Carrot (*Daucus carota* L.): Nephroprotective against gentamicin-induced nephrotoxicity in rats

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**Introduction**

Environmental pollutants, chemicals, and drugs such as antibiotics can drastically alter the anatomical and physiological functions of various organs such as kidney, heart, liver, and intestine. However, drugs such as antibiotics became the major implicating factor in the acute kidney injury due to the indigenous functions of kidney to excrete them. This acute renal injury often leads to renal failure which in turn associated to other pathological manifestations such as sepsis, cardiovascular disorders, and diabetes.

Aminoglycoside antibiotics are clinically effective in the treatment of Gram-negative bacterial infections, but they more commonly implicate the nephrotoxicity by free radical generation, loss of brush-border integrity, acute tubular necrosis, and glomerular congestion, which ultimately leads to reduced glomerular filtration rate and acute renal dysfunction. However, several pharmacological interventions such as antibiotics, calcium channel blockers, beta blockers, iNOS inhibitors, nitric oxide precursors, antianginal, hormones,...

**ABSTRACT**

**Objectives:** *Daucus carota* L.(DC) commonly known as carrot, folkorically used as ethnomedicine to treat nephrosis and other urinary disorders. Hence, the present study was aimed to investigate the nephroprotective effects of ethanolic root extract of DC against gentamicin-induced nephrotoxicity in Albino Wistar rats.

**Methods:** Nephrotoxicity in rats was induced by intraperitoneal administration of gentamicin (100 mg/kg/day) for 8 days. Rats of either sex were divided into four groups (*n* = 6). Group 1 served as control that received normal saline (i.p.) whereas Group 2 (GM) was treated with gentamicin which served as gentamicin-intoxicated group. Group 3–4 (DC200, DC 400) were pretreated with DC at doses of 200 mg/kg and 400 mg/kg (p.o.), respectively, 1 h before the gentamicin intoxication. Following treatment, the nephroprotective effects of DC were evaluated by using serum levels of urea, blood urea nitrogen (BUN), uric acid, and creatinine levels; change in body weight and wet kidney weight along with the histological observations among the experimental groups.

**Results:** Gentamicin intoxication induced elevated serum urea, BUN, uric acid, and creatinine levels which was found to be significantly (*P* < 0.01) decreased in a dose-dependent manner in groups received DC which was also evidenced by the histological observations.

**Conclusion:** DC showed a significant nephroprotective effect in a dose-dependent manner by ameliorating the gentamicin-induced nephrotoxicity and thus authenticates its ethnomedicinal use.

**KEY WORDS:** Carrot, ethnomedicine, gentamicin, nephroprotective, nephrosis
antiplatelet, statins, peroxisome proliferator-activated receptor
gamma agonists, tumor necrosis factor alpha synthesis
inhibitors, biguanides, antioxidants, and super oxygen
dismutase mimetics had the potential to halt the progression
of gentamicin-induced nephrotoxicity, but their clinical setting
needs to be debated.[31]

*Daucus carota* L. (DC) is a root vegetable commonly called
as carrot, which is a biennial plant commonly cultivated in
Northern India. The most abundant phytocomponents present
in carrot are phenolics, polyacetylenes, carotenoids, ascorbic acid,
and tocopherol.[14] It was documented that this plant had the
potentials of hypotension,[10] antifertility,[11] hepatoprotective,[12]
antispasmodic,[13] antibacterial,[14] monoamine oxidase
inhibition,[15] and cyclooxygenase enzyme inhibition.[16] However, traditionally, it was used in the treatment of nephrosis as
a nephroprotective agent.[17] However, so far, no scientific
validity has been made to establish it as a nephroprotective
agent. Hence, in the present study, an effort has been made to
authenticate its traditional use by using ethanolic root extract
of DC against gentamicin-induced nephrotoxicity in Albino
Wistar rats.

**Materials and Methods**

**Chemicals and Reagents**

Gentamicin was procured as marketed formulation (Ranbiotic)
from Ranbaxy laboratories (Batch no. 9753675), New Delhi,
India. The diagnostic kits for the estimation of uric acid (Lot
no. B12121) and blood urea nitrogen (BUN) (Lot no. B041328)
were obtained from ERBA Mannheim, Transasia bio-medicals
limited, Himachal Pradesh, India, and the diagnostic kits of
Urea (Batch no. EUB-007) and Creatinine (Batch no. ECK-087)
were obtained from Excel diagnostics, Hyderabad, India. All
the other required chemicals were of analytical grade and they
were obtained from Qualigen fine chemicals, Mumbai, India.

