

Original article:

**NEPHROPROTECTIVE ACTION OF *PEUCEDANUM GRANDE*
AGAINST CADMIUM CHLORIDE INDUCED RENAL TOXICITY
IN WISTAR RATS**

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ABSTRACT

Cadmium is a known industrial pollutant which accumulates in the kidney and its exposure leads to the production of reactive oxygen species (ROS). The present study was carried out to evaluate the protective effects of *Peucedanum grande* against CdCl₂ induced renal toxicity in Wistar rats. Wistar rats were subjected to oral pre-treatment of *P. grande* (60 and 120 mg/kg b.wt) against the renal toxicity induced by administration of CdCl₂ (3mg/kg b.wt). Efficacy of *P. grande* against the renal toxicity was evaluated in terms of biochemical estimation of antioxidant enzyme activities and histopathological changes. *P. grande* pretreatment prevented deteriorative effects induced by CdCl₂ through a protective mechanism that involved reduction of increased oxidative stress as well as by restoration of histopathological changes against CdCl₂ administration.

Keywords: Cadmium chloride, nephrotoxicity, *Peucedanum grande*, ROS

INTRODUCTION

Cadmium is a known hazardous environmental and occupational toxicant. It is present in drinking water, air and sometimes in food also (Klos, 2001). It is absorbed from the alimentary tract and in combination with thionein protein forms metallothioneins which play a significant role in further distribution of cadmium in

the different organs. Kidney is known to be the most prone organ in exposure to cadmium (Ryan et al., 2000; Yamano et al., 1999; Yiin et al., 1999). It has been evaluated that, for the treatment of cadmium toxicity chelating compounds have been used such as calcium disodium versenate, dimercaprol and mesomercaptosuccinic acid (Piotrowski et al., 1974). In recent years in scientific

investigations attention has been drawn to the “health-promoting” activity of herbal plants.

Peucedanum grande CB Clark has several names like duku, baphalle, wild carrot, hingupatri, and belongs to the family Umbelliferae. A genus of herb, shrub and rarely trees distributed chiefly in Europe, Asia, North East Africa and Western South America. Ten species occur in India (Gildemeister and Hoffmann, 1961; Kirtikar and Basu, 1987; Chopra et al., 1956).

In Unani system of traditional medicine, duku (*Peucedanum grande*) is known for its medicinal values like, diuretic (*Mudir-e-Baul*), emmenagogue (*Mudir-e-Haiz*), aphrodisiac (*Muqawwi-e-Bah*), demulcent (*Mulattif*), deobstruent (*Mufatteh*), urolithotriptic (*Mufattite-Hissat-e-Gurda-Wa-Masana*), anti-inflammatory (*Mohallil-e-Auram*), antidote (*Daaf-e-Sammyat*), maturative (*Munzija*), etc. (Sina, 1920; Ibn-e-Sina, 1927; Ibn-e-Rushad, 1980; Ghani, 1911). So far its protective effects on kidney have not been investigated.

The aim of this study was to evaluate the protective efficacy of *P. grande* against CdCl₂ induced renal toxicity. The extent of the protective effect of *P. grande* against nephroprotective effects was determined by studying serum kidney toxicity markers and biochemical estimation of antioxidant enzymes of wistar rats.

MATERIAL AND METHODS

Plant material

The drugs were purchased from Ajmal and Brothers Khari Baoli, Delhi. The botanical identity of the purchased drugs was established as Duku (*Peucedanum grande* C.B Clark Seeds) rhizome at NISCAIR (National Institute of Science Communication and Information Resources), Dr. K.S. Krishnan Marg, Pusa Gate, New Delhi, 110012 under Ref. NISCAIR/RHM/F-3/2004Consult/-486/62.

Preparation of extract of Peucedanum grande

2 kg dried seeds/fruits of the drug *P. grande* were extracted exhaustively with methanol by using a Soxhlet apparatus over boiling water bath for 3 h. It was removed from the water bath and allowed to cool at room temperature and filtered. The plant material obtained after filtration was re-extracted twice by the same procedure. All three extracts were combined together (methanolic extracts). Methanol was recovered by distillation under reduced pressure. The yield of extract was 12 % w/w in the terms of the amount of starting materials.

