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Therapeutic efficacy of Picroliv in chronic cadmium toxicity

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ABSTRACT

Cadmium (Cd), an industrial and environmental pollutant, is toxic to several tissues, most notably causing hepatotoxicity on acute administration and nephrotoxicity following chronic exposure. The therapeutic efficacy of Picroliv – a standardized fraction of *Picrorhiza kurroa*, was investigated in male rats treated with Cd as $CdCl_2$ (0.5 mg/kg, sc) 5 days/week for 24 weeks and Picroliv at two doses (6 and 12 mg/kg, p.o.) was given during the last 4 weeks.

The Cd induced levels of malondialdehyde and membrane fluidity and decreased levels of non protein sulphydryls and Na^+K^+ATPase activity of hepatic tissue, along with liver function serum enzymes were restored to near normalcy on treatment with the higher dose of Picroliv. Enhanced excretion of urinary proteins, Cd, Ca and enzymes (lactate dehydrogenase and N-acetyl-β-D-glucosaminidase) evident at 24 weeks of Cd exposure, indicated severe renal damage. Picroliv appeared less effective in causing restoration of these urinary parameters as well as oxidative stress indices in the renal tissue. Picroliv not only reduced the accumulated levels of Cd, Zn and Ca and Cd-metallothionein in liver, but also enhanced the bile flow and biliary Cd. The morphological alterations in liver caused by Cd appeared less marked on Picroliv treatment. However, the renal morphology remained uninfluenced. Our earlier data on 18 weeks of Cd and 4 weeks of Picroliv co-treatment showed significant amelioration of both hepatic and renal manifestations of Cd. The hepatic protection by Picrilov is clearly demonstrated in this study, while marginal lowering of urinary proteins and enzymes is a positive signal of renal protective efficacy of Picroliv, which could be augmented by adopting higher doses and extended regimen.

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1. Introduction

Humans are exposed to cadmium, a ubiquitous metal with no known biological function, mainly through occupation, environmental contamination and from cigarette smoke (Satarug and Moore, 2004). The toxicity of Cd, both in experimental animals and in humans, is influenced by a number of factors such as the route of administration, the dosage, the chemical form of the metal, the duration of exposure and the age of experimental animals (Casalino et al., 1997). Acute Cd exposure primarily results in accumulation of the metal in liver, causing acute hepatotoxicity (Tzirogiannis et al., 2003). Chronic exposure to Cd leading to renal proximal tubular cell injury and dysfunction in both humans and animals (Friberg, 1984), is known to be reversible during early damage conditions but becomes irreversible at an advanced stage (Goyer et al., 1984; Nomiyama and Nomiyama, 1984; Kido et al., 1988).

The deleterious effects of Cd reported to date include oxidative stress (generation of ROS, depletion of GSH, increased lipid peroxidation and altered antioxidant enzymes), modulation of apoptosis, peliosis hepatis and inhibition of DNA repair enzymes (Ercal et al., 2001; Tzirogiannis et al., 2003, 2006; Waalkes, 2003; Lee et al., 2006; Ye et al., 2007). As a result, these alterations are manifested in almost all the organs affected by Cd such as liver, kidney, testes, lungs and bones. It also increases the risk of peripheral arterial disease (Navas-Acien et al., 2004; Yadav et al., 2005; Yadav and Khandelwal, 2008).

Since Cd induced oxidative stress is reported responsible for causing organ damage, antioxidants have been successful in lowering the hepatic and renal Cd and replenishing the GSH levels (Rana and Verma, 1996; Shaikh et al., 1999a; Almazan et al., 2000). In addition to vitamins, some drugs and herbal extracts have also been evaluated in Cd induced tissue damage (Khandelwal et al., 2002; Kowalezyk et al., 2003; Yadav et al., 2005; Yadav and Khandelwal, 2008). Minophagen, a Japanese drug containing glycine, glycyrrhizin and cysteine is shown to protect chronic Cd toxicity by reducing oxidative stress (Shaikh and Tang, 1999). A pentacyclic triterpene (Lupeol) and N-acetyl cysteine are also reported as effective nephroprotective agents against toxic manifestations of chronic Cd exposure (Nagaraj et al., 2000; Shaikh et al., 1999b).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca, calcium; Cd, cadmium; Cu, copper; EDTA, ethylene diamine tetraacetic acid; GGT, gamma glutamyl transpeptidase; LDH, lactate dehydrogenase; MDA, malondialdehyde; MT, metallothionein; NAG, N-acetyl-β-D-glucosaminidase; NPSH, non protein sulphydryl; po, per oral; sc, subcutaneous; Zn, zinc.

