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Original Research Article (Experimental)

Anticalcifying effect of *Daucus carota* in experimental urolithiasis in Wistar rats

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ABSTRACT

Background: Urolithiasis is a burgeoning disease that results from pathological biomineralization. *Daucus carota* L. is a widely consumed food crop with reported nephroprotective and diuretic activity. Its potential for *Ashmari bhedan* (destruction of stone/calculi) or treatment of urinary calculi has been explored traditionally. However, no scientific evidence is available to prove its antiurolithiatic efficacy. Moreover, establishing the antiurolithiatic effects of *D. carota*, an extensively consumed commodity with numerous health benefits, would provide a beneficial dietary measure for the prevention and cure of urolithiasis.
Objective: The study aimed at investigating *in vivo* antiurolithiatic potential of hydroethanolic extract of *D. carota* roots against calcium oxalate urolithiasis.
Materials and methods: Ethylene glycol and ammonium chloride induced hyperoxaluria model of urolithiasis in male Wistar rats was used for the study. Urine and serum parameters and, kidney histopathology was used to determine the antilithic efficacy of *D. carota* root extract.
Results: *D. carota* extract significantly ameliorated abnormal urinary levels of calcium, oxalate, phosphate, magnesium, citrate, protein and uric acid in lithogenic rats. Serum BUN, creatinine and uric acid levels; and calcium, phosphate and oxalate deposition in kidney tissue were also rendered normal following *D. carota* treatment. *D. carota* extract also prevented oxidative stress mediated renal tissue degeneration both prophylactically and curatively.
Conclusion: This study suggests antiurolithiatic effect of *D. carota* roots, which can be attributed to its anticrystallization property, ability to ameliorate urine and serum biochemistry and renal cellularity.

1. Introduction

Urolithiasis is a multifactorial disorder that results from pathological biomineralization [1]. A worldwide increase in the incidences of urolithiasis has been recorded. Percent increase in the prevalence rate has been recorded to be 14.8% in Turkey, 7.4% in Taiwan, 5% in Brazil, 4.5% in UK, 4% in India, 3.6% in USA, 2.5% in China and 2.4% in Italy [2]. High recurrence rate of urolithiasis mounting to over 50% relapses in 5–10 years is exacerbating [3]. Moreover, benefits from a plethora of urological interventions available for the treatment of urinary stone disease ranging from medical expulsive therapy to extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy are compromised due to associated complications [4] and tendency to promote recurrence [5]. These treatment options for urolithiasis have emerged as one of the major contributors of chronic kidney disease [6]. Dietary and lifestyle changes that have occurred over decades promote the incidences of renal stone disease [7]. Therefore, dietary modifications and disciplined lifestyle is a key to the prevention of this disorder and others.

Health benefits of diet rich in fruits and vegetables are well known [8]. Moreover, nutraceuticals have emerged as a new venue for research [9]. Carrots or *Daucus carota* L. (Apiaceae) is a widely cultivated food crop in the world. Carrots are of high nutritional value and are a rich source of carotenes specifically α-carotenes and β-carotenes; and polyacetylenes viz. falcarinol, falcarindiol and falcarindiol 3-acetate [10]. Of these polyacetylenes, falcarinol is known to possess allergenic properties as has been reported to cause contact dermatitis [11]. Despite its allergenic potential, falcarinol is extensively explored due to its wide range of beneficial effects ranging from antitubercular, antifungal, antibacterial, anti-

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inflammatory and significant anticancer effects [12,13]. Carrots are consumed extensively as salad and are also used in food industry [14]. Carrots find application in wide range of diseases [15] and are traditionally used for the treatment of urinary stones [16]. Their use for *Ashmari bhedan* and *Mutrajanan* (diuretic) is also mentioned in Ayurveda [17,18]. Despite their established beneficial effects against numerous renal diseases, the antiurolithiatic potential of carrots still remains unexplored. Therefore, this study was conducted to establish preventive and curative role of carrot roots which would provide a simple yet beneficial means of tackling this disease.

2. Materials and methods

2.1. Plant material

Roots of *D. carota* were purchased from local market of Bhimtal region of Uttarakhand and were authenticated from Botanical Survey of India (BSI), Dehradun. A voucher specimen with accession number 116593 was also deposited in the herbarium of BSI.

2.2. Processing and extraction

Collected carrot roots were thoroughly washed under tap water, sliced to fine pieces, shade dried and then powdered. Fine powder was then extracted with 70% v/v ethanol by cold maceration and *D. carota* root extract (DCRE) thus obtained was dried in a rotary evaporator under reduced temperature and pressure [19].

2.3. Preliminary phytochemical screening

DCRE was subjected to preliminary phytochemical evaluation for qualitative estimation of phytoconstituents viz. carbohydrates, proteins, steroids, alkaloids, glycosides, saponins, flavonoids, tannins and phenolic substances [20].

