



Exploration Of Effective Anti-Urolithiatic Property Of *Carissa Carandas* L. Leaves Against Ethylene Glycol Induced Kidney Stones In Male Rats

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Abstract: Urolithiasis is a common disease that has been recognized and documented in medical literature even by the Greek and Roman physicians. *Carissa carandas* Linn., is ensconce all over India mostly in the semi-arid territory. Karonda trees are extensively cultured in the domicile gardens, farmer's fields, and orchards as hedge-row plants. The aim of the research was to evaluate the antiurolithiatic property of *Carissa carandas* Linn. leaf extract in rats. Urolithiasis in male Wistar albino rats was experimentally induced by administration of 0.75% (v/v) ethylene glycol in drinking water *ad libitum* for 28 days. Also the animals were treated with three doses of EELCC (ethanolic extract of leaves of *Carissa Carandas* Linn.) i.e., 100, 200, 400 mg/kg and Cystone 750 mg/kg b.w., p.o., respectively once daily from 15th to 28th day. On the 29th day, the body-weight difference was measured and animals was housed in individual metabolic cages, urine (pooled) collected for 24 h. Blood was collected on the same day and centrifuged. Parameters like urinary volume and pH, urinary analysis (Calcium, Oxalate, Creatinine, Uric acid, Blood urea nitrogen, and Urea) and serum analysis (Calcium, Oxalate, Creatinine, Uric acid, Blood urea nitrogen, and Urea) were performed to access the antiurolithiatic activity. The urine was subjected to microscopical study to observe the CaOx crystals. Thereafter the animals were sacrificed, kidneys excised followed by weighing the difference and estimation of homogenate parameters (Calcium, Oxalate, MDA, GSH, Catalase and SOD). Histopathological study of the kidneys were done by light microscopy, whereas the EELCC treated rats (400 mg/kg) showed no presence of CaOx crystal deposits and apparently retained normal morphology, tubular epithelial cells and glomeruli as in normal control group when compared with Cystone (750 mg/kg). Urolithiasis caused significant ($P < 0.01$) changes in all parameters in lithiatic control group rats as compared to normal control group rats., treatment with EELCC at three doses i.e. 100, 200 and 400 mg/kg and Cystone 750 mg/kg showed comparatively a significant ($P < 0.01$) restoration of all altered parameters. Based on results it can be concluded that the EELCC at dose of 400 mg/kg exhibited significant ($P < 0.01$) anti-urolithiatic activity on experimentally induced urolithiasis.

Keywords: Kidney stones, *Carissa carandas* L., Ethylene glycol, Male rats.

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1. INTRODUCTION

Urolithiasis encompasses all the renal, bladder and ureteric stones. The urinary stone disease continues to occupy an important place in everyday urological practice being the third most common urological disease worldwide affecting predominantly men.¹ The lifetime risk is about 10–15% in the developed world, but can be as high as 20–25% in the Middle East. Urolithiasis is a recurrent renal disease affecting 4–8 % in UK, 15 % in US, 20% in Gulf countries and 11% population in India with a relapse rate of 50% in 5–10 years and 75% in 20 years.² Because recurrence is common, and this condition impacts on economically active population which represents a significant health care costs burden, as it is associated with restricted activity and/or hospitalization. The prognosis of urinary stones is construct on the bolster of documentation attained from the history, physical examination, urinalysis, blood examination and radiographic investigation. The imaging techniques used for diagnosis of renal calculi are X-Ray, MRI, ultrasound, and intravenous pyelogram. The current-day medical management of urolithiasis is either costly or is linked with side-effects. Invasive methods for the therapy of urolithiasis may produce urgent issues and also take advantage of a significant burden of price on the healthcare organization. Presently, the available drug therapy for treatment of urinary stone includes the use of diuretics, antibiotics, analgesics, which do not completely cure urolithiasis rather only gives symptomatic relief. Certain herbal formulations are also available clinically like Cystone, Calcuri, etc., but they are not very effective and often require surgical interventions. The drugs temporarily relieve the symptoms but they result in autonomic, endocrine and gastrointestinal side effects whereas the procedure is expensive. The major drawback of these procedures is the recurrence of stones. In this regard, many indigenous Indian plants have been found to be useful to manage urolithiasis, one of which is *Carissa Carandas* Linn. It is commonly known as karonda or the christ's thorn (family Apocynaceae). Thus, the objective of the study was to evaluate anti-urolithiatic activity of ethanolic extract of leaves of *Carissa Carandas* Linn., (EELCC) in male albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

