help reduce weight loss due to cadmium toxicity.

Water consumption

During days 1-20, both groups consumed similar volumes of water per day. After Cd treatment commenced on day 20, both group consumed less water. However, the group provided with TL leaf extract consumed significantly more water than rats without the TL leaf extract ($p < 0.05$, Fig. 2).

Urinary and blood cadmium concentrations

Both groups of rats had extremely high urine Cd levels so the TL leaf extract had no effect on urinary Cd concentration (Table 1).

Blood cadmium concentration

The similar blood Cd concentrations in both groups of rats after 20 days exposure to Cd, indicated that *T. laurifolia* Lindl. leaf extract did not affect the concentrations of Cd in blood (Table 2).

Histopathological examination

Light microscopic examination of histopathology of the rat kidneys indicated that *T. laurifolia* Lindl. leaf extract could protect kidney from damage by Cd. The kidney cortex of rats exposed to Cd without TL leaf extract (Fig. 3B) 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 kidneys exposed to Cd and TL leaf extract (Fig. 3C) was no different from glomeruli of the normal rats (no any treatment) in Figure 3A (the result shown in Figure 3A was from our previous study with normal rat without any treatment). These plates clearly demonstrate that the kidney tubule and glomeruli structure was preserved in rats exposed to both Cd and TL leaf extract.

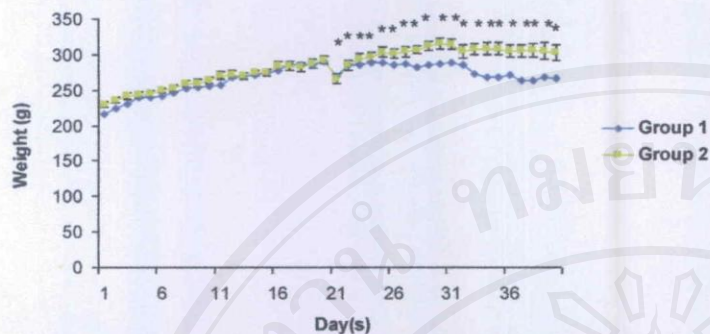


Figure 1. Rats exposed to Cd after day 20 (group 1) suffered weight loss but the loss was much more limited in rats which consumed drinking water containing TL leaf extract (group 2). All values are mean \pm SEM of 6 rats. An * indicate statistically significant differences among the two groups ($p < 0.05$).

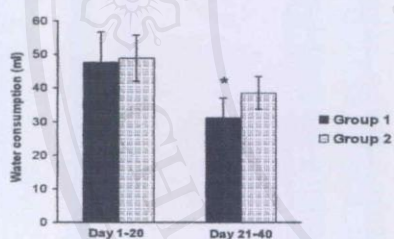


Figure 2. Water consumption of male Wistar rats in Cd treated (group 1) and pretreatment with TL leaf extract in drinking water (group 2) before (Day 1-20) and after Cd treatment (Day 21-40).

DISCUSSION

Teeyakasem et al.²⁰ reported high urinary Cd concentrations in Mae Sot residents and found they were at high risk of renal dysfunction due to chronic exposure to Cd in food and water. Our results showed rats exposed to Cd by daily subcutaneous injection for 20 days had high levels of Cd in the blood, but exposure to TL extract before and during the Cd exposure did not affect the blood or urinary Cd concentrations. Therefore, the potential use of TL tea as a medicinal herb to reduce the affect of Cd exposure in people like those living at Mae Sot district appears limited.

However, the evidence from histopathology was that TL tea may protect kidney tissue from damage caused by Cd exposure. These results are similar to previous investigations on rats dosed with Cd by oral ingestion in water for 6 weeks at 50 mg/l, which reported proximal tubular damage and glomerular swelling.¹⁵ Prozialeck et al.¹⁴ also observed proximal tubular epithelial cell with irregular shape and gaps between the cells in rats administration of Cd 0.6 mg/kg for 6 weeks. The concentrations of CdCl₂ and TL leaf extract used in this study works well to see the protective of the TL tea from Cd toxicity.