Chemicals

Reduced glutathione (GSH), 1,2-dithio-bis-nitrobenzoic acid (DTNB), 1-chloro 2,4-dinitrobenzene (CDNB), bovine serum albumin (BSA), oxidized and reduced nicotinamide adenine dinucleotide phosphate (NADP, NADPH), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich, USA. All other reagents and solvents were of a high analytical grade.

Animals

Eight week old male Wistar rats (150-200 g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi and were housed in a ventilated room at 25 ± 5 °C under a 12 h light/dark cycle. The animals were acclimatized for one week before the study and had free access to standard laboratory feed (Hindustan Lever Ltd., Bombay, India) and water ad libitum. The study was approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). CPCSEA guidelines were followed for animal handling and treatment.

Treatment regimen

Rats were divided into four groups of 6 animals each.

Test drug (methanolic extract of *P. grande*) was administered in the form of suspension using 1 % carboxymethyl cellu-

lose (CMC) as a suspending agent in distilled water.

Animals of Group 1 (control) received 1 % CMC in distilled water (10 mg/kg body weight/day) for 5 days.

Animals of group II received 1 % CMC water (10 mg/kg body weight/day) for 5 days.

Animals of group III received lower dose 60 mg/kg body weight/day of *P. grande* extract suspended in the vehicle (10 ml/kg) for 5 days.

Animals of group IV received higher dose 120 mg/kg body weight/day *P. grande* extract suspended in the vehicle (10 ml/kg) for 5 days.

A single dose of cadmium chloride (20 mg/kg body weight) (Jonker et al., 1993) was administered subcutaneously in neck region in a volume of 1 ml/kg, on fourth day to all the animals except of group 1. Group 1 received normal saline only. On the sixth day, blood samples were withdrawn from retro-orbital venous plexus and serum was separated for the estimation for urea nitrogen and creatinine concentrations. Animals were sacrificed and their kidney was isolated for histopathological studies and post mitochondrial supernatant (PMS).

Post mitochondrial supernatant preparation

Kidneys were removed quickly, cleaned free of extraneous material and immediately perfused with ice-cold saline (0.85 % sodium chloride). The kidneys were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17 %) using a Potter Elvehjem homogenizer. The homogenate was filtered through muslin cloth, and was centrifuged at 800 x g for 5 min at 4 °C by REMI Cooling Centrifuge to separate the nuclear debris. The aliquot so obtained was centrifuged at 12000 r.p.m. for 20 min at 4 °C to obtain PMS, which was used as a source of enzymes. All the biochemical estimations were completed within 24 h of animal sacrifice (Ahmad et al., 2012).

Estimation of blood urea nitrogen (BUN)

Estimation of blood urea nitrogen was carried out by the diacetyl monoxime method. Protein-free filtrate was prepared by adding serum and equal amount of 10 % TCA, then the mixture was centrifuged at 2000 r.p.m. and the supernatant was taken. To 0.5 ml of protein free filtrate 3.5 ml of distilled water, 0.8 ml diacetylmonoxime (2 %) and 3.2 ml sulphuric acid–phosphoric acid reagent were added. The reagent was prepared by mixing 150 ml 85 % phosphoric acid with 140 ml water and 50 ml of concentrated sulphuric acid. The reaction mixture was placed in a boiling water bath for 30 min and then cooled to room temperature. The absorbance was read at 480 nm (Kanter, 1975).

Estimation of creatinine

Creatinine was estimated by the alkaline picrate method. Protein-free filtrate was prepared. To 1.0 ml serum were added, 1.0 ml sodium tungstate (5 %), 1.0 ml sulphuric acid (0.6 N) and 1.0 ml distilled water. After mixing thoroughly, the mixture was centrifuged at 800 x g for 5 min. The supernatant was added to a mixture containing 1.0 ml picric acid (1.05 %) and 1.0 ml sodium hydroxide (0.75 N). The absorbance at 520 nm was read exactly after 20 min (Hare, 1950).