All Chemicals and reagents used for study were AR and Laboratory grade. Cystone, a product of Himalaya Drug Company was procured from the market. Diagnostic kits for various biochemical analysis were procured from Erba Diagnostics, Span Diagnostics, Beacon Diagnostics Pvt. Ltd.

2.2 Plant material collection and authentication

The leaves of *Carissa carandas* Linn., used in the present study were collected from the natural habitat around Nellore, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by Dr. P.V. Prasanna, Scientist 'F', Botanical Survey of India (BSI), Hyderabad, India (Ref. no: BSI/DRC/2018-19/Tech./824; Date: 29/01/2019).

2.3 Experimental animals

Male Wistar albino rats weighing 200–250g were obtained

from Adita Biosys Private Limited, Bangalore (Reg. no: 1868/PO/Bt/S/16/CPCSEA) for experimental purpose. All the animals were acclimatized for 7 days under standard husbandry condition i.e., Room temperature: $26 \pm 2^\circ\text{C}$, Relative humidity: 45–55%, Light/dark cycle: 12:12 h. All the animals were maintained in animal houses as per IAEC guidelines. The animals were housed individually in metabolic cages containing sterile paddy husk as bedding throughout the experiment. Animals were given access to standard pellet diet and water given *ad libitum*. The animal care and experimentation were in accordance with Institutional Animal Ethics Committee (IAEC), approval no: IAEC/XIII/03/RIPER/2019. Animal facility of our institution is approved by CPCSEA (Reg. no: 1736/PO/E/S/14/CPCSEA) New Delhi, India.

2.4 Preparation of ethanolic extract of leaves of *Carissa Carandas* Linn.

The fresh leaves of *Carissa Carandas* Linn., were collected, washed and dried under shade and then were coarse powdered with the help of mechanical grinder. Dried and coarsely powdered leaves were extracted with 99.9% ethanol using soxhlet apparatus for 18 h at 60°C . The extraction was carried out until colorless solvent appeared in the siphon tube. Then the material was filtered through a piece of muslin cloth and marc was pressed. The filtrate was filtered through whatman grade no.1 filter paper to get the clear filtrate. The extract was concentrated to $\frac{3}{4}$ of its original volume by distillation. The extract thus obtained was dried under reduced pressure and temperature not exceeding 40°C was maintained to obtain a semi solid extract (50 g). The concentrated extract was kept in desiccator over anhydrous calcium chloride till the constant weight of solvent free extract was attained. The extract was stored in a refrigerator at 4°C in a glass bottle throughout the study.³

2.5 Preliminary phytochemical analysis

The ethanolic extract of leaf of *Carissa carandas* Linn., was subjected to preliminary phytochemical screening.^{4,13}

2.6 Ethylene glycol induced urolithiasis in rats (Curative regimen)

2.6.1 Experimental design

Healthy male Wistar albino rats were divided into six groups containing six rats in each group and the curative study is conducted for 28 days. All animals were weighed before and after the study period. All groups received regular rat feed and drinking water *ad libitum*. Except group I, all animals received 0.75% (v/v) ethylene glycol in drinking water *ad libitum* from 1st to 28th day to accelerate lithiasis. Group I and group II served as normal control and lithiatic control respectively. Group II received normal saline from 15th to 28th day. Group III received standard anti-urolithiatic drug, Cystone (750 mg/kg body wt.) from 15th to 28th day, while Group IV, V, and VI received ethanolic extract of leaves of *Carissa carandas* Linn., at the doses of 100, 200, and 400 mg/kg body wt., respectively from 15th to 28th day. EELCC and Cystone were suspended in distilled water and 3% (v/v) Tween 80 respectively. The treatment was given orally once daily from 15th to 28th day. Various biological samples like blood, urine and kidney homogenate were collected at the end of the treatment period for the analysis of different parameters.^{5,8,9}

2.7 Evaluation parameters

2.7.1 Body weight

The body weight of each rat was measured during the experimental period, once before and after the treatment.

