การขจัดพิษของแคดเมียมที่เหนี่ยวนำให้ไตและตับหนูขาวบาดเจ็บด้วยสารสกัดใบรางจืด

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บทคัดย่อ

งานวิจัยนี้ได้ใช้ *Thunbergia laurifolia* Lindl. หรือรางจืด ซึ่งเป็นพืชสมุนไพรที่มีสรรพคุณระบุในตำราแพทย์แผนไทยว่าสามารถแก้พิษของสารพิษได้หลายชนิด มาทำการทดสอบลดพิษที่ไตและตับของหนูขาวหลังการถูกเหนี่ยวนำให้เกิดพิษด้วยสารละลายแคดเมียมคลอไรด์โดยให้สารสกัดใบรางจืดแก่หนูขาวเพศผู้ขนาด 125 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม โดยการกรอกทางปาก ก่อนและหลังการให้แคดเมียมคลอไรด์ 1.2 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัมโดยการฉีดเข้าใต้ผิวหนังทุก 5 วัน นาน 4 สัปดาห์ และเก็บปัสสาวะและเลือดของหนูขาวเพื่อตรวจหาปริมาณแคดเมียมโดยใช้เครื่องวิเคราะห์กราไฟท์เฟอเนสอะตอมมิกแอบซอบชัน สเปกโตรมิเตอร์ หลังจากนั้นนำไตและตับไปตรวจทางพยาธิวิทยา ผลการทดลองพบว่าสารสกัดใบรางจืดที่ให้ไม่สามารถช่วยให้หนูขาวที่ได้รับแคดเมียมรอดตายได้ อย่างไรก็ตามลักษณะภายนอกและพฤติกรรมที่ผิดปกติของหนูขาวลดลงเมื่อได้รับสารสกัดใบรางจืด หรือตรวจพบความผิดปกติได้น้อยกว่าหนูขาวที่ได้รับแคดเมียมเพียงอย่างเดียว องค์ประกอบของสารในใบรางจืดได้ทำการวิเคราะห์ด้วยเครื่องนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโกปี พบว่าใบรางจืดมีสารประกอบ กลุ่มอโรมาติก, เฮกซิล และกลุ่มกลูโคไซด์ ผลการวิจัยครั้งนี้แสดงให้เห็นว่าสารสกัดใบรางจืดสามารถช่วยลดพิษของแคดเมียมได้แต่ยังไม่สามารถสรุปได้ชัดเจนเนื่องจากมีการตายของหนูขาวเกิดขึ้นระหว่างการทดลอง ดังนั้นการทดลองครั้งต่อไปจะลดขนาดของแคดเมียมที่ให้กับหนูขาวและเปลี่ยนวิธีการให้สารสกัด

คำสำคัญ: แคดเมียม, การบาดเจ็บของไตและตับ, รางจืด

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Detoxification of Cadmium Induced Renal and Hepatic Injuries in Rats by *Thunbergia laurifolia* Lindl. Leaf Extract

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ABSTRACT

Cadmium can damage kidney and liver cells. *Thunbergia laurifolia* Lindl. is a herb used as an antidote for several poisonous agents in Thai traditional medicine. This study tested the effectiveness of the herb to prevent renal and hepatic injuries induced by cadmium chloride ($CdCl_2$). Male Wistar rats were fed 125 mg/kg of the leaf extract before and after administration of 1.2 mg/kg of $CdCl_2$ solution subcutaneously for 5 days/week for 4 weeks. Blood and urinary samples were collected for quantification of cadmium concentrations using graphite furnace atomic absorption spectrometer. The kidneys and livers were removed and examined for histopathological changes. The results showed that the leaf extract given to rats orally did not prevent mortality in rats exposed to cadmium. However, abnormal appearance and behaviour was less in rats fed the leaf extract prior to cadmium exposure than in those fed leaf extract after cadmium exposure. The constituents of the extract were identified as aromatic, hexyl and glucoside compounds by nuclear magnetic resonance spectroscopy. The *T. laurifolia* leaf extract may reduce some effects of cadmium toxicity, but this conclusion is uncertain due to the high mortality rate of the rats in these experiments. Future trials will use a lower cadmium dosage and alternative routes of treating the animals with the leaf extract.

Keywords: cadmium, renal and hepatic injuries, *Thunbergia laurifolia* Lindl.

