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Supplementation of *Daucus carota* L. extract prevents urolithiasis in experimental rats

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Daucus carota L. was first used for medical purposes and gradually used as a food worldwide and it has been endorsed in relic Ayurvedic scriptures in India and is being used in many Ayurvedic formulations as a chief ingredient. The present study was designed to evaluate the antiurolithiatic activity of *D. carota* L. extract against ethylene glycol (EG) and Vitamin D_3 induced urolithiasis rats. The protective effect of *D. carota* L. root extract was studied in a dose-dependent manner by using 200 and 400 mg/kg in rats for four weeks and the anti-urolithiatic potentiality was accessed by measuring the parameters like calcium, sodium, potassium, oxalate, inorganic phosphate, creatinine, blood urea nitrogen and uric acid were estimated in both serum and urine by using commercial diagnostic kits. The *in vivo* antioxidant activity of *D. carota* L. was also evaluated and histopathological changes that occurred in the kidney observed. Serum and urinary levels of calcium, creatinine, oxalate, blood urea and blood urea nitrogen level were found to be decreased significantly in groups pre-treated with *D. carota* L. extract. The animals treated with test drug showed much improvement in physical parameters like body weight, urine volume and pH of urine. Histopathology of kidney showed almost normal kidney architecture in treated groups compared to disease control rats. The biochemical and histopathological parameters studied in rats have revealed the presences of antiurolithiatic property in the roots of *Daucus carota* L. This property was dose-dependent.

Keywords: Antioxidants, Carrot, Ethylene glycol, Polyphenol, Urolithiasis.

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Introduction

Urolithiasis constitutes as a global health problem with an incidence of up to 5 % in the general population, but its prevalence is even greater in specific geographic regions such as 20 % in Gulf countries, 15 % in the United States and Turkey, 11 % in India and 4-8 % in United Kingdom[1,2]. The condition is approximately twice as common in males as in females and its incidence increases with age in adults[3]. It is a disorder of the urinary tract in which insoluble mineral and salt concretions develop and aggregate around a nidus of proteinaceous material mainly within the bladder or urethra but it can occur anywhere in the urinary tract. Abnormal microscopic precipitates in urine are known as crystalluria whereas macroscopic concretions are called uroliths[4].

One of the most significant clinical problems of urolithiasis is the high recurrence rate incidence without preventive measures after the first stone. After 3 years this is about 40 %, by 10 years up to 75 % and by 25 years virtually every patient has formed at least one more stone, due to an imbalance between promoters and inhibitors in the kidneys[2,5]. Presently, medical management of renal stone consists of lifestyle modification, calcium channel blockers, diuretics, citrate and magnesium-rich diet and surgical treatments i.e. extracorporeal shock wave lithotripsy, percutaneous lithotripsy, and transureteral lithotripsy. Medical management suffers from adverse effects and surgeries are expensive with higher complication rate than medical management, and do not affect the

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recurrence of stones[6,7]. So there is a need for medical treatment of renal stone that has curative as well as preventive action on the stone formation with insignificant adverse effect profile. Hence, the search for antilithiatic drugs from natural sources which are effective, safe and culturally acceptable nature have gained more attention compared to earlier as shown in a recent study[5,8]. Treatment of urinary stone disease with herbal remedies dates back to several centuries although scientific evidence is lacking[9]. The reasons for the use of medicinal plants as natural remedies are being cheaper (53 %) and more efficient than modern medicines, but less than 10 % of the total persons interviewed used the medicinal plants because of easy acquisition than modern drugs. On the contrary, about 72 % are satisfied with the herbal treatment than synthetic drugs. A remarkable feature in the ethno pharmacopoeia of many African populations is the use of food substances as medicinal agents (20 edible plants), such as *Daucus carota* L. in renal diseases[10].

Carrots (*D. carota* L.) belonging apiaceae family have been a favourite vegetable for a long time, due to their nutritive value and culinary uses. In specific, they are noted for their rich nutritional antioxidants such as vitamin A, C, & E and a great number of non-nutritional antioxidants, like β-carotene and polyphenol compounds[11]. Experimental and clinical studies on carrots (powder or extract) and its active constituents (mainly carotenoids) revealed that they have hypoglycemic effect[12,13], anticancer activity due to the presence of α carotene and falcarinol[14,15], protective effect against coronary heart disease[16], and hypocholesterolemic & hypolipidemic activities[17].

