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Peucedanum grande attenuates acute renal failure and oxidative stress induced by Mercuric chloride in rodents

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ABSTRACT

Peucedanum grande has been found to be associated with the multiple therapeutic properties. In the present study, we have used *P. grande* as an ameliorating agent against nephrotoxic effects of Mercuric chloride ($HgCl_2$). The rats were given pretreatment of *P. grande* orally at a dose of 60 and 120 mg/kg body weight for five consecutive days. Mercury chloride 4 mg/kg body .wt was used as renal toxicant, and injected subcutaneously in the neck region in a volume of 1 ml/kg. The modulatory effects of *P. grande* on $HgCl_2$ induced nephrotoxicity was investigated by assaying oxidative stress biomarkers, lipid peroxidation, serum kidney toxicity markers and by histopathological examination of kidney. The $HgCl_2$ induced nephrotoxicity by depleting antioxidant levels, elevating the level of serum creatinine and BUN, as well as damaging the normal architecture of kidney. *P. grande* pretreatment prevented deteriorative effects induced by $HgCl_2$ through a protective mechanism that involved reduction of increased oxidative stress as well as by restoration of histopathological change against $HgCl_2$ administration.

Keywords: *Peucedanum grande*, Mercuric chloride, Kidney, histopathology, oxidative stress.

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INTRODUCTION

Mercury (Hg) is a naturally occurring metal that is present everywhere all through the environment. It occurs in inorganic forms including metallic (Hg^0), mercurous (Hg^+), and mercuric (Hg^{2+}) valence states [1]. Mercury is found in household products as barometers, blood pressure instruments, and switches in automobiles. It is also found in switches in children's shoes that light up, thermometers, as well as in dental restoration as an amalgam. Its mercuric form is found in fluorescent lights [2]. Various studies on the reproductive effects of inorganic Hg have been done such as reproductive failures, menstrual cycle disorders, primary subfecundity, and adverse pregnancy outcomes [3, 4]. Recent study has been done on females working in an Hg exposed environment which exposed to elevated frequency of undesirable reproductive outcomes, particularly congenital anomalies [5]. It has been evaluated that exposure to inorganic Hg in animal models cause an ovulation in hamsters [2]. It has been studied in both male and female rats when exposed to mercuric chloride ($HgCl_2$) and then mated together, made known significant differences in implantation efficiency, fertility, live births and litter size [6]. Recent studies have been done on the role of oxidants and free radicals in the kidney injury induced by $HgCl_2$. For example, $HgCl_2$ increases the production of hydrogen peroxide [7], depletes glutathione (GSH), and reduces free radical scavenging systems such as SOD [8].

In $HgCl_2$-induced nephrotoxicity, the reduction in renal content of antioxidants such as GSH, as well as the decrease of thiol groups, has been advanced as evidence of the role of ROS in this pathology [9,10].Since ancient times herbal plants have been used for the cure of human diseases and are attaining more attention due to less toxicity and high efficiency. *Peucedanum grande* have several name like Duku, Baphalle, Wild carrot, Hingupatri, belongs to family Umbelliferae [13]. In Unani system Duku (Peucedanum grande) is known to its medicinal values like, Diuretic (Mudir–e-Baul), Emmenagogue (Mudir-e-Haiz), Aphrodisiac (Muqawwi-e-Bah), Demulscent (Mulattif), Deobstruent (Mufatteh), Urolithotriptic (Mufattite–Hissat-e-Gurda-Wa-Masana), Anti-Inflammatory (Mohallil-e-Auram), Antidote (Daaf-e-Sammyat), Concoctive/Maturative (Munzij), etc [14,15,16,17].

The aim of this study was to determine if *P. grande* shows protection against $HgCl_2$ induced renal toxicity. The extent of the protective effect of *P. grande* against nephroprotective effects were determined by studying serum marker enzymes 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*

The 2 kg dried seeds/fruits of the drug *Peucedanum grande* was extracted exhaustively extracted with methanol by using a Soxhlet apparatus over boiling water bath for 3 hr. 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 method under reduced pressure. The yield of extract was 12 % w/w in the terms of starting materials. The yield of extract was calculated with respect to starting material.