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INTRODUCTION

Environmental contamination by cadmium (Cd) is a subject of serious international concern as the metal can enter the food chain and be bioaccumulated, endangering human health.[1] The main pathways of Cd exposure to humans are by inhalation of particles or fumes during industrial operations or in cigarette smoke and by ingestion of Cd in food or water.[1-4] The extremely long biological half-life of Cd essentially makes it a cumulative toxin in the liver and kidney which makes up the bulk of total body burden.[5] It has been reported that after absorption, Cd is taken up by hepatocytes and then circulates in blood as a metallothionein complex (Cd-Mt). Because of its small molecular size, Cd-Mt can pass easily through the glomerular membrane and be taken up by renal tubular cells. The methallothionein is then catabolized releasing Cd^{+2} into the cytoplasm. This induces synthesis of new methallothionein molecules, which in turn, bind and retain Cd in the kidney for a long period of time[6,7] causing toxicity.

In the Mae Sot District, Tak Province of Thailand, farmers irrigating crops using water Mae Tao and Mae Ku creeks, which have a zinc mine in the catchment, have been exposed to Cd contaminated rice and water over a long period of time. An epidemiological study revealed that persons who mainly consumed rice grown in contaminated fields around Mae Sot had higher urinary Cd than those who did not.[8,9] Cd intoxication has been managed by metal chelating compounds, but there are numerous undersirable side effects.[10] Medical plants such as ginger, onion, and garlic have also been used for Cd detoxification.[11-13] There is no report of the use of *Thunbergia laurifolia* Lindl. as a detoxifying agent.

Thunbergia laurifolia Lindl. or "Rang Jerd" (in Thai) has various uses in Thai traditional medicine. Aqueous extract of fresh and dried leaves, dried root and bark are used as an antidote for insecticide poisoning,[14,15] and dried root is used as an anti-inflammatory and antipyretic agent.[16,17] Other reported uses of the plant are for treatment of amphetamine addiction[18], reducing the toxicity of parathion insecticide[15], and as antimicrobial agent[19] and antioxidant.[20] Other study has reported that the aqueous extract of *T. laurifolia* Lindl. in the massive single dose was not toxic to rats, did not affect the behaviour of rats, did not induce free radical formation and was not mutagenic for bacteria.[21]

Therefore, the aim of this study was to use a rat model induced renal and hepatic injuries by Cd exposure in order to study the effectiveness of *T. laurifolia* Lindl. leaf extract as a detoxifying agent.

MATERIALS AND METHODS

Induction of rat model

Animals : Male Wistar rats purchased from the National Laboratory Animal Centre, Mahidol University, weighing between 275-300 g were maintained under standardized laboratory conditions (temperature 22 ± 2˚C, 12/12 hr light/dark cycle) for one week acclimatization. They were allowed free access to drinking water and commercial standard rodent pellets. The study protocol including number of animal use was approved by the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University.

Induction of renal and liver injuries by cadmium chloride : Six of male Wistar rats were randomized into two groups of three. The control group (n=3) was injected subcutaneously with normal saline solution (0.9% NaCl). The treated group (n=3) was injected with $CdCl_2$ solution at the concentration of 1.2 mg/kg BW subcutaneously for 5 days/week for 4 weeks (28 days, d1-d28; 20 doses of $CdCl_2$). The dose of $CdCl_2$ and the period of treatment were modified from the study of Prozialeck.[22]

Collection of rats' urine and blood : A urinary sample was collected from each rat over 24 hr using metabolic cage on day 0 and day 28 and a blood sample was collected by cardiac puncture on the last day of the experiment after the rats were anesthetized intraperitoneally with sodium phenobarbital solution. After perfusion of the whole animal with physiological saline via the portal vein, the kidneys and livers were removed, washed with physiological saline solution and fixed in 10% neutral-buffered formalin for 48 hr for histopathological examination.

Determination of blood and urinary cadmium and urinary creatinine **:** Blood and urinary Cd concentrations were quantified by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman-GFAAS background correction (Varian, SpectraA800Z) using 5% monobasic ammonium phosphate as a modifier. Blood samples were digested in 5% nitric acid solution with a ratio of 1:3 (v/v), then mixed, and centrifuged after standing for an hour. The supernatant was removed for analysis by GFAAS. Urine samples were diluted in 0.1% nitric acid solution with a ratio of 1:1 (v/v). Urinary creatinine levels were measured based on the Jaffe reaction.[23]

Histopathological study : The rat kidneys and livers were processed and individually embedded in the paraffin wax. Sections (5 μm) were cut and stained with haematoxylin and eosin (H&E) dye for examination under a light microscope to determine the morphological changes.