Shashi Alok *et al*. stated that one glass of *D. carota* L. extract is given for night to remove kidney stone[18]. Indian Materia Medica mentions carrot has a beneficial influence on the kidneys and dropsy and prevents the brick-dust sedimentation sometimes found in the urine[19]. *D. carota* L. root extract is prescribed in painful urination, urine retention, and other bladder disorders[20].

However, no studies have so far been reported on antiurolithiatic effects of *D. carota* L. extract. Hence, in the present study, an effort has been made to establish the scientific authenticity for the antiurolithic property of *D. carota* L. extract against ethylene glycol (EG) and Vitamin D_3 induced hyperoxaluria in rats.

Materials and Methods

Plant material

The roots of *D. carota* L. for the proposed study were collected in January 2014 from the surroundings of Tirupati and was authenticated by N Savithramma, Professor, Department of Botany, S V University, Tirupati, India and a voucher specimen (SVUHA 2175) was deposited in the department.

Preparation of the root extract

The fresh juice extract of roots of *D. carota* was prepared according to Bishayee *et al*.[21] with slight modifications. The fresh roots of *D. carota* L. were peeled, washed, cut into small pieces and homogenized using a grinder. The homogenate was squeezed and filtered through a muslin cloth to yield *D. carota* L. extract (DCE). The extract was subjected to lyophilization to get powder and it was stored at -20 °C for further experimental use.

Animals

Healthy male albino rats of Wistar strain of about 180±20 g with age of 120 days were procured from Raghavendra enterprise Bangalore for the study. The animals were kept in polypropylene cages and maintained at a room temperature of 25±2 °C with 55±5 % relative humidity and 12 hours light/dark cycle. They were fed with standard rat pellet and drinking water *ad libitum.* The experimental protocol was approved by the Institutional Animal Ethical Committee of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati (SPSP/IAEC 1016/a/2014/003).

Preliminary phytochemical screening

The preliminary phytochemical screening of the fresh juice of *D. carota* L.was carried out according to the methods described by Khandelwal *et al*.[22], and all the chemicals used in the present study were of indigenous and analytical grade.

Standardization of DCE by HPLC

The phenolic compounds were determined by high-performance liquid chromatography (HPLC). The HPLC system consisted of a Shimadzu (Waters Associates, Milford, MA, USA), a MCPD-3600 UV detector (Otsuka Electronics, Osaka, Japan) and C_{18} column (GL Science, Tokyo, Japan). Elution was performed at a flow rate of 1 mL/min using Mobile phase A (methanol: water 75:25), Mobile phase B (methanol: water 85:15) and Mobile phase C (methanol: water 95:5) for 15 minutes. The universal UV detector was set at 254 nm. Gallic acid was used as an internal standard. The HPLC fingerprint

chromatogram of DCE produced 5 peaks in phenolic compounds (Fig. 1 and 2).

Acute toxicity studies

An acute toxicity study has been performed as per OECD 423 guidelines. Single doses of fresh juice extract of *D. carota* ranging from 5, 50, 300, and 2000 mg/kg body weight was administered. All the behavioural, motor and autonomic changes were observed as per Irvin scale.

Experimental protocol

Urolithiasis in the experimental animals was induced by administering the ethylene glycol (EG) (0.75 v/v %, daily, p.o) and Vitamin D_3 (5 µg/kg, alternate day, p.o) for 28 days of treatment schedule[23].

The rats were divided into five groups of six animals in each group. The standard drug cystone treatment at a dose of 250, 500, and 750 mg/kg b.wt. in rats revealed a dose-related response. Cystone treatment at dose levels of 500 and 750 mg/kg b.wt. showed a better protective effect against glycolic acid induced urolithiasis in rats[24]. However, there was no significant difference observed between 500 and 750 mg/kg b.wt. of cystone treatment. With the above reference cystone at 750 mg/kg b.wt was used as the standard treatment in the present study and the treatment schedule was fixed for 28 days.

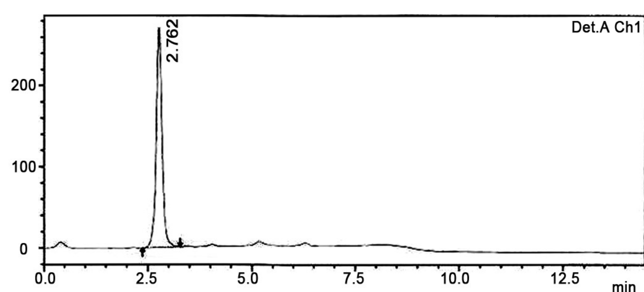

Fig. 1 — High-performance liquid chromatography profile of gallic acid (254 nm). Mobile phase B (methanol: water, 85:15), 1 mL/min flow rate.