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), trichloroacetic acid (TCA) etc were obtained from Sigma-Aldrich, USA. India. All other reagents and solvents were of a high analytical grade.

Animals

Eight week old male wistar rats (150-200g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi and were housed in a ventilated room at 25 ± 5 ^{0}C under a 12 hr 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 *adlibitum.* 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% CMC as a suspending agent in distilled water.

- Animals of Group 1 (Control) received orally 1% CMC in distilled water (10 mg/kg b.wt) for 5 consecutive days.
- Animals of group II (Toxicant) received orally 1% CMC in distilled water (10 mg/kg b.wt) for 5 consecutive days. Mercury chloride 4 mg/kg b.wt was injected subcutaneously in the neck region in a volume of 1 ml/kg b.wt on 6th day.
- Animals of group III received a single lower dose (60 mg/kg b.wt) suspended in the vehicle (10 ml/kg b.wt) for 5 consecutive days. Mercury chloride 4 mg/kg b.wt was injected subcutaneously in the neck region in a volume of 1 ml/kg b.wt on 6th day.
- Animals of group IV received higher dose (120 mg/kg b.wt) suspended in the vehicle (10 ml/kg b.wt) for 5 consecutive days. Mercury chloride 4 mg/kg b.wt was injected subcutaneously in the neck region in a volume of 1 ml/kg b.wt on 6th day.

Mercury chloride 4 mg/kg b.wt was used as nephro toxicant and was injected subcutaneously in the neck region in a volume of 1 ml/kg b.wt. The blood samples withdrawn from retro-orbital venous plexus were centrifuged, and sera thus obtained were investigated to determine the concentrations of blood urea nitrogen (BUN) and serum creatinine. Animals were sacrificed and their kidney were isolated for histopathological studies and post-mitchondrial 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 KCI (1.17%) using a Potter Elvehjen homogenizer. The homogenate was filtered through muslin cloth, and was centrifuged at 800 × g for 5 min at 4 ^{0}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 ^{0}C to obtain PMS, which was used as a source of enzymes. All the biochemical estimations were completed within 24 hr of animal sacrifice [18.]

Estimation of Blood Urea Nitrogen

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 mixture was centrifuged at 2000 r.p.m. and supernatant was taken. To 0.5 ml of protein free filtrate, were added 3.5 ml of distilled water, 0.8 ml diacetylmonoxime (2%) and 3.2 ml sulphuric acid–phosphoric acid reagent (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[19].

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[20].

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 1ml (10%) and TBA 1.0 ml. Test tubes having reaction mixture were kept in boiling water for about 45 minutes and transferred on to ice cooled water and then centrifuged at 2500 x g for 10 minutes. The malondialdehyde formation in each sample was detected as optical density observed at 532 nm. The results were expressed as nmole of MDA formed per minutes per gram of tissue using molar extension coefficient 1.56 x 10^5 M^{-1} cm^{-1} [21].

Estimation of reduced glutathione

For reduced GSH, 1.0 ml of 10% PMS mixed with 1.0 ml of 4% sulphosalicylic acid, Then incubated at 4 ^{0}C for a minimum time period of 1 hr and then centrifuged at 4 ^{0}C at 1200×g for 15min.Briefly reaction mixture having 0.4 ml supernatant, 2.2 ml phosphate buffer (0.1M, pH 7.4) and 0.4 ml DTNB (4 mg/ml) making a total volume of 3.0 ml. The yellow colour 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 [22].

Assay for superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured by the method of Beauchamp and Fridovich, (1971). The reaction mixture of total volume 1.0 ml consisted of 0.5 M phosphate buffer pH 7.4, 0.1 ml PMS, 1.0 mM xanthine, and 57 µM NBT. It was incubated for 15 min at room temperature and reaction was initiated by the addition of 50-mU xanthine oxidase. The rate of reaction was measured by recording change in the absorbance at 550 nm due to formation of formazan, a reduction product of NBT [23]

Assay for Catalase activity

In case of catalase activity, the reaction mixture comprised of 0.05 ml PMS, 1.0ml hydrogen peroxide (0.019M), 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 change in absorbance was calculated as nmol H_2O_2 consumed per min per mg protein [24].