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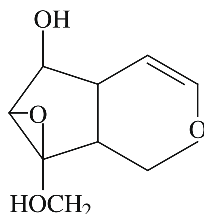
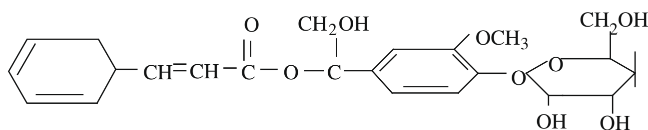
Limited literature on effective herbals against heavy metal toxicity and our previous expertise in the management of acute (Yadav et al., 2005) and early Cd nephrotoxicity (Yadav and Khandelwal, 2006) by Picroliv – a standardized alcoholic extract of root and rhizomes of *Picrorhiza kurroa* (family – Scrophulariaceae) in rat model, prompted us to explore the potential of this herbal extract in Cd induced chronic renal damage. Picroliv contains about 60% picroside I and kutkoside in the ratio of 1:1.5 along with several other uncharacterized glycosides as minor constituents.



Kutkoside



Picroside

Structure of Picroliv

To attain this objective, we treated male rats with $CdCl_2$ for 24 weeks (chronic exposure) and hepatic and renal damage were ascertained by organ specific functional parameters in serum and urine. Toxic and essential metal uptake in the two organs along with oxidative stress markers and morphological alterations were also studied. Picroliv, when administered concurrently during the last month of Cd exposure was able to mitigate hepatotoxicity successfully with some indication of renal protection.

2. Materials and methods

2.1. Chemicals

Cadmium chloride was obtained from Sigma (St, Louis, MO, USA). Picroliv was obtained from Central Drug Research Institute, Lucknow.

2.2. Animals and treatment

Young male albino rats of Druckrey strain weighing 180–200 g were maintained in our animal house under controlled conditions (12 h light/dark cycle with relative humidity of 50–70%, temperature 25 ± 2 °C and 12–15 air changes per h). They were fed with standard rodent pellet and water *ad libitum*. Our animal house and breeding facility are registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and CPCSEA guidelines were followed (IAEC approval obtained). The animals were grouped in batches of 20.

The grouping chart is as below:

- Group I – Control saline (1 ml/kg/day, sc) for 24 weeks.
- Group II – Saline for 24 weeks and Picroliv (6 mg/kg, po) from week 21–24.
- Group III – Saline for 24 weeks and Picroliv (12 mg/kg, po) from week 21–24.
- Group IV – Cadmium chloride as Cd (0.5 mg/kg/day, sc) for 24 weeks.
- Group V – Cd for 24 weeks and Picroliv (6 mg/kg, po) from week 21–24.
- Group VI – Cd for 24 weeks and Picroliv (12 mg/kg, po) from week 21–24.

2.3. Urine and bile collection

At periodic intervals (week 20, 21, 22, 24), rats were placed in steel metabolic cages individually for 18 h and urine was collected over ice. Prior to sacrifice, the animals were anaesthetized by urethane and their bile duct cannulated for bile collection.

Urine samples were centrifuged at 300*g* (5 min, 0 °C) and the volume recorded. The clear supernatant was used for the estimation of total protein, lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase (NAG), creatinine, cadmium and calcium.

2.4. Collection of blood and tissue samples

All the animals were sacrificed at the end of week 24. Blood was collected from jugular vein for serum separation and liver and kidney were dissected and cleaned free of extraneous material. A part of both the tissues was immediately put in 10% neutral buffered formalin for histopathological examination. A piece of liver and half kidney was kept at −20 °C for the estimation of Cd and other metals. The remaining kidney and a portion of liver was used for the assay of lipid peroxidation, NPSH, membrane fluidity, Na^+ K^+ATPase and metallothionein.