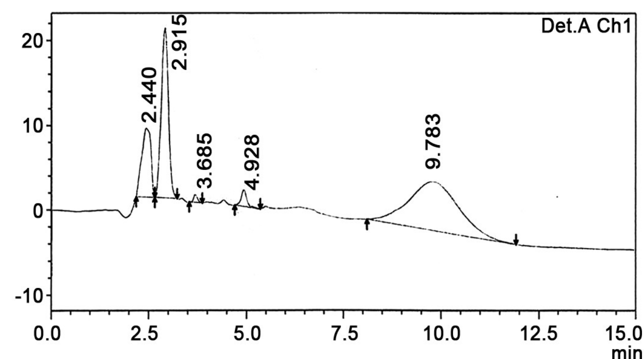

Fig. 2 — High-performance liquid chromatography profile of DCE (254 nm). Mobile phase B (methanol: water, 85:15), 1 mL/min flow rate.

Group-I served as normal control, treated with distilled water in the entire period of study schedule, Group-II served as disease control, treated with ethylene glycol (0.75 v/v %, daily, p.o) and vitamin D_3 (5 µg/kg, alternate day, p.o), Group-III, treated with cystone (750 mg/kg), and EG (0.75 v/v %, daily, p.o) and vitamin D_3 (5 µg/kg, alternate day, p.o). Group-IV and Group-V rats respectively received DCE at the dose of 200 and 400 mg/kg, as low and high doses along with EG (0.75 v/v %, daily, p.o) and vitamin D_3 (5 µg/kg, alternate day, p.o). At the end of the experiment, urine samples were collected from all groups for 24 hrs by keeping rats in individual metabolic cages. Rats had free access to drinking water during the urine collection period. The volume of urine collected from each animal was recorded, and collected urine was analyzed for various parameters by using standard methods. Blood was collected from retro-orbital plexus in plain EDTA coated tubes and was subjected to centrifugation to separate the serum for estimation of various biochemical parameters.

Haematological and biochemical analyses in blood and urine samples

At the end of the experiment, blood samples were collected from the retro-orbital plexus into plain EDTA coated tubes and were centrifuged at 3000×G for 10 minutes to obtain serum fordetermination of biochemical parameters.

The serum parameters like blood urine nitrogen (BUN), creatinine, uric acid, inorganic phosphate, oxalate and calcium were evaluated by using commercial diagnostic kits (Agappe Pvt. Ltd, Kerala, India). Haematological analyses were carried out by Mispa Excel semi auto analyzer.

At the end of the experiment, urine samples were collected from all groups for 24 hours by keeping rats in individual metabolic cages. Rats had free access to drinking water during the urine collection period. The volume of urine collected from each animal was recorded, and collected urine was analyzed for various parameters by using standard methods.

Urinary oxalate, uric acid, creatinine and levels of some elements like Ca_2^+ Na^+ and K^+ were measured from the urine samples of the last day. All biochemical parameters were determined by Mispa Excel semi auto analyzer, whereas oxalate levels were measured by Shimadzu spectrophotometer.

Determination of tissue antioxidants

At the end of the experimentation, kidneys were excised from rats and homogenate in 0.1M tris buffer and the separated homogenates were used for estimation of kidney antioxidants like superoxide dismutase (SOD)[25], reduced glutathione (GSH) [26], Catalase[27] and lipid peroxidation (LPO)[28].

Histological examinations

The kidneys excised from rats were washed thoroughly with normal saline to remove blood and then fixed in 10 % buffered formalin, and embedded in paraffin; sections were cut at 5 µm and stained with hematoxylin and eosin. These sections were then examined under a light microscope with plane polarized light for histological changes and the photographs were taken by using an Olympus Digital Camera. Histopathological changes, aggregation of calcium oxalate crystals and stones in the kidney tissues were recorded.

Statistical analysis

The data represent mean ±SEM. Results obtained on these experiments were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparisons using prism graph pad 5.0 software. The statistical difference $p < 0.05$ was considered significant.