2.4. Quantification of total phenolic and flavonoid content

Total phenolic content (TPC) of DCRE was determined by Folin–Ciocalteau method. Briefly, two milliliters of 10% Folin–Ciocalteau reagent was added to 0.5 ml of 1 mg/mL DCRE. After 5 min, 2 mL of 7.5% sodium carbonate solution was added to the mixture and allowed to stand at room temperature for 1 h. Absorbance of the reaction mixture was then recorded at 760 nm wavelength. A standard calibration curve was plotted for gallic acid (5–100 mg/l) on similar grounds with R^2 value 0.988. TPC was then determined using the standard calibration curve and expressed in terms of mg of gallic acid equivalent (GAE) per g of dry weight of extract [21].

Total flavonoid content (TFC) was determined by aluminium chloride ($AlCl_3$) colorimetric method. To the reaction mixture containing 0.5 ml DCRE (1 mg/mL) and 1.5 mL ethanol was added 0.1 mL $AlCl_3$ solution and 0.1 mL potassium acetate solution. Finally, 3 mL distilled water was added to the final mixture and its absorbance was measured at 415 nm wavelength after incubation at room temperature for 1 h. A standard calibration curve was plotted for quercetin (5–100 mg/L) on similar grounds with R^2 value 0.983. TFC was then determined using the standard calibration curve and expressed in terms of mg of quercetin equivalent (QE) per g of dry weight of extract [21].

2.5. HPLC fingerprinting

Qualitative HPLC fingerprinting analysis was performed on Agilent 1200 series HPLC system equipped with a HPLC quaternary pump, auto sampler, degasser, UV detector and column oven controlled by EZchrome software. RP-C18 column (4.66 × 250 mm; particle size 5 mm; Agilent, Zorbax Eclipse plus) was used for the fingerprinting with 45 °C column oven temperature using a gradient elution of eluent A (water) and B (acetonitrile) for the separation of analytes. The following gradient program was used: 0–5 min, 5% B; 5–20 min, 10% B; 20–25 min, 100% B; 25–30 min 100% B; 30–35 min, 5% B; 35–40 min, 5% B. The solvent flow rate was of 1.0 mL/min and the injection volumes were in range of 2.0–10.0 mL. The HPLC analysis was conducted at room temperature at 254 nm wavelength and run time was 40 min.

2.6. Experimental animals

Male and female Wistar rats (150–200 g) were housed in polypropylene cages and maintained at 12 h light/dark cycle under controlled temperature (22–25 °C) and relative humidity (50–60%). They were allowed free access to standard pellet food and water *ad libitum*. Approval for this study was obtained from Institutional Animal Ethics Committee (IAEC) (Approval number KUDOPS/21 dated 22/08/2015). The experiment was carried out in compliance to the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

2.7. Acute toxicity study

Acute toxicity study of DCRE was performed using acute toxic class method of acute oral toxicity determination proposed in Organization for Economic Co-operation and Development (OECD) 423 guidelines [22]. Considering the safety profile of DCRE from previously reported studies [23], limit test was performed and the highest dose of DCRE (2000 mg/kg body weight, p.o.) was tested in nulliparous and non-pregnant female Wistar rats fasted overnight. Dosed rats were kept under observation for clinical signs of toxicity for duration of 14 days. Highest tolerated dose of DCRE was then determined based on Globally Harmonised Classification System for Chemical Substances and Mixtures (GHS) classification [22].

2.8. In vivo antiurolithiatic activity

Screening of antiurolithiatic potential of DCRE was performed on male Wistar rats using ethylene glycol (EG) and ammonium chloride (NH_4Cl) induced hyperoxaluria model of urolithiasis. Forty-two male Wistar rats were divided into seven groups (n = 6). Vehicle control group received distilled water *ad libitum*. Urolithiatic control group received urolithiatic treatment that consisted of 0.75% v/v EG with 1% w/v NH_4Cl in drinking water *ad libitum* for five days to accelerate stone formation, then switching to only 0.75% v/v EG in drinking water for twenty-three days. Standard control group received urolithiatic treatment daily along with Cystone 750 mg/kg per day, per orally (p.o.) from 15th day till 28th day. Preventive groups received urolithiatic treatment daily along with DCRE 200 mg/kg and 400 mg/kg per day, p.o., respectively. Curative groups received urolithiatic treatment daily along with DCRE 200 mg/kg and 400 mg/kg per day, p.o., respectively from 15th day till 28th day. Distilled water was used as vehicle for suspending Cystone and DCRE for the preparation of respective doses and the standard and test drugs were administered in experimental rats by oral gavage [24].

2.8.1. Food and water intake

Food and water consumption of each group was measured daily and expressed as relative food (g/100 g body weight) and water intake (mL/100 g body weight) [25].