Lipid peroxidation

Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) formation which is the major product of membrane lipid peroxidation. The reaction mixture - a total volume of 3 ml - contained mainly TCA 1 ml (10 %) and TBA 1.0 ml. Test tubes with the reaction mixture were kept in boiling water for about 45 min and transferred to ice cooled water and then centrifuged at 2500 x g for 10 minutes. Malondialdehyde formation in each sample was quantified as optical density observed at 532 nm. The results were expressed as nmol of MDA formed per minutes per gram of tissue using a molar extinction coeffi-

cient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (Wright et al., 1981).

Estimation of reduced glutathione

For determination of reduced GSH, 1.0 ml of 10 % PMS was mixed with 1.0 ml of 4 % sulphosalicylic acid and incubated at 4 °C for a minimum time period of 1 hr and then centrifuged at 4 °C at $1200 \times g$ for 15 min. Briefly reaction mixture contained 0.4 ml supernatant, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml DTNB (4 mg/ml) making a total volume of 3.0 ml. The yellow color developed, was read immediately at 412 nm on spectrophotometer (Perkin Elmer, lambda EZ201). The reduced glutathione concentration was calculated as nmol GSH conjugates/g tissue (Jollow et al., 1974).

Assay for superoxide dismutase activity

The assay mixture consisted of 50 mM glycine buffer, pH 10.4, 20 mg/ml epinephrine, and cytosolic fraction (10 % w/v) in a total volume of 1.0 ml. Enzyme activity was recorded at 480 nm and the activity was calculated as μM epinephrine oxidized /min/mg protein (Stevens et al., 2000).

Assay for catalase activity

The reaction mixture for determination of the catalase activity consisted of 0.05 ml PMS, 1.0 ml hydrogen peroxide (0.019 M), 1.95 ml phosphate buffer (0.1M, pH 7.4), in a total volume of 3 ml. Changes in absorbance were recorded at 240 nm and the activity was calculated as nmol H_2O_2 consumed per min per mg protein (Claiborne, 1985).

Histopathological examination

The kidneys were quickly removed after sacrifice of rats and were fixed in 10 % neutral buffered formalin solution for histopathological processing. Sections were stained with hematoxyline and eosin before being observed under an Olympus microscope at 400X magnification.

RESULTS

Effect of *Peucedanum grande* on lipid peroxidation

The effect of methanolic extract *P. grande* on the levels of LPO in kidney PMS is shown in Table 1. Cadmium chloride treatment alone raised renal LPO by about 9.50 folds as compared to vehicle treated control. Co-administration of methanolic extract (60 mg/kg body weight) with cadmium chloride inhibited the rise in blood LPO (76.0 %). There was 89 % inhibition in the rise of LPO with high dose (120 mg/kg body weight).

Effect of *Peucedanum grande* on GSH levels

The effect of methanolic extract *P. grande* on the levels of GSH in kidney PMS is shown in Table 1. Cadmium chloride treatment alone exhausted renal GSH by about 46.52 % as compared to vehicle treatment. However, co-administration of methanolic *P. grande* extract with cadmium chloride dose dependently prevented GSH depletion significantly.

Effect of *Peucedanum grande* on SOD activity

The cadmium chloride (alone) treatment diminished the SOD activity to 50.37 % in the kidney compared to vehicle treated control (Table 1). Co-treatments of rats with methanolic extract *P. grande* with cadmium chloride, dose dependently enhanced the reduced level of SOD activity significantly.

Effect of *Peucedanum grande* on catalase activity

The cadmium chloride (alone) treatment diminished the level of catalase activity to 29.77 % in the kidney compared to vehicle treated control (Table 1). Co-treatments of rats with methanolic *P. grande* extract with cadmium chloride, dose dependently enhanced the reduced level of catalase activity significantly.