Plant extract

Thunbergia laurifolia Lindl. leaf extract : T. laurifolia Lindl. (Acanthaceae) leaves were collected from Ob Khan National Park, Hangdong District, Chiang Mai Province in July and October 2009. Taxonomy of the plant has been identified at the Queen Sirikit Botanic Garden, Mae Rim District, Chiang Mai Province.[24] The leaves were washed with tap water, dried and grounded to powder, then stored in an amber glass bottle at room temperature before extraction.

The leaves 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.

Characterization of T. laurifolia Lindl. leaf extract : Lyophilized residue of the *T. laurifolia* Lindl. leaf extract was dissolved in D_2O as aqueous solvent and the constituents were characterized by nuclear magnetic resonance spectroscopy (NMR) using a Bruker AVANCE-400 spectrometer with water suppression technique.

Detoxification experiments

Detoxification effects of T. laurifolia Lindl. against cadmium toxicity : Eighteen rats were randomized into three groups of six rats. The positive control group (group 1) was injected subcutaneously with 1.2 mg/kg BW $CdCl_2$ solution for 5 days/week for 4 weeks followed by water orally for the next 28 days. The leaf extract treatment group (group 2) was injected with $CdCl_2$ solution as for the positive control group. Then after $CdCl_2$ injection was completed, the group was fed daily with the leaf extract for 4 more weeks. Rats in group 3 were injected with $CdCl_2$ solution and fed the leaf extract twice a day at the same frequency as the $CdCl_2$ injections (20 doses over 28 days). This experimental design was expected to test the antagonistic effect of the leaf extract.

To investigate the prophylactic effect of the leaf extract, eighteen rats were separated into three equal groups and treated as above except the group 2 and 3 were fed the leaf extract before and during the $CdCl_2$ treatment. Urine, blood, kidneys and livers were collected and removed from all rats for Cd quantification and

histopathological examination. The appearances and behaviour of the rats was also observed and recorded daily.[21]

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 12.0. Values were considered statistically significant when $p<0.05$.

RESULTS

Induction of rat model

Body, kidneys and liver's weight of rats after treatment with Cd : Body weight of the control rats gradually increased during the experiment. The body weight of the rats treated with $CdCl_2$ did not increase but at the conclusion of the experiment, the mean weights of both groups were different (Figure 1). The weights of kidneys and liver's were not significantly different between the control and the Cd treated rats eventhough the data appeared that Cd treated rats had larger organs than the control rats without $CdCl_2$ treatment (Table 1).

Blood and urinary Cd in cadmium treated rats : After the Cd exposure, the blood Cd concentration of treated rats was approximately 5,000 times greater than in the control rats (Table 2). Likewise, the urinary Cd concentration in treated rats was much higher than for the control rats.

Histopathological study of cadmium treated rats : The kidneys of the control rats had normal structure of glomerulus (G) and proximal convoluted tubules (T) (Figure 2A). The kidneys of Cd treated rats had proximal renal tubular damage (Figure 2B and 2C). The cuboidal shaped cells were disrupted with undefined epithelial cell lining. The intracellular space or lumen (L) increased. Protein casts (arrow) were present in collecting tubules. Some glomeruli had increased cellular appearance and diminished capsule space.

Histological examination indicated the hepatic architectures in the control group was normal (Figure 3A). The liver of rats exposed to Cd (Figure 3B and 3C) showed degenerative changes like swollen hepatocytes with pale cytoplasm and condensed nuclear chromatins with pyknosis. Single cell necrosis (white arrow), marked by contracted cells detached from the others and densely hyperchromatic nuclear chromatins, together with sinusoidal (black arrow) widening was also observed in all animals with Cd treatment.

Plant extract

Characterization of the constituents of T. laurifolia Lindl. Leaves : The NMR spectra of the aqueous extract of *T. laurifolia* Lindl. leaves collected in the rainy season (July) and cool season (October) were similar (Figure 4A and 4B). The major constituents were aromatic, hexyl and glucoside compounds.

Detoxification experiments

Detoxification effects of T. laurifolia Lindl. against Cd toxicity : Most rats treated with Cd died during the Cd exposure period in both the antagonistic and prophylactic studies. The appearance and behaviour of group fed the leaf extract during the Cd exposure (prophylactic study) was different to positive control group ($CdCl_2$ treatment only). Abnormal behaviours included, such as bleeding nose, hunched back, falling hair, passive head tap, sensitive body touch and statue position.

The mortality of animals in the positive control group commenced from day 5 while animals fed the leaf extract from commencement of the study started to die after day 10. At the conclusion of the experiment, there was one survivor in the positive control group and two survivors in group 3 in the prophylactic study.