2.8.2. Body weight

Body weight of each rat was measured weekly and expressed as relative body weight (RBW). RBW was calculated from initial body weight (IBW) and weekly absolute body weight (ABW) of each rat using the following formula [26]:

$$RBW = \frac{ABW(g)}{IBW(g)} \times 100$$

2.8.3. Urine analysis

After 28 days experimental period, rats were individually placed in metabolic cages. Their 24 h urine samples were collected in the presence of thymol crystals to prevent microbial growth [27]. Volume and pH of urine samples was determined followed by dipstick urine analysis prior to centrifugation at 2500 rpm for 5 min. Urine samples were then subjected to microscopic evaluation of crystalluria and quantitative estimation of calcium, phosphorus, uric acid, protein and magnesium on a Carelab 200 autoanalyzer using respective diagnostic kits [28]. Oxalate and citrate content in urine was determined by the methods of Hodgkinson, 1970 [29] and Şeker et al., 2009 [30], respectively.

2.8.4. Serum analysis

Following urine collection, blood was collected from the retro-orbital plexus of the rats under mild anesthesia. Serum was separated from the blood by centrifugation at 3000 rpm for 15 min. The serum thus obtained was then subjected to the quantitative estimation of blood urea nitrogen (BUN), creatinine and uric acid using respective diagnostic kits on Carelab 200 autoanalyzer [31].

2.8.5. Kidney analysis

Rats were euthanized under mild anesthesia by cervical dislocation and both of their kidneys were resected. Both kidneys of each rat were weighed and expressed as relative organ weight (ROW) using the formula:

$$ROW = \frac{AOW(g)}{FBW(g)} \times 100$$

where, AOW represents absolute organ weight and FBW represents final body weight of rats on the day of sacrifice [26]. Right kidney of each rat was fixed in 10% neutral buffered formalin and subjected to histopathological examination. Left kidney was homogenized in 0.15 M KCl using Remi's glass homogenizer for quantitative estimation of calcium, oxalate and phosphorus [31].

2.9. Statistical analysis

Statistical computations were performed using software GraphPad Prism 6.0. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test was used to analyze the data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Preliminary phytochemical screening

DCRE showed the presence of carbohydrates, saponins, flavonoids, tannins and phenolic compounds.

3.2. Total phenolic and flavonoid content

Total phenolic and flavonoid content of DCRE was found to be 1.427 ± 0.016 mg GAE/g of the extract and 1.24 ± 0.013 mg QE/g of the extract, respectively.

3.3. HPLC fingerprinting

HPLC fingerprinting analysis revealed the presence of total 17 compounds with 5 major compounds having area % more than 10. The fingerprinting analysis revealed the plausible presence of retinol (retention time 2.2 min) and caffeoylquinic acid (retention time 11.685 min) as their retention time was found to be similar to that has been reported in earlier studies [32,33]. However, the estimation of marker compound was the limitation of the study (Fig. 1).

3.4. Acute toxicity study

No sign of toxicity was observed at the highest administered dose (2000 mg/kg) of DCRE. Therefore, 2000 mg/kg body weight dose of DCRE (GHS category 5) was considered the highest tolerated dose. One tenth of the highest tolerated dose i.e. 200 mg/kg was selected as lower therapeutic dose and two times the lower therapeutic dose i.e. 400 mg/kg was selected as higher therapeutic dose for the antiurolithiatic study [23].

3.5. Food and water intake

All the groups except the urolithiatic control group showed a significant increase in food consumption over a 28 days experimental period. Urolithiasis inducing treatment produced a significant reduction in food intake ($P < 0.05$), and increase in water consumption ($P < 0.001$) of the rats of urolithiatic control group (UCG). Groups that received Cystone and DCRE treatment showed significant resistance to these effects of EG on feed and water intake (Table 1).

3.6. Relative body weight

A gradual increase in RBW was observed in rats of all the groups except the UCG. Urolithiatic treatment produced a significant reduction in RBW ($P < 0.01$) of the rats of UCG. This detrimental effect of disease progression was largely prevented by Cystone and DCRE treatment (Table 1).

3.7. Urine analysis

Urolithiasis inducing treatment did not produce any prominent changes on urinary output and pH of the treated rats. However, significant ($P < 0.05$) reduction in specific gravity of urine with presence of comparatively higher number of red blood cells (RBC) and white blood cells (WBC) was observed in rats from UCG. This was not the case with Cystone and DCRE treated groups (Table 2). Moreover, crystalluria in UCG showed large agglomerates of numerous calcium oxalate monohydrate (COM) crystals of

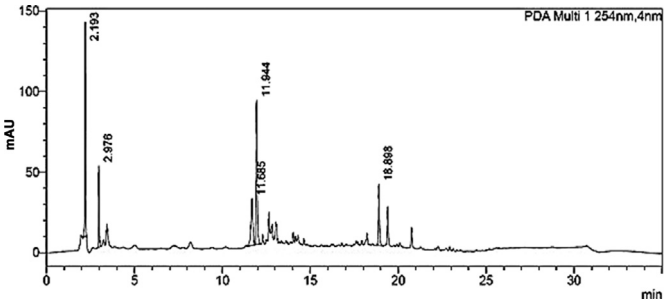

Fig. 1. HPLC fingerprint of DCRE.