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 400 x magnification.

Statistical analysis

Differences between groups were analyzed using analysis of variance followed by Dunnet's multiple comparisons test. All data points are presented as the treatment groups mean ± S.E.

RESULT AND DISCUSSION

Mercuric chloride ($HgCl_2$) administration to experimental animals is a well-known model of nephrotoxic acute renal failure and is widely employed to study its pathophysiology. The functional alterations, histological changes and hydrodynamic alterations induced in this model are well understood [25, 26]. Some of the major cellular and biochemical lesions induced by $HgCl_2$ include impairment of mitochondrial function, hampering of oxidative phosphorylation, derangement of plasma membrane phospholipids and the underlying cytoskeleton, impaired calcium sequestration by the mitochondria and induction of calcium dependent injury, complexation with critical thiols in the cell etc. Although the mechanism underlying $HgCl_2$ toxicity is not clearly understood, several hypotheses have been proposed. Of these, a critical one includes induction of oxidative stress. A number of evidences advocate the role of oxidative stress in Hg (II) induced renal toxicity. For instance, $HgCl_2$ exposure augments H_2O_2 production by the mitochondria in renal epithelial cells [27]. $HgCl_2$ leads to attenuation of antioxidant armory depleting GSH and inhibiting the activities of antioxidant enzymes in kidney tissues [28, 29]. $HgCl_2$ exposure has also been shown to induce lipid peroxidation both in vitro and in vivo [30, 31]. Furthermore, reports also exist advocating complexation of Hg (II) to cellular thiols to promote ROS formation [32]. Considering oxidative stress to play a crucial role in $HgCl_2$ induced nephrotoxicity, compounds with antioxidant potential are expected to have a protective role against it. In notion to this, melatonin, a known lipid peroxidation retarder, has been found to have a protective effect against $HgCl_2$ induced acute renal toxicity [33, 34]. Likewise, a number of plant extracts with antioxidant properties have been shown to inhibit $HgCl_2$ induced renal toxicity [35, 36, 37]. Protective effect of *P. grande* on serum BUN and creatinine level was observed as shown in table 1. Significant protection ($p < 0.01$, $p < 0.001$) in these marker enzymes were

observed in the *P. grande* pretreatment group and found to be effective in the normalization of these markers when compared to $HgCl_2$ treated group.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 $HgCl_2$ thus ameliorating nephrotoxicity on $HgCl_2$ toxicity in rats.

Table.1 Results of pre-treatment of Peucedanum grande on GSH, catalase, LPO and SOD on administration of HgCl2 in kidney of wistar rats.

Treatment regimen per group	**GSH (n mol CDNBConjugate formed /g tissue)**	**Catalase (nmol H_2O_2consumed/ min/mg protein)**	**SOD** (Units/mg of protein)	**(n mol TBARS / mg protein)**
Group I (control 10 ml/kg)	0.302 ± 0.005	179.0 ± 1.4	6.67 ± 0.08	3.6 ± 0.12
Group II ($HgCl_2$)	0.235 ± 0.006***	146.0 ± 1.5***	4.50 ± 0.15***	10.1 ± 0.13***
Group III (*P.grande* D1+ $HgCl_2$)	0.264 ± 0.003$^{\#}$	164.5 ± 1.4$^{\#\#}$	5.37 ± 0.14$^{\#}$	6.3 ± 0.10$^{\#}$
Group IV (*P.grande* D2+ $HgCl_2$)	0.285 ± 0.002$^{\#\#}$	167.7 ± 1.1$^{\#\#\#}$	5.94 ± 0.02$^{\#\#}$	4.62 ± 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 HgCl2 treated group (#P < 0.05), (##P < 0.01) and (###P < 0.001). D1 = 60 mg/kg b wt; D2 = 120 mg/kg b wt.