DISCUSSION

Chronic exposure to Cd leads to damage to several organs and systems primarily the kidney.[1,2] Our results showed a significant increase in Cd level in blood and urine as has been reported elsewhere.[10,25,26] Cd exposure increased body, kidneys and liver weight. The high concentrations of Cd in blood and urine indicated a high level exposure. This high exposure and organ weight increase indicated Cd toxicity could have resulted from Cd-associated pathologies in renal tubular epithelium such as calcuria, magnesuria and proteinuria as well as bone demineralization and anemia.[13] In this study, we observed the degenerative changes in rat kidney and liver accorded with the high blood Cd concentrations.

Lyophilized *T. laurifolia* Lindl. leaf extract administrated to rats at 500 mg/kg BW for 28 days did not affect rat behaviour[21] and the lower dosage used in this study also had no abnormal effect on the animals prior to $CdCl_2$ injection.

The high mortality rate of rats during the Cd exposure period indicated the Cd dose was too high or the experimented rats were more sensitive to Cd than rats in our previous experiments when we established a rat model for renal and liver injuries induced by Cd. The antagonistic and prophylactic effects of the leaf extract were not defined at the Cd dose used in this study because of the high mortality rate. However, rats fed the leaf extract before and during Cd exposure had a slightly lower mortality rate than the positive control group treated with $CdCl_2$ alone. Administration of the *T. laurifolia* Lindl. leaf extract by gavage may reduce Cd toxicity but not antagonize or prevent the lethality caused by high Cd exposure. Therefore, our next study will be focus on reducing Cd dosages and pretreatment experimented rats with *T. laurifolia* Lindl. leaf extract by other routes of administration, such as drinking water.

The compounds in the NMR spectra in our study were similar to those previously reported from *T. laurifolia* Lindl. leaves that consisted of two iridoid glycosides; 8-*epi*-grandiforic and 3'-*O*-β-glucopyranosyl-stibericoside along with seven known compounds; benzyl β-glucopyranoside, benzyl β-2-*O*-β-glucopyranosyl, glucopyranoside, grandifloric acid, *E*-2-hexynyl β-glucopyeanoside, hexanol β-glucopyranoside, 6-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosylapigenin.[27]

In conclusion, we have established a renal and hepatic injuries model of rats by $CdCl_2$ subcutaneously treatment for studying the antagonistic and prophylactic effects of *T. laurifolia* Lindl. leaf extract. This study could not demonstrate that the leaf extract could not be used to treat Cd toxicity. However, the leaf extract may provide some protection if administrated prior to Cd exposure. The routes of the leaf extract administration to reduce Cd toxicity and/or the dosage of $CdCl_2$ need to be modified in future.

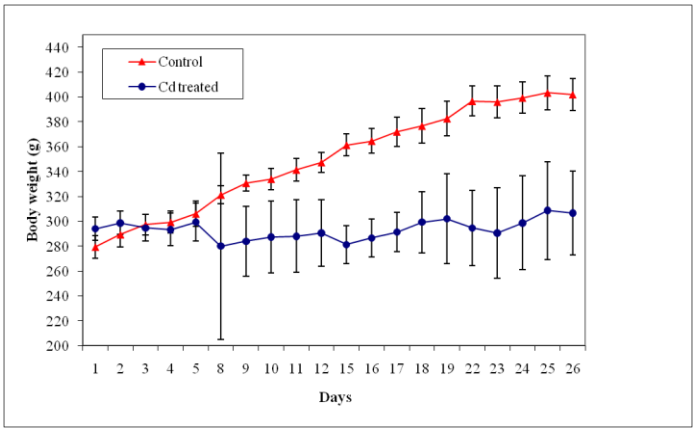

Figure 1 The effect of Cd on body weight. Each point represents mean ± SEM of 3 rats.

Table 1 Left and right kidneys and the liver's weight of cadmium (1.2 mg/kg $CdCl_2$) treated rats compared to the control rats

Rats	Left kidney weight (g)	Right kidney weight (g)	Liver weight (g)
Control	1.16 ± 0.09	1.28 ± 0.04	11.75 ± 0.25
Cd treated	1.30 ± 0.11	1.34 ± 0.13	18.96 ± 1.91

Values represent mean ± SEM of 3 rats.