Results

The present study 'the therapeutic effects of *D. carota* L. extract on stone formation' were investigated. Ethylene glycol and vitamin D_3 was administered to the experimental animals for 28 days to induce stone formation in the kidney. The phytochemical evaluation of *D. carota* L. extract reveals the presence of plant secondary metabolites like flavonoids, polyphenols, alkaloids, carbohydrates, proteins and tannins.

HPLC result (Fig. 1 and 2) reveals that the peaks of phenolic compounds were identified by comparison of retention times with gallic acid as authentic reference compounds with a retention time of 2.762 minutes. Whereas DCE revels 5 peaks with different retention times 2.440, 2.915, 3.685, 4.928 and 9.783 minutes. The first two peak's retention time is near to the standard gallic acid to support the extract contains polyphenol substances.

The results of acute toxicity studies have revealed that there was no toxicity found at all the doses like 5, 50, 300, and 2000 mg/kg body weight. Thus the dose has been selected 1/10th of maximum dose as low (200 mg/kg body weight) dose and double of low dose (400 mg body weight) was considered as high dose.

Effect of DCE on various physical parameters Table 1 in EG and vitamin D_3 induced urolithiasis in rats. There is a significant decrease in body weight, and urine volume in disease control rats when compared to normal control rats. DCE treatment at a dosage of 200 mg/kg and 400 mg/kg showed a significant increase in body weight, and urine volume when compared with the disease control group. Urinary pH was found to be alkaline in the disease control group when compared with normal control group and was observed near to the neutral in DCE treated rats at a dose 200 mg/kg and 400 mg/kg body weight respectively.

Effect of DCE on various serum biochemical parameters in EG and vitamin D_3 induced urolithiasis in rats

The changes in serum biochemical parameters were shown in Table 2. A significant ($p < 0.05$) increase in serum creatinine, urea, uric acid, blood urea nitrogen (BUN), calcium and oxalate levels in disease control rats when compared with normal control rats. These alterations were significantly ($p < 0.05$) reduced by treatment with DCE at doses of 200 mg/kg and 400 mg/kg body weight respectively when compared to disease control rats.

Effect of DCE on urinary parameters in EG and vitamin D_3 induced urolithiasis in rats

The changes in urinary parameters are shown in Table 3. There was a significant ($p < 0.05$) increase in urinary excretion of calcium, oxalate and inorganic phosphate and decrease in urinary excretion of

Table 1 — Effect of *Daucus carota* L.extract on various physical parameters in EG and Vitamin D_3 induced urolithiasis in rats

Group	Body weight on 0 day in g	Body weight on 28th day in g	Volume of urine (mL)/24 h	pH of urine
Normal control	182.5±1.118	188.33±1.382	6.35±0.162	6.5±0.088
Disease Control	195.0±2.236	186.0±2.129	4.51±0.070^{a}	8.2±0.044^{a}
Standard	187.5±1.118	193.16±1.869	10.28±0.070	7.2±0.073
DCE(200mg/kg)	185.0±1.826	191.17±1.833	8.20±0.073^{b}	7.4±0.044^{b}
DCE(400mg/kg)	188.3±3.801	194.0±3.615	9.25±0.114^{b}	7.5±0.044^{b}

All value are mean±SEM (*n*= 6) One way analysis of variance test (ANOVA) followed by Tukey's multiple comparison test.

Table 2 — Effect of *Daucus carota* L. extract on various biochemical parameters in serum in EG and vitamin D_3 induced urolithiasis in rats.

Group	Creatinine (mg/dL)	BUN (mg/dL)	Inorganic phosphate(mg/dL)	Uric acid (mg/dL)	Calcium (mg/dL)	Oxalate (mg/dL)
Normal control	0.65±0.057	6.5±0.342	3.78±0.338	1.5±0.252	7.51±0.395	0.82±0.012
Disease control	2.5±0.065[a]	17.58±0.472[a]	7.21±0.460[a]	6.83±0.326[a]	14.96±0.259[a]	3.26±0.11[a]
Standard	0.98±0.092	9.25±0.597	5.19±0.160	1.96±0.308	11.45±0.387	0.55±0.02
DCE (200 mg/kg)	0.89±0.114[b]	11.72±0.829[b]	4.34±0.214[b]	2.80±0.249[b]	6.15±0.684[b]	0.82±0.006[b]
DCE (400 mg/kg)	0.72±0.093[b]	10.01±0.567[b]	3.32±0.391[b]	2.01±0.215[b]	8.76±0.207[b]	0.72±0.003[b]

All value are mean±SEM (*n*= 6) One way analysis of variance test (ANOVA) followed by Tukey's multiple comparison. DCE= *Daucus carota* L. extract. [a]indicates $p < 0.01$ when compared to normal group, [b]indicates $p < 0.05$ when compared to control group.