Table 1
Effect of DCRE on RBW, food and water intake in urolithiasis induced rats.

	Vehicle Control	Urolithiatic Control	Cystone 750 mg/kg	Preventive 200 mg/kg	Preventive 400 mg/kg	Curative 200 mg/kg	Curative 400 mg/kg
Feed intake (g/100 g b.w.)							
Week 1	73.36 ± 2.9	72.02 ± 2.1	79.59 ± 2.8	78.86 ± 3.2	78.36 ± 2.9	73.54 ± 2.2	79.33 ± 1.9
Week 2	78.98 ± 0.78	74.61 ± 2.3	80.5 ± 2.04	82.7 ± 1.9	83.5 ± 1.7^{*b}	74.7 ± 2.1^{*d}	76.6 ± 2.1
Week 3	83.2 ± 0.9$^{\#\#}$	74.7 ± 1.5^{*a}	82.69 ± 1.05^{*b}	86.3 ± 2.6^{***b}	87.47 ± 1.6$^{***b,\#}$	79.42 ± 2.8^{*d}	81.67 ± 1.2
Week 4	85.22 ± 0.4$^{\#\#\#}$	76.75 ± 1.3^{*a}	87.73 ± 1.5$^{***b,\#}$	89.2 ± 2.2$^{****b,\#}$	91.26 ± 1.97$^{****b,\#\#}$	85.2 ± 1.5$^{*b,\#\#}$	87.4 ± 2.01$^{***b,\#}$
Water intake (ml/100 g b.w.)							
Week 1	58.62 ± 6.2	69.22 ± 5.9	63.02 ± 4.2	69.65 ± 4.5	67.2 ± 5.2	70.86 ± 4.6	61.39 ± 4.6
Week 2	64.04 ± 5.1	74.26 ± 3.8	72.1 ± 5.2	64.71 ± 4.2	67.43 ± 4.0	74.7 ± 6.4	71.01 ± 3.5
Week 3	58.9 ± 3.9	70.5 ± 4.6	64.2 ± 2.9	64.6 ± 3.03	58.9 ± 3.02	64.2 ± 2.2	62.4 ± 2.8
Week 4	58.5 ± 3.9	78.8 ± 2.9^{**a}	57.7 ± 4.2^{***b}	59.8 ± 3.9^{**b}	62.3 ± 2.9^{*b}	65.9 ± 2.5	61.8 ± 1.9^{*b}
RBW (%)							
Week 1	113.1 ± 3.4	100.7 ± 3.1	105.6 ± 2.5	98.97 ± 4.1	113.9 ± 4.9	100.5 ± 6.2	104.8 ± 2.8
Week 2	128.8 ± 5.5	100.0 ± 3.8^{*a}	110.7 ± 2.5	118.8 ± 8.5	120.2 ± 8.5	118.2 ± 1.6	121.8 ± 3.6,$^{\#\#}$
Week 3	137.8 ± 5.5$^{\#}$	102.6 ± 3.9^{**a}	121.4 ± 5.2	129.0 ± 9.2	148.5 ± 9.1$^{****b,*c,\#}$	132.0 ± 2.2$^{*b,\#\#\#}$	129.6 ± 3.4$^{*b,\#\#\#}$
Week 4	142.4 ± 6.8$^{\#\#}$	106.6 ± 3.9^{**a}	130.0 ± 5.4$^{\#\#}$	138.6 ± 9.4$^{*b,\#}$	147.8 ± 9.1$^{**b,\#}$	135.9 ± 6.6$^{*b,\#\#\#\#}$	140.6 ± 3.1$^{*b,\#\#\#\#}$

Values are expressed as mean ± SEM. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. week 1 of respective groups; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.
[a]vs. vehicle control. [b]vs. urolithiatic control. [c]vs. standard control. [d]vs. preventive 400 mg/kg.

rectangular habit (Fig. 2). Significantly higher number ($P < 0.0001$) of significantly large sized ($P < 0.0001$) crystals were found in the urine of UCG rats as compared to small and fewer crystals found in urine of DCRE and Cystone treated groups (Table 2). Urolithiasis disease progression was marked by significantly raised urinary levels of calcium ($P < 0.0001$), oxalate ($P < 0.0001$), phosphate ($P < 0.0001$), uric acid ($P < 0.001$) and protein ($P < 0.05$) and reduced citrate ($P < 0.05$) and magnesium concentration in urine of UCG. Cystone and DCRE treatment largely prevented these detrimental changes of urolithiasis progression and enhanced urinary levels of magnesium and citrate (Table 2). Effect of DCRE on all the urine parameters followed a dose dependent trend and showed comparatively better outcomes with preventive protocol.