The protective effect of the TL leaf extract on kidney tissues may be due to antioxidant properties of the phenolic compounds or other anti-oxidants or anti-inflammatory constituents in the leaves.^{10,26,27}

We conclude that TL leaf extract can prevent or reduce Cd induced structural damage in the kidney of rats. The major chemical constituents of the TL leaf extract will be isolated and identified then tested for Cd protective properties in rats to elucidate whether they can also help prevent or reduce toxicity from high Cd exposure. The results will be very applicable advantage to unavoidable of Cd exposure population in the polluted area such as Mae Sot district.

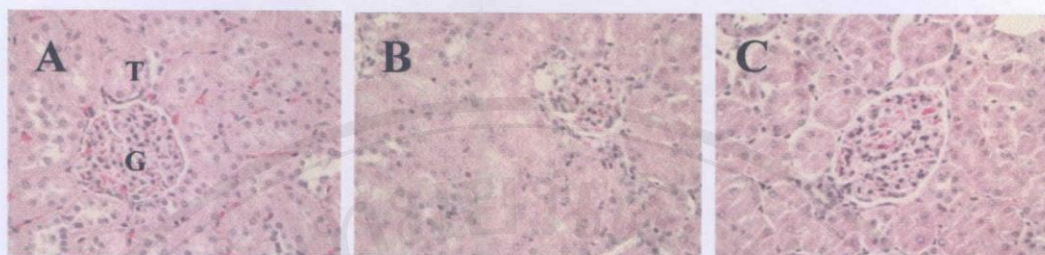


Figure 3. Histopathology (H&E, x400) of tubule (T) and glomeruli (G) in the kidney cortex of a normal, untreated rat (A); a rat exposed to cadmium chloride at 1.0 mg/kg for 20 days (B); and a rat exposed to both *T. laurifolia* leaf extract and CdCl₂ (C).

Table 1. Urinary Cd concentration in rats exposed to Cd with (group 2) and without (group 1) exposure to *T. laurifolia* leaf extract.

Rats	U-Cd		
	Day 0 (µg/gCr)	Day 20 (µg/gCr)	Day 40 (µg/gCr)
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

Table 2. Comparison of blood concentrations of rats treated with Cd only (group 1) and the concentrations of rats pretreatment with *T. laurifolia* Lindl. leaf 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

ACKNOWLEDGEMENT

The authors thank to Dr. Peter R. Hawkins for proofreading the manuscript and for his valuable comments. This study was supported by the Faculty of Medicine Endowment Fund, Faculty of Medicine, Chiang Mai University.

REFERENCES

- Thongsaard W, Marsden CA, Morris P, Prior M, Shah YB. Effect of *Thunbergia laurifolia*, a Thai natural product used to treat drug addiction, on cerebral activity detected by functional magnetic resonance imaging in the rat. *Psychopharmacology* 2005; 180: 752-60.
- Oonsivilai R, Cheng C, Bomser J, Ferruzzi M, Ningsanond S. Phytochemical profiling and phase II enzyme-inducing properties of *Thunbergia laurifolia* Lindl. (RC) extracts. *J Ethnopharmacol* 2007; 114: 300-6.
- Tejasen P, Thongthapp C. The study of the insecticide antitoxicity of *Thunbergia laurifolia* Linn. *Chiang Mai Bulletin*. 1980; 19: 105-14.

