การดื่มน้ำสกัดใบรางจืดช่วยป้องกันการเกิดพิษของแคดเมียมต่อไตหนูขาว

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บทคัดย่อ

รางจืด หรือชื่อทางวิทยาศาสตร์คือ *Thunbergia laurifolia* Lindl. เป็นสมุนไพรไทยที่มีสรรพคุณในการถอนพิษต่างๆ ปัจจุบันพบว่าการตกค้างแคดเมียมในสิ่งแวดล้อมเป็นปัญหาสำคัญของประเทศ โดยพบมากบริเวณอำเภอแม่สอด จังหวัดตาก ประชากรที่อาศัยอยู่ในบริเวณนี้เป็นกลุ่มที่มีความเสี่ยงสูงต่อการได้รับแคดเมียมแบบเรื้อรังอันอาจก่อให้เกิดความผิดปกติต่อไตและกระดูกได้ ดังนั้นเพื่อศึกษาว่าสารสกัดใบรางจืดสามารถป้องกันการเกิดพิษของแคดเมียมต่อไตได้หรือไม่ งานวิจัยนี้ได้ใช้หนูขาวเพศผู้ 12 ตัว แบ่งเป็นสองกลุ่ม ๆ ละ 6 ตัว โดยกลุ่มแรกเป็นกลุ่มควบคุมให้น้ำกลั่นเป็นน้ำดื่ม 20 วันก่อนการให้สารละลายแคดเมียมคลอไรด์ความเข้มข้น 1.0 มิลลิกรัมต่อกิโลกรัมโดยการฉีดเข้าใต้ผิวหนังเป็นเวลา 20 วัน กลุ่มที่สองให้สารสกัดใบรางจืด 0.1 มิลลิกรัมต่อมิลลิลิตรในน้ำดื่มก่อนการให้สารละลายแคดเมียมคลอไรด์ 20 วัน และให้ต่อเนื่องไปพร้อมกับการได้รับสารละลายแคดเมียมคลอไรด์ เช่นเดียวกับหนูกลุ่มที่หนึ่ง ผลการศึกษาพบว่าหนูในกลุ่มที่ได้รับสารสกัดใบรางจืดมีน้ำหนักตัวสูงกว่าหนูกลุ่มควบคุมที่ได้รับสารละลายแคดเมียมคลอไรด์เพียงอย่างเดียวอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) อย่างไรก็ตามแม้ว่าสารสกัดใบรางจืดไม่สามารถลดปริมาณแคดเมียมที่สะสมในเลือดและปัสสาวะของหนูขาวที่ได้รับแคดเมียมได้ แต่สามารถช่วยป้องกันความผิดปกติที่ตรวจพบทางจุลพยาธิวิทยาที่ไตของหนูขาวได้ ผลการวิจัยครั้งนี้แสดงให้เห็นว่าสารสกัดใบรางจืดสามารถป้องกันการเกิดพิษของแคดเมียมต่อไตหนูขาวได้ และยังลดพิษที่ระบบอื่นๆของร่างกาย ผลการศึกษาครั้งนี้อาจเป็นประโยชน์สำหรับการนำไปประยุกต์ใช้ลดความเป็นพิษของแคดเมียมในกลุ่มประชากรที่อาศัยอยู่ในบริเวณที่มีความเสี่ยงจากการได้รับแคดเมียมปนเปื้อนในอาหารและน้ำดื่ม

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

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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 ($CdCl_2$) 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 $CdCl_2$ 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

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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 insecticide[3-5], ethyl alcohol, arsenic and strychnine poisoning.[6] The dried root was also used as an anti-inflammatory[7] 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 glucose[14] 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 district[18] reported that residents had high level of Cd exposure with 9.2% of subjects having urinary Cd between 5-10 µg/gCr and 2.5% with urinary Cd >10 µg/gCr, compared to the maximum recommended level of the urinary Cd of 2 µg/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 day[20] 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 $CdCl_2$ 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 $CdCl_2$ 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 reaction[23] 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 ± 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.

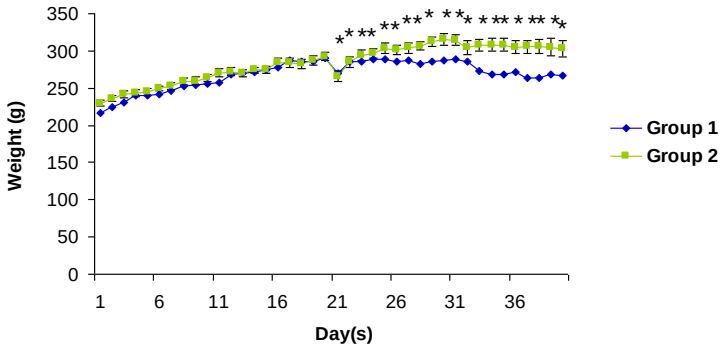

Figure 1. Rats exposed to Cd after day 20 (group 1) suffered weight loss but the loss was much more limited in rats which consumed drinking water containing TL leaf extract (group 2). All values are mean ± SEM of 6 rats. An * indicate statistically significant differences among the two groups ($p<0.05$).

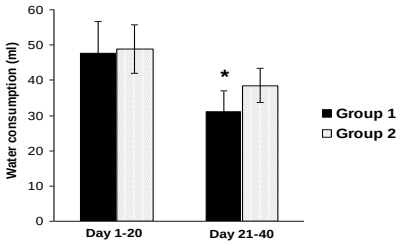

Figure 2. Water consumption of male Wistar rats in Cd treated (group 1) and pretreatment with TL leaf extract in drinking water (group 2) before (Day 1-20) and after Cd treatment (Day 21-40).

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 $CdCl_2$ 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.

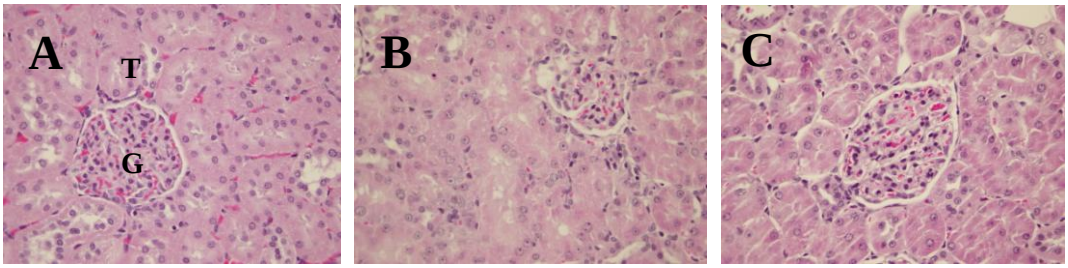

Figure 3. Histopathology (H&E, x400) of tubule (T) and glomeruli (G) in the kidney cortex of a normal, untreated rat **(A);** a rat exposed to cadmium chloride at 1.0 mg/kg for 20 days **(B);** and a rat exposed to both *T. laurifolia* leaf extract and $CdCl_2$ **(C)**.

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

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