Table 1: Effects of pre-treatment with *Peucedanum grande* extract on GSH, catalase activity, LPO and SOD activity in kidney of Wistar rats after administration with cadmium chloride

Treatment regimen per group	GSH (n mol CDNB Conjugate formed /g tissue)	Catalase activity (nmol H ₂ O ₂ consumed/min/ mg protein)	SOD activity (Units/mg of protein)	LPO (nmol TBARS/mg protein)
Group I (control 10 ml/kg)	0.316±0.01	180±3.64	6.69±0.44	2.8±0.057
Group II (CdCl ₂)	0.169±0.01***	126.4±4.4***	3.32±0.13***	26.6±0.127***
Group III (<i>P. grande</i> D1+ CdCl ₂)	0.294±0.002###	163±4.35##	4.48±0.13#	7.38±0.135###
Group IV (<i>P. grande</i> D2+ CdCl ₂)	0.289±0.005###	168.2±4.93###	5.36±0.12###	5.36±0.09###

Results represent mean ± SE of six animals per group. Results obtained are significantly different from Control group (***P < 0.001). Results obtained are significantly different from CdCl₂ treated group (#P < 0.05), (##P < 0.01) and (###P < 0.001). D1 = 60 mg/kg body weight; D2 = 120 mg/kg body weight

Effect of *Peucedanum grande* on blood urea and creatinine level

In the present study, cadmium chloride treatment caused nephrotoxicity as evidenced by marked elevation in blood urea (237.6 %) and creatinine (222.15 %) shown in Table 2. Co-administration of methanolic extract (60 mg/kg body weight) with cadmium chloride, inhibited the rise in blood urea (96.5 %) and serum creatinine (96 %). There were 97 % inhibition in the rise of BUN and 95 % inhibition in the rise of serum creatinine with high dose (120 mg/kg body weight). The present finding clearly indicates the protective effect of *P. grande* at both doses (60 mg and 120 mg/kg body weight) on cadmium toxicity in rats.

Effect of *Peucedanum grande* pretreatment on the histology of rats

The histological examination of kidney tissue of rats is shown in Figure 1. Control group showed normal glomerular and tubular histology of kidney. The tubules were largely intact without the presence of any mononuclear infiltrates in the interstitium and blood vessels were also unremarkable. Histological sections from CdCl₂ group showed acute tubular necrosis and glomerular widening. Lower dose of *P. grande* showed focal necrosis of the proximal convoluted tubular lining epithelial cells with areas of desquamation of the cells in the tubular lumina where as higher dose of *P. grande* showed less necrosis of the proximal tubular lining epithelial cells along with cellular swelling, desquamation and loss of brush border.

Table 2: Effects of pre-treatment with *Peucedanum grande* extract on serum markers of Wistar rats after administration of cadmium chloride

Treatment regimen per group	BUN (mg/dl)	Creatinine (mg/dl)
Group I (control 10 ml/kg)	13.6±0.7	0.167±0.02
Group II (CdCl ₂)	32.32±1.6***	0.371±0.009***
Group III (<i>P. grande</i> D1+ CdCl ₂)	14.26±1.3###	0.187±0.001##
Group IV (<i>P. grande</i> D2+ CdCl ₂)	14.73±1.4###	0.173±0.007###

Results represent mean ± SE of six animals per group. Results obtained are significantly different from Control group (***P < 0.001). Results obtained are significantly different from CdCl₂ treated group (#P < 0.01) and (###P < 0.001). D1 = 60 mg/kg body weight; D2 = 120 mg/kg body weight