Table 3 — Effect of *Daucus carota L.* extract on various biochemical parameters in urine in EG and Vit-D_3 induced urolithiasis in rats.

Group	Creatinine (mg/kg/dL)	Uric acid (mg/dL)	Inorganic phosphate (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Calcium (mg/dL)	Oxalate (mg/dL)
Normal control	2.18±0.080	0.92±0.290	4.74±0.067	20.29±2.367	3.80±0.042	3.41±0.196	3.37±0.090
Disease Control	8.52±0.238[a]	13.97±0.785[a]	11.72±0.349[a]	12.77±4.881[a]	1.240±0.245[a]	6.48±0.202[a]	9.42±0.065[a]
Standard	2.54±0.246	10.19±0.342	4.86±0.262	41.57±0.428	9.36±0.432	5.63±0.140	6.47±0.142
DCE(200mg/kg)	3.15±0.195[b]	8.61±0.352[b]	8.06±0.236[b]	24.12±1.187[b]	5.63±0.100[b]	4.82±0.202[b]	5.48±0.101[b]
DCE(400mg/kg)	2.98±0.076[b]	6.49±0.424[b]	5.96±0.133[b]	34.77±0.410[b]	6.84±0.0.230[b]	4.12±0.297[b]	4.72±0.088[b]

All value are mean±SEM (n= 6) One way analysis of variance test (ANOVA) followed by Tukey's multiple comparison. DCE= *Daucus carota* L. extract. [a]indicates $p < 0.05$ when compared to normal group, [b]indicates $p < 0.05$ when compared to control group.

Table 4 — Effect of *Daucus carota* L.extract on kidney antioxidant studies in EG and Vit-D_3 induced urolithiasis in rats.

Group	SOD (U/mg Protein)	GSH (µg of GSH/mg protein)	Catalase (µM H_2O_2 consumed/mg protein	LPO (nM of MDA/mg protein)
Normal control	14.11±0.593	3.19±0.333	51.47±0.830	1.45±0.276
Disease control	6.65±0.839[a]	1.42±0.025[a]	23.01±2.783[a]	5.25±0.388[a]
Standard	19.65±1.498	3.41±0.366	47.90±6.652	1.89±0.191
DCE (200 mg/kg)	11.85±0.869[b]	2.13±0.406[b]	39.76±3.185[b]	2.85±0.170[b]
DCE (400 mg/kg)	12.13±1.059[b]	2.64±0.249[b]	41.28±1.598[b]	2.06±0.042[b]

All value are mean±SEM (n= 6) One way analysis of variance test (ANOVA) followed by Tukey's multiple comparison.
DCE= *Daucus carota* L. extract. [a]indicates $p < 0.05$ when compared to normal group, [b]indicates $p < 0.01$ when compared to control group.

sodium and potassium ions in disease control rats when compared with normal control rats. There was a significant ($p < 0.05$) increase in urinary excretion of creatinine, and uric acid in disease control rats when compared with normal control rats. DCE treatment at doses of 200 mg/kg and 400 mg/kg has significantly reduced the altered urological changes in EG and vitamin D_3 induced urolithiasis in rats.

Effect of DCE on tissue antioxidant levels

The changes in kidney antioxidants are presented in Table 4. In urolithiasis induced rats, there was a significant ($p < 0.05$) decrease in GSH, catalase, SOD and a significant increase in LPO compared to normal control group and pre-treatment with DCE at doses of 200 and 400 mg/kg respectively brought all these changes back to normalcy.

Plate 1 shows that the histopathological investigations of kidney sections of rats treated with EG and vitamin D_3 a significant deposition of the crystalline components in the renal tissue when compared to normal rats. However, significantly less deposition of the crystalline components in the renal tissue in the animals pretreated with DCE 200 and 400 mg/kg body weight respectively.