In the process of lipid peroxidation there is a production of malondialdehyde which is formed by the conversions of polyunsaturated fatty acid or lipid peroxides. They damage the membranes, cells and even tissues. Malondialdehyde react with thiobarbituric acid and generate red coloured products[38] namely which was significantly increased in mercuric chloride treated rats as compared to controls. A significant ($p < 0.001$) increase of the MDA formation was found in the $Hgcl_2$ treated group when compared with control group as shown in table 2. It has been observed that pretreatment with *P. grande* at both doses D1 and D2 leads to significant ($p < 0.05$ and $p < 0.01$ respectively) prevention of membrane damage by reducing the elevated level of LPO in liver when compared to only $Hgcl_2$ treated group.

Table.2 Results of pre-treatment of *Peucedanum grande* on serum marker enzymes.

Treatment regimen per group	**BUN** (mg/dl)	**Creatinine** (mg/dl)
Group I (control 10 ml/kg)	11.91 ± 1.1	0.160 ± 0.018
Group II ($HgCl_2$)	80.0 ± 7.26***	0.558 ± 0.026***
Group III (*P.grande* D1+ $HgCl_2$)	51.38 ± 6.12$^{\#\#}$	0.470 ± 0.019$^{\#\#}$
Group IV (*P.grande* D2+ $HgCl_2$)	61.61 ± 6.15$^{\#\#\#}$	0.340 ± 0.46$^{\#\#\#}$

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 $HgCl_2$ treated group ($^{\#\#}$P < 0.01) and ($^{\#\#\#}$P < 0.001). D1 = 60 mg/kg b wt; D2 = 120 mg/kg b wt.

In the present study the methanolic extract of *P. grande* dose dependently reduced the increased lipid peroxidation level. Modulation in the antioxidant enzyme activity was also observed. Reduced glutathione known to be a first line of defence neutralizes the hydroxyl radical and plays a key role against inflammatory responses and oxidative stress [39]. Protective effect of *P. grande* on GSH level was observed as shown in table 2. GSH level was depleted significantly ($p < 0.001$) in $Hgcl_2$ treated group as compared to the control group. The GSH level in *P. grande* pre-treated groups is increased significantly ($p < 0.05$, $p < 0.01$) as compared to $Hgcl_2$ treated group A significant restoration of reduced glutathione, to normal levels in the dose dependent manner in *P. grande* pre-treated groups was observed.

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 $HgCl_2$ treatment diminished the level of catalase in the kidney compare to vehicle treated control ($P<0.001$) as shown in table 2. Pretreatment of rats with *P. grande* dose dependently enhanced the reduced level of catalase significantly ($p < 0.01$, $p < 0.001$).However, pretreatment of *P. grande* dose dependently enhanced catalase activity significantly. SODs are a class of enzymes which catalyze the dismutation of superoxide into hydrogen and peroxide oxygen. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Only $HgCl_2$ treatment group diminished the level of SOD as compared to vehicle treated control. The kidney superoxide level decreased in $HgCl_2$ treated rats as compared to control animals ($P<0.001$) as shown in table 2. The SOD level in *P. grande* pre-treated groups is increased significantly ($p < 0.05$, $p < 0.01$) as compared to $HgCl_2$ treated group. Pretreatment of rats with methanolic extract *P. grande* dose dependently enhanced the reduced level of SOD significantly. The main histological finding of this study was that $HgCl_2$ group showed acute tubular necrosis and glomerular widening, however pretreatment with *P. grande* protected the kidney architecture from damage induced by $HgCl_2$. Normal glomerular and tubular histology was seen both in cortical and medullary regions of kidney in control rats. Mercuric chloride was found to cause severe glomerular and peritubular congestion. There was also severe invasion of inflammatory cells seen in both cortical and medullary section from the Mercuric chloride treated group. In addition to these features of Mercuric chloride nephrotoxicity, necrosis of tubular structure was seen in proximal and distal portions. The inner cortical and outer medullary regions exhibited more damage than the inner medullar regions of the kidney. In contrast, renal sections obtained from rats that were pre-treated with *Peucedanum grande* at a dose of 60 mg/kg b.wt. Showed partial reduction of the histological features of renal injury (Figure 1). Pretreatment of

Peucedanum grande at 120 mg/kg b.wt was associated with more reduction in injury almost similar to control rat kidney. In conclusion, mechanism of the protective action of *P. grande* against nephrotoxicity of mercury might be due to its free radical scavenging activity.