4. Ussanawarong S, Thesiri T. Effect of *Thunbergia Laurifolia* Linn. on detoxication of parathion in rat. *Khon Kaen Univ Res J*. 2000; 6: 3-13.
5. Ussanawarong S, Thesiri T, Mahakunakorn T, Parasupattana S. Effect of *Thunbergia Laurifolia* Linn. on detoxication of paraquat. *Khon Kaen Univ Res J* 2001; 5: 11-7.
6. Sunyapridakul L. Pharmacologic neuromuscular blocking effect of Rang Jert (*Thunbergia laurifolia* Linn.). Proceedings of the 4th Asian Symposium on edical Plants Species, Bangkok, Thailand, 1980: p 132.
7. Charumane S, Vejabhikul S, Taesotikul T, Netsingha W, Sirisaad P, Leelapornpisit P. Development of Topical Anti-inflammatory Preparations from *Thunbergia laurifolia* Linn. Phase 1 Research Report. Faculty of Pharmacy. Chiangmai University Chiangmai Thailand, 1998.
8. Chamreondarassame B. The effect of Rang Jert leaves on the body temperature. Thesis of Chiang Mai University, 1978.
9. Wisitpongpan W, Ruangyuttikarn W, Rujjanavet C. Toxicity tests of Rang Jert (*Thunbergia laurifolia* Linn.) leaves aqueous extracts in rats. *Thai J Phytopharm* 2003; 10(2): 23-36.
10. Oonsivilai R, Ferruzzi MG, Ningsanond S. Antioxidant activity and cytotoxicity of Rang Chuet (*Thunbergia laurifolia* Lindl.) extracts. *As J Food Ag-Ind* 2008; 1(02): 116-28.
11. Haouem S, Hmad N, Najjar MF, Hani AE, Sakly R. Accumulation of cadmium and its effects on liver and kidney functions in rats given diet containing cadmium-polluted radish bulb. *Exp Toxicol Pathol* 2007; 59: 77-80.
12. Chowdhury BA, Friel JK, Chandra RK. Cadmium-Induced Immunopathology Is Prevented by Zinc Administration in Mice. *J Nutr* 1987; 117: 1788-94.
13. Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Public Health Service. Toxicological Profile for Cadmium. Atlanta, Geneva, 1998.
14. Prozialeck WC, Edwards JR, Vaidya VS, Bonventre JV. Preclinical evaluation of novel urinary biomarkers of cadmium nephrotoxicity. *Toxicol Appl Pharm* 2009; 238: 301-5.
15. Aughey E, Fell GS, Scott R, Black M. Histopathology of early effects of oral cadmium in the rat kidney. *Environ Health Persp* 1984; 54: 153-61.
16. Jihen EH, Imed M, Fatima H, Abdelhamid K. Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: Histology and Cd accumulation. *Food Chem Toxicol* 2008; 46: 3522-7.
17. Simmon RW, Pongsakul P, Saiyasitpanich D, Klinphoklap S. Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: implications for public health. *Environ Geochem Hlth* 2005; 27: 501-11.
18. Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, Krintratun S, Padungtod C. Cadmium-exposed population in Mae Sot District, Tak Province: 1. prevalence of high urinary cadmium levels in the adults. *J Med Assoc Thai* 2007; 90(1): 143-8.

19. WHO, "Environmental health criteria 134: Cadmium", World health Organization, Geneva, 1992.
20. Teeyakasem W, Nishijo M, Honda R, Satarug S, Swaddiwudhipong W, Ruangyuttikarn W. Monitoring of cadmium toxicity in a Thai population with high-level environmental exposure. *Toxicol Lett* 2007; 169: 185-95.
21. Nambunmee K, Honda R, Nishijo M, Swaddiwudhipong W, Nakagawa H, Ruangyuttikarn W. Bone resorption acceleration and calcium reabsorption impairment in a Thai population with high cadmium exposure. *Toxicol Mech Method* 2010; 20(1): 7-13.
22. Chaityasing K. Effect of *Thunbergia laurifolia* Lindl. Leaf extract on methomyl-induced cholinesterase inhibition. Thesis of Chiang Mai University, 2005.
23. Lauhachinda B, Heuseugcharern A. Chemistry, Clinical-Laboratory manuals. Chiang Mai, Tanabun Printing, 2004: 197-207.
24. Thongnoon S. Lymphocyte micronucleus induction by lead, cadmium and particulate matters in Chiang Mai ambient air. Thesis of Chiang Mai University, 2004.
25. Singhatong S. Toxicity and oxidative stress after subchronic low carbon monoxide exposure in rats. Thesis of Chiang Mai University, 2004.
26. Srida C, Hankete J, Khunkitti W, Aromdee C, Pese M. Antioxidant activity of *Thunbergia laurifolia* ethanolic extract. *Thai J. Pharm. Sci.* 2002; 26.
27. Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. *Planta Med* 1991; 57(1): 1-7.