Discussion

Research has shown that fruits and vegetables contain many antioxidants, and the major part is polyphenols[29]. Major polyphenols in carrots include chlorogenic acid and p-hydroxybenzoic acid along with numerous cinnamic acid derivatives. The absorption and metabolism of carrot antioxidants are important factors that affect their function *in vivo*. Chlorogenic acid had the highest content among all phenolic acids in the carrot[30]. The phytochemical analysis of the present study reveals the presence of polyphenols, flavonoids and in order to authenticate

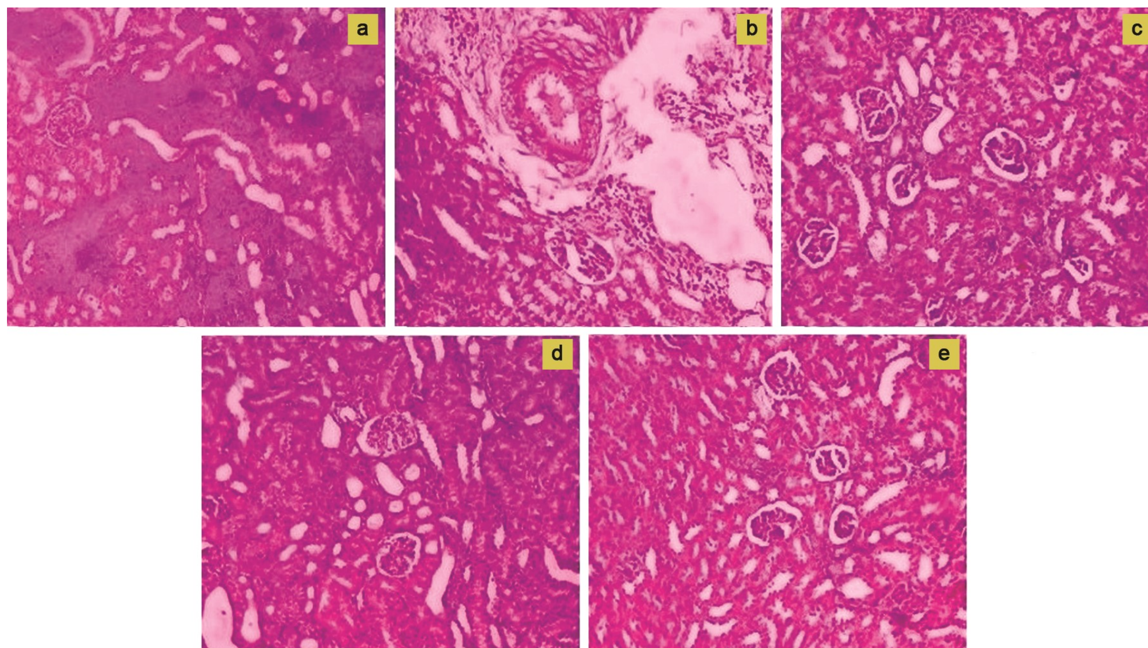

Plate 1 — Photomicrographs of sections of kidney at 40X magnification a) Normal control rats show a normal structure of kidney and renal tubules, b) Disease control rat kidney shows oxalate deposition in renal tubules and glomerular haemorrhage along with damage to renal tubules was noticed, d)Standard treatment with Cystone 750 mg/kg, d and e) Pre-treatment DCE 200 and 400 mg/kg shows marked reduction in the deposition of oxalate crystals and inflammatory cell infiltration in the renal tubule spaces and reduces the cellular damage with oxalate toxicity.

these components, the extract was subjected to HPLC analysis by using gallic acid as the reference standard. The polyphenols were identified by comparing peak retention times with the peak obtained by gallic acid, the peak retention time of the extract was near to gallic acid. Thus the DCE must contain polyphenolic substances like chlorogenic acid and caffeic acid.

No significant change in the body weight was found during the study in control and test groups. Whereas significant diuresis and neutral pH was observed in the DCE treated groups. Alkaline pH promotes the crystallization in urine leading to the stone formation and the neutral pH of urine would help in the reduction of stone formation[31].

As mentioned earlier, *D. carota* extract is given for night to remove kidney stone[18]. Many studies demonstrated that administration of EG and vitamin D_3 causes hyperoxaluria which results in proximal renal tubular damage and shedding of brush border cells in kidney[23]. It is known that nearly 80 % of all kidney stones are composed of calcium and oxalate. Sodium oxalate injection is one of the best ways to induce experimental oxalate stones in animals[32].