**Experimental Animals**

The experimental protocol was carried out by using Albino
Wistar rats of both sex weighing about 180–220 g procured
from Sri Venkateshwara Enterprises, Bengaluru, India, and
1-week acclimatization was done as per CPCSEA guidelines
before the study was carried out. Animals were housed in clean
polypropylene cages and they were fed with standard pellet
diet and water *ad libitum* during the study. This experimental
study was approved by Institutional Animal Ethical Committee
of Sree Vidyanikethan College of Pharmacy, Tirupati, India, with
the approval no. SVCP/IAEC/1-006/2013-14.

**Plant Material**

The fresh roots of DC were obtained from the local market
of Tirupati, Andhra Pradesh, India, in the month of May, 2014,
and they were authenticated by Prof. P. Jayaraman at the Plant
Anatomy Research Centre (PARC), Chennai, India. A voucher
specimen of this plant material was deposited in the herbarium
of PARC with voucher specimen no. PARC/2014/2290.

**Extraction**

The roots were shade dried, powdered, and sieved (mesh
no. 40) to get coarse powder. Then, this powdered plant
material was subjected to soxhletation using absolute ethanol
as solvent for 72 h at a temperature of 40°C. After filtration,
it was evaporated by using rotary vacuum evaporator at a
temperature not exceeding 40°C to get crude extract. The yield
was found to be 16.9% w/w.

**Preliminary Phytochemical Analysis**

A preliminary phytochemical analysis was carried out for the qualitative identification of phytocomponents in DC.[11,19]

**Acute Oral Toxicity Studies**

Acute oral toxicity study was carried out in accordance
of OECD guidelines No. 423 by using female Wistar rats.
Four dosing levels (5, 50, 300, and 2000 mg/kg) (p.o.) were
considered to carry out acute oral toxicity study. Three animals
were selected for single escalating testing dose to observe the
signs of toxicity and mortality for a period of 14 days.[20]

**Gentamicin-Induced Nephrotoxicity**

Twenty-four healthy Albino Wistar rats of either sex were
weighed and grouped randomly into four groups (n = 6). Group 1
served as normal control (CON) receiving normal saline (i.p.) and
0.5% carboxymethyl cellulose (CMC) orally for 8 days whereas
Group 2 considered as gentamicin-intoxicated group (GM)
receiving gentamicin (i.p.) at a dose of 100 mg/kg/day and
0.5% CMC (p.o.) for 8 days. Group 3 (DC200) and group 4 (DC
400) were considered as treatment groups that received DC at
doses of 200 mg/kg/day and 400 mg/kg/day (p.o), respectively,
an hour before gentamicin intoxication for a period of 8 days.[1]

**Evaluation of Nephroprotective Potential**

**Biochemical analysis**

After 24 h of experimental protocol, the animals were
euthanized by cervical dislocation under mild ether anesthesia
and blood was collected by cardiac puncture. Then, the blood
was made to coagulate by leaving them undisturbed for 1 h at
4°C and it was centrifuged (REMI, R-8°C laboratory centrifuge)
at 3000 rpm for 15 min to separate serum. The serum was
stored at -5°C until the analysis of serum urea, BUN, uric acid,
and creatinine using diagnostic kits.

**Change in body weight and wet kidney weight**

At the final day of the experimental procedure, the body
weights were measured to evaluate the change in the body
weight from the initial body weight.[21,22] After the blood
collection, the kidneys were excised, weighed, and the result was
expressed as wet kidney weight/100 g of body weight to assess
the change in kidney weight among the experimental groups.[23]

**Histopathology**

The excised kidneys from sacrificed animals were fixed
in 10% neutral formalin solution immediately for a period of
24 h. Then, they were processed for dehydration using absolute ethanol, cleaned in xylene, and embedded in paraffin.
The sectioning was made by using microtome apparatus at a
thickness of 4 µm and stained with eosin and hematoxylin. The histopathology changes of each section were observed and
photographed (×45) by using light microscope equipped with
digital camera (OLYMPUS BX51TRF, China).

**Statistical Analysis**

The results were expressed as mean ± standard error
of mean (n = 6) and the statistical analysis was made by
one-way ANOVA followed by Dunnett’s comparison test using
computerized Graphpad prism (version 5.0, trial version,
Graphpad soft ware, USA) software at a level of significance
of P < 0.01.
Results

Preliminary Phytochemical Analysis

The preliminary qualitative phytochemical analysis revealed the presence of carbohydrates, amino acids, polyphenols, terpenoids, and phytosterols.