2.5. Estimation of urinary enzymes, total protein, serum enzymes, lipid peroxidation, non protein sulphydryls (NPSH), membrane fluidity, Na^+ K^+ATPase, metallothionein (MT) and metal (Cd, Cu, Zn, Ca) levels

The N-acetyl-β-D-glucosaminidase (NAG) activity in urine was measured by the method of Bourbouze et al. (1984) and urinary creatinine by Jaffe's method as modified by Bartels and Boehmer (1971) using creatinine as standard. The lactate dehydrogenase (LDH) activity in urine was measured on Autolab (Autoanalyser of Boehringer Mannheim, Germany) using their commercial kit (Wacker et al., 1956). Total protein was determined by the protein–dye binding method of Bradford (1976) by using BSA as standard.

Serum was refrigerated and used for biochemical estimations the very next day. The clinical biochemical analysis of serum was performed on Autolab (Autoanalyser of Boehringer Mannheim, Germany). Analysis was carried out at 37 °C as per the instructions of the manufacturer (Boehringer Mannheim, Application sheets, 1995). Briefly, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the International Federation of Clinical Chemistry Kinetic method (Bergmeyer and Hoerder, 1980), alkaline phosphatase (ALP) using AMP buffer, following the Kinetic method of Bowers and McComb (1972), gamma glutamyl transpeptidase (GGT) assay was according to the kinetic method of Szasz (1974) and lactate dehydrogenase (LDH) according to the recommendations of the German Society for Clinical Chemistry (DGKC) using lactate as substrate and NAD as coenzyme (Wacker et al., 1956). Urea was estimated by the kinetic UV method (Neumann and Ziegenhorn, 1977) and hemoglobin by cyanmethemoglobin method (Van Kampen and Zijlstra, 1961).

A 20% (w/v) homogenate of liver and kidney was prepared in 1.15% KCl for assay of lipid peroxidation, membrane fluidity and Na^+K^+ATPase and a 10% (w/v) homogenate in 0.02 M EDTA was used under ice cold conditions for same day estimation of NPSH levels. A part of 1.15% KCl homogenate was spun at 10,000*g* (10 min, 0 °C) and the supernatant fractions were used for MT determination.

The estimation of lipid peroxidation was monitored by malondialdehyde (MDA) formation by the method of Ohkawa et al. (1979) and NPSH levels by Sedlak and Lindsay (1968).

For membrane fluidity, the liver and renal homogenate (20% w/v) were centrifuged at 100,000*g* (60 min, 0 °C) and the membrane was treated with 2 mM diphenylhexatriene (DPH) in tetrahydrofuran as fluorescent probe and incubated for 60 min at 37 °C. Fluorescence polarisation as a measure of membrane fluidity was determined on spectrofluorometer with perpendicular and parallel polarizers at excitation wavelength of 350 nm and emission wavelength of 432 nm (Shinitzky and Barenhoiz, 1978).

The activity of Na^+K^+ATPase was determined in liver and kidney supernatants obtained from 20% (w/v) homogenate spun at 800*g* (10 min, 0 °C). The activity was measured as net difference in the rate of release of inorganic phosphate in presence of 4 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl and 4 mM ATP and the rate of release in the same medium with 1 mM ouabain added (Colowick and Kaplan, 1974). In the supernatant, inorganic phosphate (Pi) was measured by the method of Fiske and Subba Row (1925). Protein in the samples was estimated by the method of Lowry et al. (1951) using BSA as standard.

Metallothionein (MT) in liver and kidney was measured by the method of Eaton and Toal (1982).

Wet acid digestion procedure of Parker et al. (1967) for metal quantitation was followed. Cadmium, Ca, Cu and Zn were analyzed on flame atomic absorption spectrophotometer (Varian 250 plus) at 228.8 nm for Cd, 422.7 nm for Ca, 324.7 nm for Cu and 213.9 nm for Zn. Respective metal standards were used.

2.6. Histopathological studies

For histopathological assessment of hepatic and renal damage, tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (McManus and Mowry, 1965) and assessed under a light microscope. The extend of necrosis in liver and kidney was analyzed semi-quantitatively by using a scale of severity on a five point score of 0–4; 0 = no necrosis, 1 = necrosis of 1–5%, 2 = necrosis of 6–25%, 3 = necrosis of 26–50%, 4 = necrosis of >50% in all the groups.

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