Table 2 Cadmium concentrations in blood and urine of the cadmium (1.2 mg/kg $CdCl_2$) treated rats compared to the control rats

Rats	Blood Cd (µg/L)	Urinary Cd (µg/gCr) Day 0	Urinary Cd (µg/gCr) Day 20
Control	1.67 ± 0.33	56.00 ± 31.34	4.67 ± 1.67
Cd treated	5,114.33 ± 1,081.98*	202.33 ± 73.13	220,792.33 ± 81,714.00

Values represent mean ± SEM of 3 rats. *p<0.05

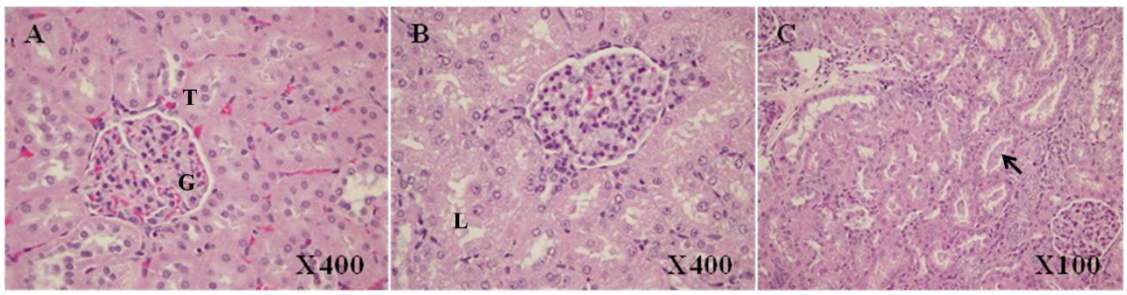

Figure 2 Kidney histopathology of control rat (A) showed normal structure of glomerulus (G) and proximal convoluted tubules (T), and of rat exposed to cadmium (B and C) showed proximal tubular damage with dilation of lumen (L) and protein casts (arrow).

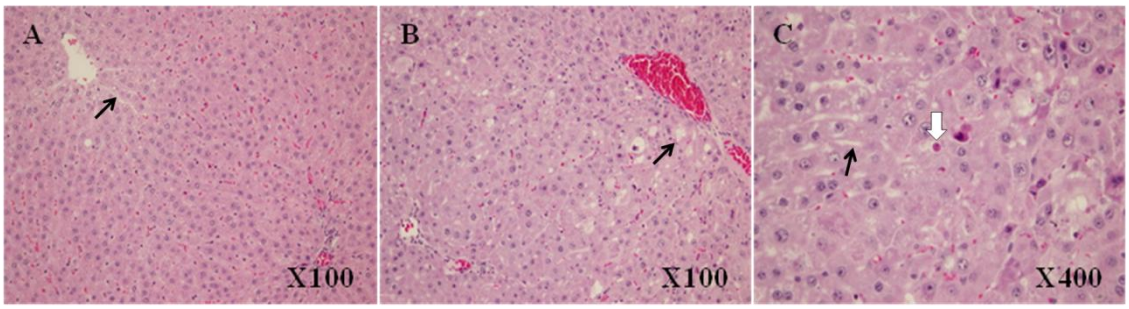

Figure 3 Liver histopathology of control rat (A) and of rat exposed to cadmium showed degenerative hepatocytes with sinusoidal (arrow) widening and the presence of single cell necrosis (white arrow) (B and C).

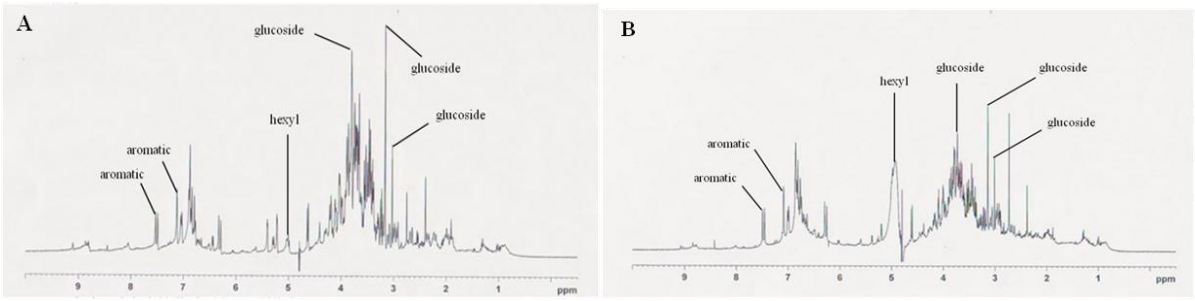

Figure 4 NMR spectra of the aqueous extract of *Thunbergia laurifolia* Lindl. leaves collected in July (A) and October 2009 (B) showing similar constituents

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