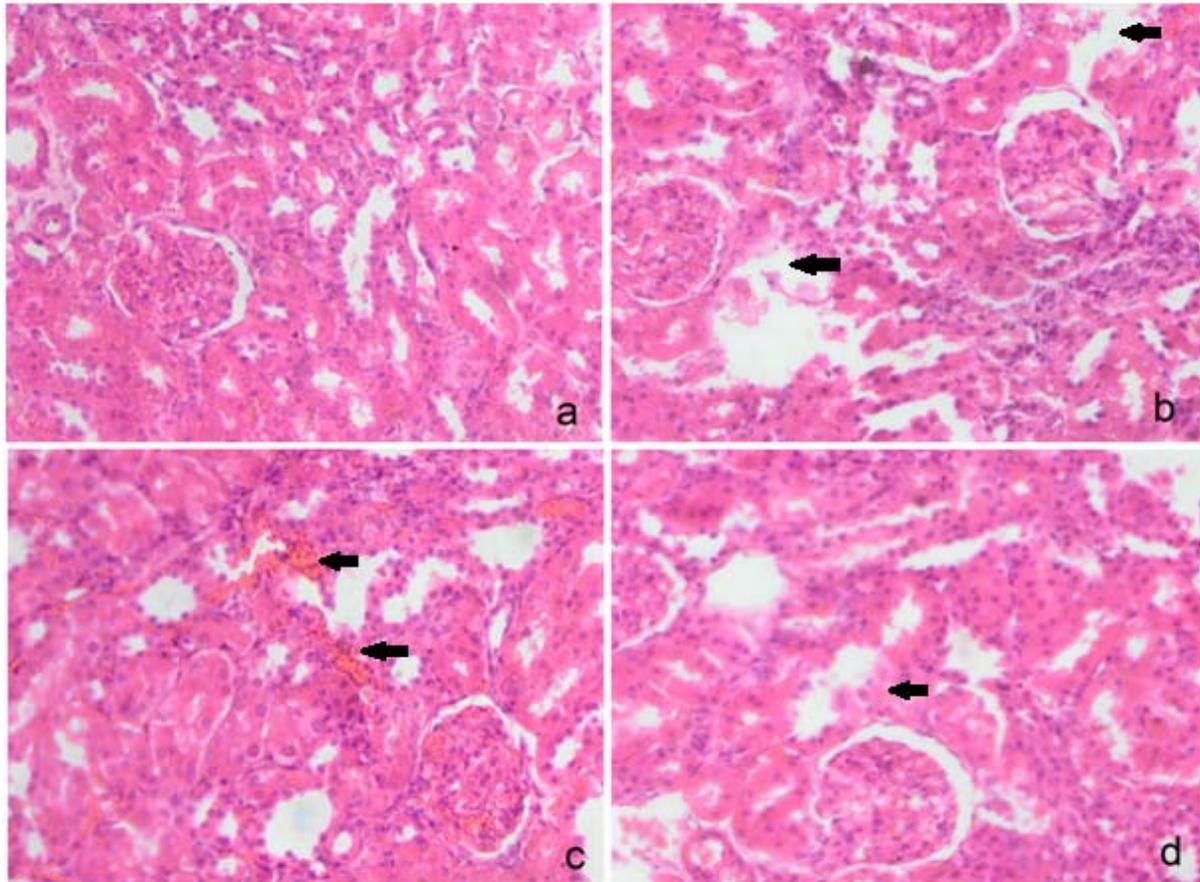


Figure 1: Kidney histology of rats (x 400 magnification)

(a) Control group showed normal glomerular and tubular histology. The tubules were largely intact without the presence of any mononuclear infiltrates in the interstitium and blood vessels were also unremarkable. **(b)** Toxicant (cadmium chloride) group showed acute tubular necrosis and glomerular widening (black solid arrows). **(c)** Low dose of *Peucedanum grande* and cadmium chloride showed focal necrosis of the proximal convoluted tubular lining epithelial cells with areas of desquamation of the cells in the tubular lumina (black solid arrows). **(d)** High dose of *Peucedanum grande* and cadmium chloride showed that focal areas of necrosis of the proximal tubular lining epithelial cells were still seen along with cellular swelling, desquamation and loss of brush border (black solid arrows).