CURRICULUM VITAE

Name Ploypailin Chattaviriya

Date of birth April 9, 1987

Education

2004 High school
Prince Royal College, Chiang Mai

2008 Bachelor of Science (Medical Technology)
Faculty of Associated Medical Sciences,
Chiang Mai University

Publication

1. **Chattaviriya P**, Morkmek N, Lertprasertsuke N, Ruangyuttikarn W. Drinking *Thunbergia laurifolia* Lindl. leaf extract helps prevent renal toxicity induced by cadmium in rats. *Thai J Toxicol* 2010; 25(2): 124-32.
2. Morkmek N, **Chattaviriya P**, Lertprasertsuke N, Chuncharunee S, Ruangyuttikarn W. Detoxification of cadmium induced renal and hepatic injuries in rats by *Thunbergia laurifolia* Lindl. leaf extract. *Thai J Toxicol* 2010; 25(2): 115-23.

Oral Presentation

Ploypailin Chattaviriya. Reduction of cadmium toxicity in rats by drinking *Thunbergia laurifolia* Lindl. leaf extract. Presented at the Department of Forensic Medicine Research Seminar VIII, Faculty of Medicine, Chiang Mai, Thailand, August 24, 2011

Poster Presentation

Chattaviriya P, Morkmek N, Lertprasertsuke N, Ruangyuttikarn W. Drinking *Thunbergia laurifolia* Lindl. leaf extract helps prevent renal toxicity induced by cadmium in rats. Presented at The 3rd National Conference in Toxicology “Critical Issue in Toxicology for Thailand Development”. The IMPACT Exhibition and Convention Center, Nonthaburi, Thailand, November 25-26, 2010

Professional experience (attending conference / seminar/ special lecture)

Date	Title	Place
November 2-4, 2009	The 50 th Anniversary Academic Conference, Faculty of Medicine, Chiang Mai University	The Empress International Convention Center, Chiang Mai, Thailand
December 17-18, 2009	The 2 nd National Conference in Toxicology “ The Challenge in the 21 st Century”	Miracle Grand Convention Hotel, Bangkok, Thailand
December 21-25, 2009	Special lecture of Cellular and Molecular Toxicology by Assoc. Prof. Dr. Nongnit Laytragoon-Lewin from Dept. of Oncology, Rudbeck Laboratory, Uppsala University Hospital, Sweden	Dept. of Forensic Medicine, Fac. of Medicine, Chiang Mai University, Thailand
January 12, 2010	Special seminar in infection & immunity and translational medicine hosted by Dept. of Microbiology, Fac. of Medicine and Fac. of Associated Medical Sciences	Dept. of Microbiology Fac. of Medicine Chiang Mai University, Thailand

Date	Title	Place
January 14, 2010	Department Research Seminar VII	Dept. of Forensic Medicine, Fac. of Medicine, Chiang Mai University, Thailand
October 7, 2010	The special seminar of “Laboratory Safety and Economics”	UNISERV Chiang Mai University, Thailand
November 25-26, 2010	The 3 rd National Conference in Toxicology “Critical Issue in Toxicology for Thailand Development”	IMPACT Exhibition and Convention Center, Nonthaburi, Thailand
December 13-21, 2010	Hands on workshop on writing a scientific paper for international standard journal	Dept. of Forensic Medicine, Fac. of Medicine, Chiang Mai University, Thailand

Date	Title	Place
April 28-29, 2011	Special lecture on Global Health Impact of Exposure to Cadmium in Food and Cigarette Smoke and Cadmium and Liver Effects: Cytochrome P450 Enzymes and Cancer by Dr. Soisungwan Satarug from National Research Centre for Environmental Toxicology, The University of Queensland, Australia	Dept. of Forensic Medicine, Fac. of Medicine, Chiang Mai University, Thailand
June 16-17, 2011	Forensic Toxicology Network Seminar “Homicidal poisoning and Special Cases in Forensic Toxicology”	Furama Hotel, Chiang Mai University, Thailand
August 24, 2011	Department Research Seminar VIII	Dept. of Forensic Medicine, Fac. of Medicine, Chiang Mai University, Thailand