Administration of DCE has significantly decreased the elevation of plasma and urine biochemical nitrogenous bases (creatinine, BUN and uric acid) caused by urolithiasis. Additionally, urinary calcium and oxalate levels have been decreased with pre-treatment of DCE. Thus, *D. carota* L. has the ability to alter calcium and oxalate excretion and its deposition may be due to the disintegration of mucoproteins, which are actually promoters of crystallization. A similar mechanism for anti-urolithiatic agents was reported previously by Grases *et al.*[33].

The antioxidants derived from fruit and vegetables are believed to maintain health and afford protection from coronary heart disease[34]. In the present study treatment with EG and vitamin D_3 shows decline levels of antioxidants like SOD, catalase, and GHS in the kidney. Superoxide dismutase (SOD) renovates superoxide radicals into the hydrogen peroxide, which sequentially removed by catalase and glutathione peroxides. It is commonly accepted that SOD safeguards against the free radical injury by transforming oxygen radical to hydrogen peroxide (H_2O_2) and leads to the generation of OH^- radicals by oxygen radical driven reaction and the catalase can remove H_2O_2[35]. SOD levels in the DCE pre-treated groups were significantly increased indicates *D. carota* L. a potential vegetable with a good antioxidant property.

Reduced glutathione (GSH) and catalase serve as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of the system[35]. Restoration of GSH and Catalase levels were noticed in DCE treated groups when compared to respective disease control, is almost normal, and this effect was dose-dependent.

Oxalate promotes oxidative stress, which is substantially retarded by antioxidants[36]. Moreover, oxalate enhances the influx of leukocytes into the kidney and change phagocytes, especially macrophages and monocytes, which produce various reactive oxygen metabolites by activating the complement system in the kidney[37].

In the present study treatment with EG and vitamin D_3 shows a significant increase in LPO levels. Evidence for the involvement of oxalate in free radical-mediated LPO reactions related to membrane injury is further strengthened by subsequent observations made by Scheid *et al.*[38]. These increased levels of LPO levels may be attributed to the increase in the deposition and excretion of oxalate in the kidney and urine, respectively.

Wilhelm *et al*. stated that carotenoids are efficient antioxidants which are present in carrot like vegetables are protecting plants, animal and humans against oxidative damage[39]. Due to their unique structure, it can be suggested that they possess specific tasks in the antioxidant network such as protecting lipophilic compartments or scavenging reactive oxygen species generated in oxidative processes. HPLC findings of the present study confirm the presence of polyphenolic substances like chlorogenic acid, caffeic acid and p-hydroxybenzoic acids in *D. carota* L.are responsible for its antioxidant and antiurolithiatic activity. Thus carrot may serve as one of the potential dietary sources of natural antioxidants for human nutrition and health. Bajaj *et al.*[40] reported that the content of 5-O-transcaffeoylquinic acid was the highest in polyphenols of fresh carrots, while other isomers took few proportions. Due to the above reason, the fresh carrot juice extract was selected to carry out the study. Thus, fresh juice of *D. carota* L. which has highest polyphenolics, supplementation of the same juice may effectively prevent the EG and vitamin D_3 induced urolithiasis in rats.

Histopathological changes of the kidney (Plate 1), were also supported the above results. Renal epithelial injury promotes crystal retention and adhesion of crystal adhesion molecules on epithelial surfaces.

Histopathological sections of animals treated with EG and vitamin D_3 showed deposition of the crystalline components in the renal tissue. There was a marked dilation of the tubules and degeneration of epithelial lining with infiltration of inflammatory cells into the interstitial spaces of renal tubules. This effect might be attributed due to oxalate toxicity when compared to the normal group. Pre-treatment with *D. carota* L. extract at low and high doses respectively has shown a marked reduction in the deposition of oxalate crystals and inflammatory cell infiltration in the renal tubule spaces and reduces the cellular damage with oxalate toxicity. Thus, the present investigation suggests that prior administration of *D. carota* L. extract significantly reduce the oxalate salt crystal deposition in kidney and also averted the oxalate toxicity induced kidney damage.

Conclusion

In conclusion, the data of present experiment suggest that the *D. carota* L. extract used in rats might prevent and possibly eliminate pre-existing kidney stones, this might be due to the presences of polyphenolics like chlorogenic acid in the extract. Further experimentations are required to elucidate the chemical constituents of the extracts and the mechanisms that are responsible for the pharmacological activities.

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