3.8. Serum analysis

Significantly elevated levels of BUN ($P < 0.001$), creatinine ($P < 0.01$) and uric acid ($P < 0.05$) were observed in serum of UCG. DCRE treatment produced a significant reduction in elevated serum levels of BUN ($P < 0.01$) and creatinine ($P < 0.01$) with preventive regimen and significantly reduced elevated serum BUN ($P < 0.05$) with curative regimen. Efficacy of DCRE (curative regimen) was comparable to that of Cystone (Table 3).

3.9. Kidney analysis

Urolithiasis that surfaced due to EG treatment resulted in significantly higher ($P < 0.01$) relative kidney weight (ROW) of rats (Table 3), and significantly higher calcium ($P < 0.0001$), phosphate ($P < 0.0001$) and oxalate ($P < 0.001$) levels in kidney homogenate of rats from UCG (Fig. 3). Moreover, histopathological interpretations of renal tissue from UCG revealed marked degenerative changes and intratubular crystal deposition (Fig. 4). DCRE and Cystone significantly attenuated elevated ROW and, calcium, phosphate and oxalate depositions in renal tissue and ameliorated renal cellularity.

4. Discussion

CaOx urolithiasis is the most prevalent type of urolithiasis which compared to other types of urinary stone diseases is constantly on rise [34]. Therefore, CaOx urolithiasis was addressed in the present study to demonstrate the *Ashmaribhedan* potential of *D. carota*. EG and NH_4Cl induced hyperoxaluria model of urolithiasis was selected for the study due to its simplicity, popularity [35] and the fact that it induces hyperoxaluria which is a crucial risk factor for CaOx urolithiasis in clinical setting [36]. Cystone was used as a standard control in the present study considering it is a polyherbal

Table 2
Effect of DCRE on urine parameters in urolithiasis induced rats.

	Vehicle Control	Urolithiatic Control	Cystone 750 mg/kg	Preventive 200 mg/kg	Preventive 400 mg/kg	Curative 200 mg/kg	Curative 400 mg/kg
Volume (mL/24 h/100 g b.w.)	1.2 ± 0.32	3.5 ± 1.0	2.8 ± 0.70	2.4 ± 0.52	2.03 ± 0.29	2.3 ± 0.39	2.2 ± 0.48
pH	7.5 ± 0.23	6.7 ± 0.36	7.05 ± 0.25	7.12 ± 0.2	7.64 ± 0.19	6.97 ± 0.18	7.1 ± 0.16
Specific gravity (dipstick test)	1.018 ± 0.003	1.006 ± 0.002^{*a}	1.013 ± 0.003	1.013 ± 0.001	1.014 ± 0.002	1.016 ± 0.001	1.014 ± 0.005
RBC/μL (dipstick test)	0.0 ± 0.0	53.33 ± 40.06	16.67 ± 10.54	8.33 ± 8.33	1.67 ± 1.67	16.67 ± 10.54	1.67 ± 1.67
WBC/μL (dipstick test)	0.0 ± 0.0	20.83 ± 11.93	12.50 ± 12.50	0.0 ± 0.0	0.0 ± 0.0	4.17 ± 4.17	0.0 ± 0.0
Crystal size in μm (n = 100)	–	7.79 ± 0.33	4.46 ± 0.23^{****b}	4.09 ± 0.36^{****b}	3.84 ± 0.18^{****b}	5.07 ± 0.23^{****b,*c}	4.07 ± 0.25^{****b}
No. of crystals (n = 5 fields)	3.0 ± 1.3	120.0 ± 16.24^{****a}	23.6 ± 2.38^{****b}	15.6 ± 1.89^{****b}	11.6 ± 0.87^{****b}	19.4 ± 2.25^{****b}	15.2 ± 1.24^{****b}
Calcium (mg/dL)	2.08 ± 0.79	15.85 ± 2.01^{****a}	3.32 ± 0.74^{****b}	4.10 ± 0.81^{****b}	2.59 ± 0.52^{****b}	8.04 ± 2.35^{*b}	4.61 ± 1.88^{****b}
Oxalate (mg/dL)	1.53 ± 0.24	6.7 ± 1.17^{****a}	1.93 ± 0.36^{****b}	2.27 ± 0.30^{****b}	1.97 ± 0.24****	3.77 ± 0.28^{**b}	3.53 ± 0.22^{**b}
Phosphate (mg/dL)	14.36 ± 1.4	56.71 ± 3.5^{****a}	28.89 ± 3.05^{*a,****b}	20.66 ± 2.81^{****b}	21.32 ± 2.98^{****b}	29.06 ± 2.54^{*a,****b}	27.48 ± 2.81^{*a,****b}
Protein (mg/dL)	2.87 ± 0.39	9.12 ± 2.21^{*a}	5.25 ± 1.26	5.93 ± 1.68	4.31 ± 0.73	7.37 ± 0.67	5.56 ± 0.80
Uric acid (mg/dL)	0.79 ± 0.09	3.33 ± 0.85^{***a}	0.88 ± 0.13^{***b}	0.79 ± 0.05^{***b}	0.79 ± 0.09^{***b}	1.31 ± 0.35^{**b}	1.10 ± 0.23^{**b}
Citrate (mg/dL)	52.02 ± 6.9	24.38 ± 4.9^{*a}	49.64 ± 4.2^{*b}	56.38 ± 2.4^{**b}	64.24 ± 5.7^{****b}	60.53 ± 4.6^{***b}	61.22 ± 6.4^{***b}
Magnesium (mg/dL)	0.96 ± 0.21	0.88 ± 0.12	1.21 ± 0.15	1.44 ± 0.33	2.22 ± 0.34^{**a,b}	1.22 ± 0.23	1.09 ± 0.14^{*c}

Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.
[a]vs. vehicle control. [b]vs. urolithiatic control. [c]vs. preventive 400 mg/kg.

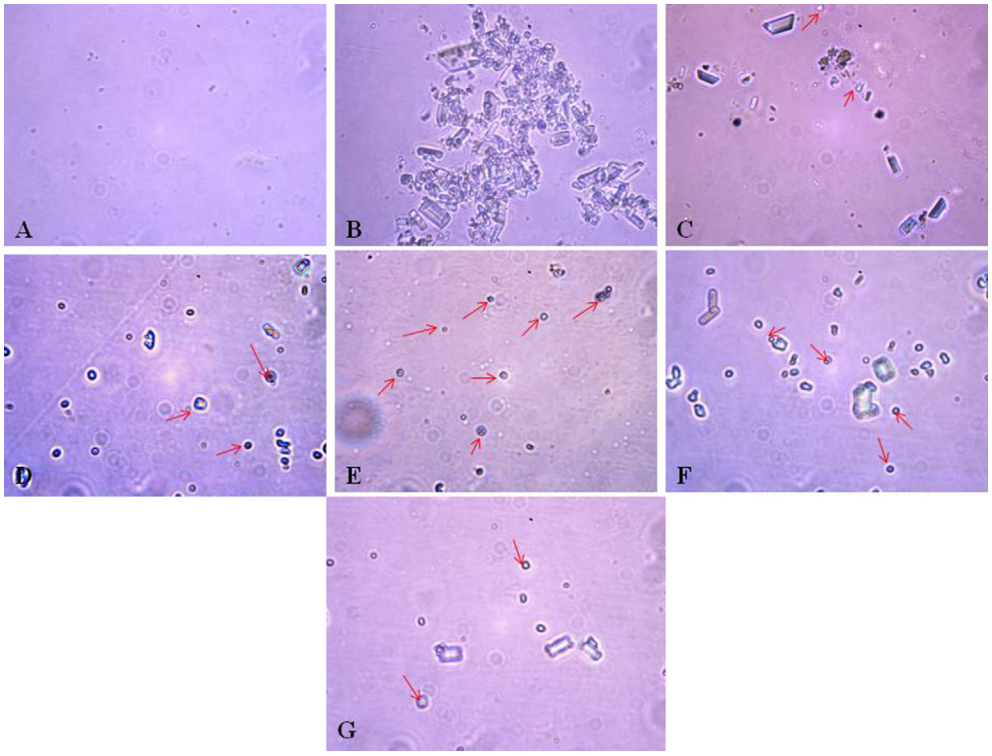

Fig. 2. Representative photographs of CaOx crystals from urine samples of experimental rats as observed under light microscope (x1000) in **A** Normal Control group, **B** Urolithiatic control group, **C** Cystone treated group, **D** Preventive 200 mg/kg DCRE treated group, **E** Preventive 400 mg/kg DCRE treated group, **F** Curative 200 mg/kg DCRE treated group, **G** Curative 400 mg/kg DCRE treated group. Red arrows indicate typical tetrahedral or octahedral COD crystals.

Table 3
Effect of DCRE on serum and kidney parameters in urolithiasis induced rats.

	Vehicle Control	Urolithiatic Control	Cystone 750 mg/kg	Preventive 200 mg/kg	Preventive 400 mg/kg	Curative 200 mg/kg	Curative 400 mg/kg
Serum parameters							
BUN (mg/dL)	16.03 ± 3.81	60.75 ± 6.45^{***a}	34.62 ± 5.79	25.89 ± 5.45^{**b}	23.23 ± 6.23^{**b}	28.84 ± 5.63^{*b}	29.71 ± 5.79^{*b}
Creatinine (mg/dL)	0.47 ± 0.04	0.86 ± 0.09^{**a}	0.68 ± 0.02	0.54 ± 0.08^{*b}	0.49 ± 0.02^{**b}	0.78 ± 0.06^{*a,*,c}	0.63 ± 0.09
Uric acid (mg/dL)	1.08 ± 0.13	2.16 ± 0.14^{*a}	1.17 ± 0.09	1.65 ± 0.12	1.37 ± 0.17	1.19 ± 0.07	1.45 ± 0.07
Kidney parameters							
ROW left (%)	0.38 ± 0.01	0.49 ± 0.01^{**a}	0.41 ± 0.01^{*a}	0.43 ± 0.02	0.41 ± 0.02^{**b}	0.41 ± 0.01	0.43 ± 0.03
ROW right (%)	0.39 ± 0.01	0.49 ± 0.02^{**a}	0.41 ± 0.015^{*a}	0.43 ± 0.02	0.42 ± 0.02^{*b}	0.41 ± 0.01^{*b}	0.42 ± 0.02^{*b}

Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
^{a}vs. vehicle control. ^{b}vs. urolithiatic control. ^{c}vs. preventive 400 mg/kg.

formulation which is extensively used for the treatment of urolithiasis in clinical practice [37]. Its antiurolithiatic efficacy is produced by multiple mechanisms that include its crystal disintegrating ability, diuretic and urinary smooth muscle relaxing activity [28]. Therefore, it provides numerous ways for comparing the efficacy and inferring the mechanism of action of the test drug.

Alterations in food and water consumption serve as early markers of disease progression [38], hence these parameters were taken into account. Aversion towards food was observed in the EG treated rats. This also reflected in the RBW of rats from UCG as has also been reported earlier in previous studies [26]. On contrary, gain in food consumption and RBW of rats was observed in Cystone and DCRE treated groups showing their efficacy in preventing or reversing the progression of disease. Water consumption increased significantly as a result of EG treatment in UCG although increase in urine output and reduction in urinary pH was not significant. Specific gravity of urine of rats from UCG was found to be significantly low which could be the result of excessive hydration or due to renal impairment [39]. Renal impairment as a result of renal tissue injury in UCG could be a possibility because RBC and WBC were found to be in a comparative excess in UCG urine samples. DCRE and Cystone treated groups showed the presence of fewer RBC/μL and WBC/μL urine.

Extent and exhibit of crystalluria is a key determinant of stone formation and recurrence [40]. In the present study EG treatment advocated the formation of numerous large calcium oxalate monohydrate crystals (COM) of monoclinic, rhombic or rectangular habit in urine of UCG rats. While significantly fewer and smaller tetrahedral or octahedral shaped calcium oxalate dihydrate crystals (COD) that are less adhesive and less injurious to the renal epithelium than COM crystals [41] were predominant in the urine of rats that received DCRE treatment. This reveals the anti-crystallization activity of DCRE that can be attributed to the saponins, tannins and polyphenolic compounds present in the extract as was confirmed through the phytochemical evaluation. The presence of polyphenolic compounds was also confirmed through HPLC fingerprinting. It has been established through previous reports that saponins and flavonoids are known to have stone

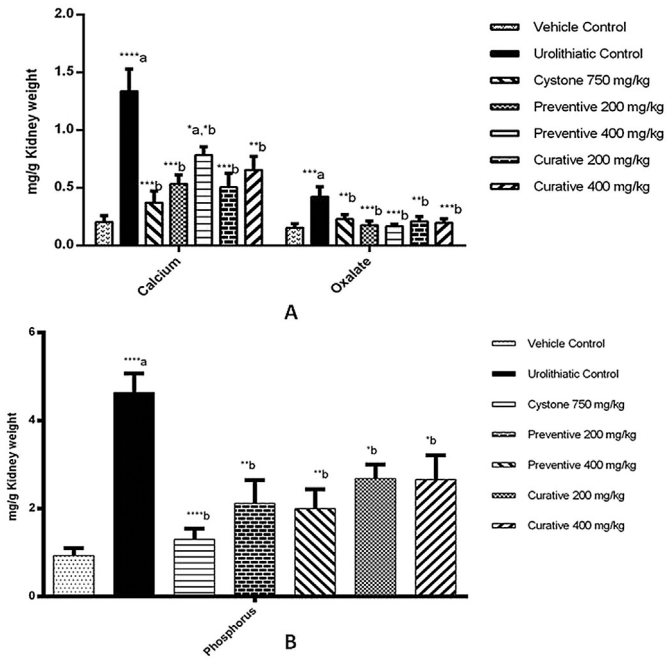

Fig. 3. Effect of DCRE on kidney homogenate parameters in urolithiasis induced rats (A, B). Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$,$^{***}P < 0.001$,$^{****}P < 0.0001$; **a** when compared to vehicle control; **b** when compared to urolithiatic control.

disintegrating and CaOx crystal dissolution potency, respectively [31]. Tannins on the other hand exhibit anticrystallization activity by aiding calcium complexation and thus deterring CaOx crystal formation [42].

Hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia and hypomagnesuria are common findings in CaOx urolithiasis that exacerbate and accelerate renal stone disease [43]. Excessive proteinuria promotes CaOx crystallization and serves as indicator of renal tubular dysfunction. Elevated phosphate level in urine promotes CaOx stone formation by precipitating as calcium phosphate (CaP) and providing nidus for CaOx deposition [44]. Cystone and DCRE treatment significantly reduced elevated urinary calcium, phosphate, oxalate, protein and uric acid and restored the diminished urinary levels of magnesium and citrate that accompanied stone formation thus restoring the disrupted equilibrium between promoters and inhibitors of stone formation. Kidney homogenates of rats from DCRE and Cystone treated groups also showed significantly reduced calcium, phosphate and oxalate deposition as compared to the extremely elevated levels of these constituents in kidney homogenates of rats from UCG.