DISCUSSION

Cadmium is a non-essential trace element, which is toxic to many plants and animals. Its technological utilization has led to increasing levels in the environment and also in the human body. Mammalian organisms are exposed to cadmium, either from vapor or food constituents and/or water (National Institute for Safety and Health, 1984.) Epidemiological studies have revealed that cadmium is one of the most toxic of the heavy metals to humans (Abu-Hayyeh et al., 2001). 70 % of the ultrafiltered cadmium is taken up, largely by the proximal tubules of the kidney and is

accumulated mainly in kidney cortex leading to proximal tubule lesions (Järup, 2002). The most sensitive cellular targets of cadmium seem to be ion transport and signal transduction. These include intracellular mobilization of second messengers such as inositol triphosphate and calcium (Rasheed et al., 1984), inhibition of plasma membrane calcium channels (Huang et al., 1989) and inhibition of Ca^{2+} -ATPases of the sarcoplasmic reticulum (Herak-Kramberger et al., 2000). At the gene level, cadmium induces the expression of metallothionein and inhibits the repair of DNA damage (Ganguly et al., 1996; Jahangir et

al., 2005). Large numbers of enzymatic activities are influenced by cadmium and the mechanisms of these effects have been hypothesized to be due to, either displacement of a beneficial metal from the active site or through binding to the active site in the enzyme itself (Stillman and Zelazowski, 1988). Cadmium is thought to induce lipid peroxidation and this has often been considered to be the main cause of its deleterious influence on membrane-dependent function (Jahangir et al., 2005; Yiin et al., 1998; Eybl et al., 2006). In the present study, pre-treatment with *P. grande* depleted the serum toxicity markers significantly in modulating kidney toxicity. Rats pretreated with *P. grande* had BUN, creatinine significantly lower than those receiving only CdCl₂ thus ameliorating nephrotoxicity on cadmium toxicity in rats. Lipid peroxidation refers to the oxidative degradation of lipids. The products of lipid peroxidation are highly destructive. They damage the membranes, cells and even tissues. Most of the products of lipid peroxidation are unstable e.g. carbonyls, esters, alkanes, alkenes, 2-alkenal and malondialdehyde. The latter is often used to assess oxidative stress. Malondialdehyde and other aldehydes react with thiobarbituric acid and produce red colored products which can be measured photometrically (thiobarbituric acid reactive substance, TBARS). In the present study we have observed a noticeable increase of the LPO in CdCl₂ administered group and further it was found that there is a significant attenuation of LPO by *P. grande* pretreatment. The present finding clearly indicates the dose dependent protective effect of *P. grande*. Glutathione reductase, also known as GSR or GR, is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. The activity of glutathione reductase is used as indicator for oxidative stress. CdCl₂ treatment alone exhausted renal GSH as compared to vehicle treated. However, the methanolic *P. grande* extract significantly diminished the cadmium

chloride dependent GSH depletion in the kidney in a dose dependent manner. Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen. The CdCl₂ alone treatment, diminished the level of catalase activity in the kidney as compared to the vehicle treated control. Co-treatments of rats with methanolic extract *P. grande* with cadmium chloride dose dependently enhanced the reduced level of catalase significantly. Dukku (*P. grande*) has been described having carminative stimulant and diuretic properties. Mammalian organisms are exposed to cadmium, either from vapor or food constituents and/or water (National Institute for Safety and Health, 1984). Cadmium toxicity severely depleted GSH levels in the kidneys of Wistar rats. However, co-administration of methanolic extract of *P. grande* with cadmium chloride, dose dependently enhanced GSH contents significantly. SODs are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. The CdCl₂ treatment diminished the level of SOD activity as compared to vehicle treated control. Co-treatments of rats with methanolic extract *P. grande* along with CdCl₂, dose dependently enhanced the reduced level of SOD activity significantly. Both applied doses of *P. grande* extract were effective in maintaining the normal level of antioxidant enzymes. The main histological finding of this study was that CdCl₂ group showed acute tubular necrosis and glomerular widening and that pre-treatment with *P. grande* extract recovers the kidney architecture induced by CdCl₂. However, it is important to mention that higher dose of *P. grande* extract was more effective against the architectural damage induced by cadmium nephrotoxicity.

In conclusion, mechanism of the protective action of *P. grande* might be due to its free radical scavenging activity. How-

ever more studies are required before the clinical application of *P. grande* can be recommended for the treatment of cadmium induced nephrotoxicity in the affected population.

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