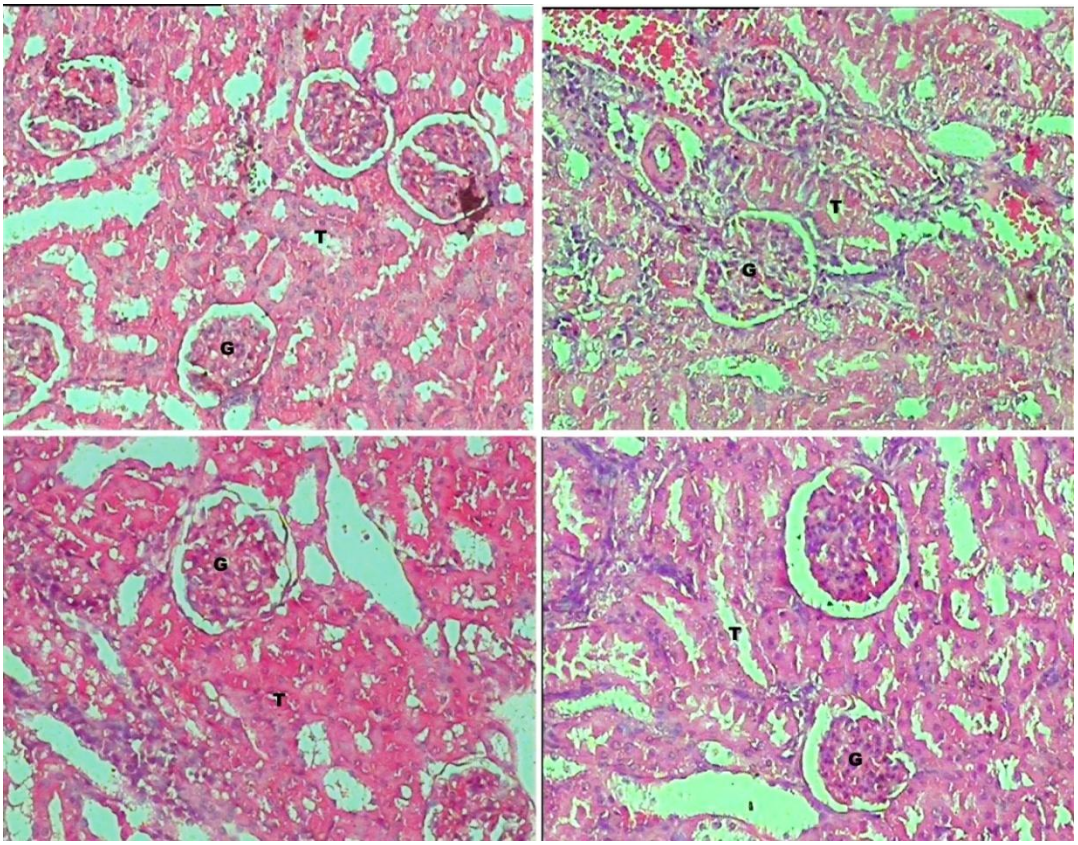

Figure1. Liver histology of Rats (x 400 magnifications): G – Glomerulus and T – Tubules.

(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 ($HgCl_2$) group showed acute tubular necrosis and glomerular widening.
(C) Low 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.
(D) High dose of *P. grande* showed less necrosis of the proximal tubular lining epithelial cells along with cellular swelling, desquamation and loss of brush border.

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