Urinary obstruction due to large stones or crystals results in diminished glomerular filtration and subsequent accumulation of nitrogenous wastes such as creatinine, BUN and uric acid in blood [26,44]. This was also evident in the present study. Cystone and DCRE treatment reduced the elevated serum levels of BUN, creatinine and uric acid thus showing efficacy in ameliorating impaired renal function.

DCRE and Cystone treatment also significantly inhibited renal tissue damage and inflammation which is a common finding associated with urolithiasis and surfaces due to hyperoxaluria and crystalluria mediated generation of reactive oxygen species [45]. Renal tissue from DCRE and Cystone treated groups presented mild inflammation with extremely few CaOx crystals if any. Histopathological interpretation of renal tissues from UCG showed presence of crystal aggregates in renal tubules and within the urothelium of the renal pelvis along with interstitial inflammation, and tubular damage in renal cortex and medulla. The protective effect of DCRE

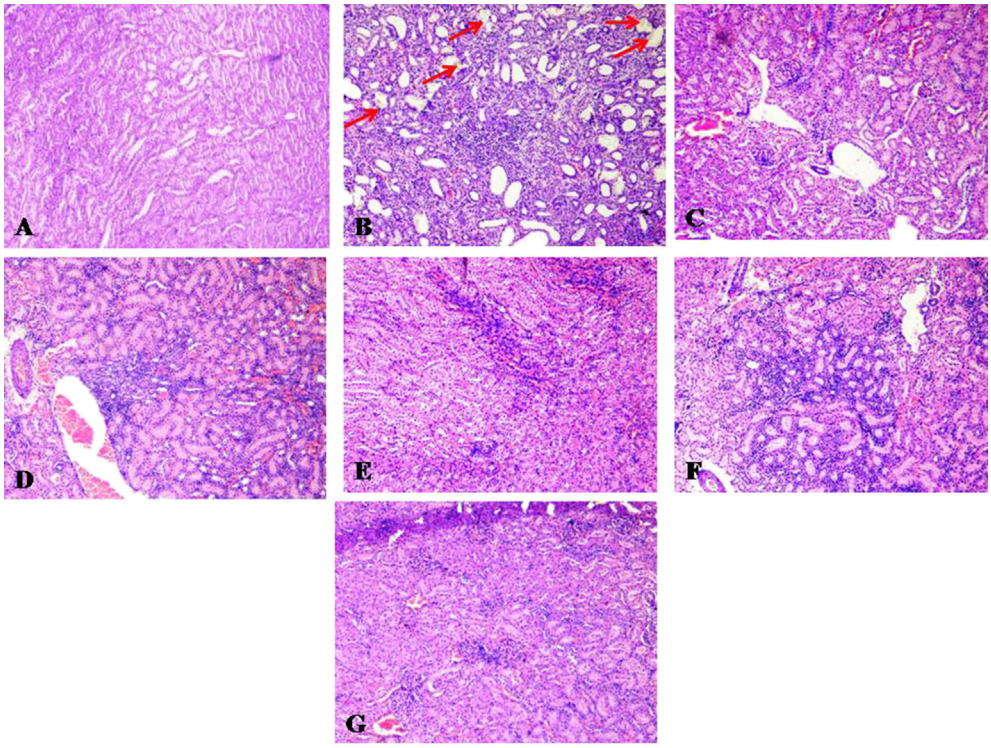

Fig. 4. Representative images of Haematoxylin and Eosin stained kidney sections (x400) from **A** Normal Control group**, B** Urolithiatic control group, **C** Cystone treated group, **D** Preventive 200 mg/kg DCRE treated group, **E** Preventive 400 mg/kg DCRE treated group, **F** Curative 200 mg/kg DCRE treated group, **G** Curative 400 mg/kg DCRE treated group. Red arrows indicate CaOx crystal depositions.

against oxidative and crystal induced damage of renal tissue can be attributed to its carotenoid and polyacetylene content as carotenoids are known to be potent antioxidants [46] and polyacetylenes like falcarinol provide protection against inflammation [12,13].

5. Conclusion

Present study for the first time provided scientific credence to the folklore claiming antiurolithiatic potential of *D. carota* roots. Antiurolithiatic activity of *D. carota* can be attributed to its anti-crystallization property, its ability to ameliorate urine and serum biochemistry and renal cellularity. This may prove to be beneficial in developing safer and economic alternatives for prevention and cure of urolithiasis. This study also paves path for unraveling the exact molecular events involved in the antiurolithiatic activity of *D. carota*.

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Conflicts of interest

None.

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