Acute Oral Toxicity Study

It was observed that there were no clinical signs of toxicity and mortality for a period of 14 days at a testing dose of 2000 mg/kg. As a result of this, one-tenth of the maximum tolerated dose of DC was selected as therapeutic low dose (200 mg/kg, DC200) and double of this low dose was considered as highest dose (400 mg/kg, DC400) for this study.[1]

Biochemical Analysis

Gentamicin intoxication in GM group significantly (P < 0.01) increased the serum levels of urea, BUN, uric acid, and creatinine compared to CON group. However, with the co-administration of DC at doses of 200 mg/kg/day and 400 mg/kg/day, the elevated serum urea, BUN, uric acid, and creatinine levels were curtailed to a great extent in DC200 and DC400 groups in a dose-dependent fashion [Table 1].

Effect on Change in Body Weight and Wet Kidney Weight

At the end of the study, gentamicin intoxication made a significant (P < 0.01) weight loss as compared with CON group and the concurrent treatment of DC ablated the gentamicin-induced weight loss significantly in DC200 and DC400 [Figure 1]. On the other hand, the wet kidney weight of GM group significantly increased (P < 0.01) compared with CON group [Figure 2]. However, the treatment curtails the gentamicin-induced renal weight gain significantly in DC200 and DC400 groups.

Histopathology

The histopathology changes of each kidney section were interpreted in terms of presence of hyaline casts, glomerular congestion, mononuclear cell infiltration, tubular necrosis and degeneration, and intertubular hemorrhage microscopically and they were depicted in Table 2. The microscopical investigation of CON group revealed the normal renal tubular morphology with intact glomerulus [Figure 3], whereas the GM showed the majority of the histopathological events of gentamicin intoxication [Figure 4]. It was observed that treatment groups of DC remarkably ameliorate the gentamicin-induced acute renal damage manifestations in histology [Figures 5 and 6]. Indeed, DC400-treated group showed normal renal morphology even comparable to that of CON group with slight intertubular hemorrhage and sporadic hyaline casts.

Discussion

Despite the use of gentamicin was limited with nephrotoxicity, it becomes a promising therapeutic antibiotic due to its potent bactericidal and less bacterial resistance properties. Accumulation of drug tends to be a primary key pathological event in gentamicin-inducing nephrotoxicity and subsequent renal dysfunction.[24] Being as cationic in nature, gentamicin has a strong affinity toward negatively charged brush-border membrane components of proximal tubule where it forms drug receptor complex with megalin, a cationic drug receptor. Then, pinocytosis translocates the drug to lysosomes, where phospholipidosis takes place to interrupt various intracellular renal functions leading to renal injury.[25,26] This renal injury in turn manifests the migration of monocytes and macrophages to the site of injury by stimulating intercellular adhesion molecule-1 and monocyte chemoattractant protein[27,28] while several other studies reported the role of reactive oxygen species in implicating the pathogenesis of gentamicin-induced nephrotoxicity.[29]

Gentamicin-intoxicated nephrotoxicity is functionally evident by the elevated serum levels of urea, BUN, uric acid, and creatinine; structurally characterized by tubular necrosis, glomerular atrophy, mononuclear cell infiltration, intertubular hemorrhage, and hyaline casts. Similar sort of alterations were

Table 1:

Effect of Daucus carota L. on serum levels of urea, blood urea nitrogen, uric acid, and creatinine in gentamicin-induced nephrotoxicity in Wistar rats

| Parameter (mg/dl) | CON | GM | DC200 | DC400 |
|------------------|-----|----|-------|-------|
| Urea             | 38.4±1.60 | 79.1±2.21** | 57.26±1.60** | 45.96±2.37** |
| BUN              | 16.69±2.48 | 37.23±1.36** | 24.54±2.85** | 20.21±3.38** |
| Uric acid        | 2.25±0.44 | 4.87±0.29** | 3.14±0.21** | 2.83±0.35** |
| Creatinine       | 0.74±0.06 | 2.03±0.08** | 1.51±0.25** | 1.04±0.08** |

Values are expressed as mean±SEM; *P<0.05, **P<0.01, *Compared to CON, 1Compared to GM. BUN=Blood urea nitrogen, CON=Control, GM=Group 2, DC=Daucus carota L., SEM=Standard error of mean

Figure 1: Effect of Daucus carota L. on body weight of gentamicin-intoxicated rats; data were expressed as mean ± standard error of mean (n = 6); **P < 0.01 versus control, *P < 0.05 versus control, ##P < 0.01 versus GM

Figure 2: Effect of Daucus carota L. on wet kidney weight of gentamicin-intoxicated rats; data were expressed as mean ± standard error of mean (n = 6); **P < 0.01 versus control, *P < 0.05 versus control, **P < 0.01 versus GM
observed with the gentamicin treatment in GM and treatment groups. As an indicative of decrease in glomerular filtration rate, there was an increase in the serum creatinine levels in the GM group, whereas the serum urea and BUN were found to be increased as an indicative of parenchyma tissue injury after tubular necrosis. The serum uric acid levels were found to be increased because of accumulation by the decrease in glomerular filtration rate in gentamicin-intoxicated rats, but with the supplementation of DC, it dose dependently ameliorated the gentamicin-induced elevated serum levels of urea, BUN, uric acid, and creatinine in DC200 and DC400 groups. However, in DC400 group, the nephroprotective property was found to be very prominent with high dose when compared with DC200 group. These results notified the improved renal function by the effective clearance of urea, BUN, creatinine, and uric acid.

Moreover, gentamicin induced weight loss that resulted from renal tubular injury and academia‑impaired dehydration, and anorexia was ablated to a great extent by DC treatment. The increased wet kidney weight of gentamicin‑treated rats due to edema induced by acute tubular necrosis was even normalized by the DC treatment. Thus, the results notified that DC may act by antagonizing the gentamicin‑implicated acute renal tubular necrosis and academia to attenuate the nephrotoxicity. The histopathological observations also revealed the protective role of DC in parallel to the results of biochemical analysis. After the gentamicin administration, the GM groups showed the extensive renal tubular necrosis, glomerular atrophy, mononuclear cell infiltration, hyaline casts, and intertubular hemorrhage. However, DC supplementation showed the marked attenuation of histopathological implications induced by gentamicin, especially in DC400 group with slight presence of intertubular hemorrhage and sporadic hyaline casts. Thus, the histopathology results demonstrated the ablated inflammatory events by the cellular anti‑inflammatory properties of polyacetylenes present in carrot.

By considering the oxidative stress in the pathophysiology of nephrotoxicity, antioxidants therapy opted to be an alternative choice in its management. Polyphenolic compounds reported to possess nephroprotective property by promoting antioxidant enzyme system, thereby attenuating ROS generation and lipid peroxidation. In evidence of this, the polyphenolic compounds of DC can contribute to nephroprotection by its antioxidant activity. The natural antioxidants such as β‑carotene, a terpenoid constituent of the crude extract can ameliorate the nephrotoxicity by its free radical scavenging activity.

Thus, all together from the results of biochemical and morphologic pathology, DC had the ability to halt the gentamicin nephrotoxicity dose dependently by having rich antioxidant and cellular anti‑inflammatory property. However, the other mechanisms such as N‑balancing properties and antagonizing principles against phospholipidosis in proximal tubules cannot be ruled out.

### Conclusion

Concurrent administration of ethanolic extract of DC dose dependently attenuated the pathological implications of gentamicin nephrotoxicity to a great extent. Being as a source of rich carotenoid, polyphenolic and polyacetylene constituents in carrot, the principle mechanism to elucidate

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**Table 2:** Effect of *Daucus carota* L. on kidney histopathological changes induced by gentamicin‑induced nephrotoxicity in Wistar rats

| Histopathological changes                  | Treatment     |
|-------------------------------------------|---------------|
|                                          | CON  | GM  | DC200 | DC400 |
| Hyaline casts                             | -    | +++ | +     | +     |
| Glomerular congestion and degeneration    | -    | +++ | ++    | -     |
| Tubular necrosis and degeneration         | -    | +++ | ++    | -     |
| Mononuclear cells infiltration            | -    | +++ | ++    | -     |
| Intertubular hemorrhage                    | -    | +++ | +     | +     |

The severity of histopathological changes were graded as mild (+), moderate (++) and severe (+++), and none (−). BUN=Blood urea nitrogen, CON=Control, GM=Group 2, DC=Daucus carota L.

**Figure 4:** Kidney histopathology of gentamicin‑intoxicated group (x45): A, B-cortex showing glomerular degeneration and atrophy (g), mononuclear cells infiltration (m), renal tubular desquamation (rt), presence of hyaline casts (h)and intertubular hemorrhage (ih); C-medulla showing hyaline casts (h) along with intertubular hemorrhage (ih)
the nephroprotective potentiality possibly attributed due to its antioxidant and cellular anti-inflammatory properties. Thus, it validated the traditional use as an ethnomedicine against nephrosis. However, further studies are needed to identify and characterize the phytoconstituents from DC; and also to explore the exact mechanism to act as nephroprotective, before being establish it in clinical setting.

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Conflicts of Interest

There are no conflicts of interest.

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