2.7.2 Collection and analysis of urine^{6,10}

For this purpose the animals were kept on fasting for 24 h, on 29th day of the experiment, animals were placed in individual metabolic cages for 24 h and urine samples were collected. Animals had free access to drinking water during the urine collection period. Inner surface of the urine collecting container was smeared with liquid paraffin to avoid the chance of evaporation of urine. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for Calcium, Oxalate, Creatinine, Uric acid, BUN, and Urea content.

2.7.3 Urine volume¹¹

On the 29th day of the experiment, Animals were placed in separate metabolic cages for 24 h and total urinary volume was measured using the measuring cylinder and reported in mL.

2.7.4 Urine pH¹²

Calcium oxalate crystals were found to deposit most frequently in the concentrated acidic urine. Thus, the acidity of the animals urine was tested on the 29th day of the experiment using the pH meter.

2.7.5 Microscopic studies⁷

On 29th day of the experiment, urine microscopy of all the animals was done. Microscopic examination should be performed on centrifuged samples.

2.7.6 Collection and analysis of serum¹⁴

After the experimental period, 1 mL of blood was collected retro-orbitally under anaesthetic conditions and animals were sacrificed by cervical dislocation. Serum was separated by centrifugation at 15000 rpm for 20 min. and analyzed for Calcium, Oxalate, Creatinine, Uric acid, Urea, and BUN content.

2.7.7 Kidney weight¹⁵

After urine and blood collection, all rats were sacrificed by cervical dislocation; the abdomen was cut open to carefully excise both the kidneys then weighed.

2.7.8 Kidney homogenate analysis¹⁵

Rats were sacrificed by cervical dislocation at the end of the experimental period. The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue, one half of the right kidney was dried at 80°C in a hot air oven and weighed. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min., and homogenized. The homogenate was

centrifuged at 10000 rpm for 20 min., and the supernatant was separated and analyzed for various biochemical parameters like Calcium, and Oxalate. The other half of the right kidney (100 mg) used to prepare homogenate in (pH 7) phosphate buffer for estimation of MDA, GSH, Catalase and SOD. The left kidneys selected from each group were preserved in 10% neutral formalin for histopathology.

3. STATISTICAL ANALYSIS

All the datas were expressed as mean \pm SEM (standard error of mean) of six rats in each group (n=6). The data was analyzed using Graphpad prism software version 7.0. Statistical significance was analyzed using one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparisons test using GraphPad Instat 3 and $P < 0.05$ considered as statistical significance.

4. RESULTS AND DISCUSSION

Phytochemical analysis revealed the presence of tannins, flavonoids, saponins, triterpenes phytosterol, phenols in the ethanolic extract of *Carissa carandas* L. leaves.

4.1 Effect of EELCC on physiological parameters

The ethylene glycol (0.75% v/v in drinking water) induced urolithiasis produced a significant ($P < 0.01$) reduction in weight of body¹⁶ (g), day to day in a lithiatic control group as compared to a normal control group. These changes were significantly ($P < 0.01$) decreased in the EELCC (100, 200, and 400 mg/kg) and Cystone (750 mg/kg) curative treated groups as compared to a lithiatic control group (Table I). Furthermore, the EELCC (400 mg/kg) was more significant ($P < 0.01$) in the prevention of body weight reduction. A significant ($P < 0.01$) decrease in 24 h urine volume¹⁷ (mL) was observed in lithiatic control group as compared to a normal control group, while EELCC (100, 200, and 400 mg/kg) and Cystone (750 mg/kg) curative treated groups showed significant ($P < 0.01$) improvement in urinary output as compared to a lithiatic control group (Table I). Furthermore, the EELCC (400 mg/kg) was more significant ($P < 0.01$) increase in the urine volume and restored it to near normal value than Cystone (750 mg/kg). A significant ($P < 0.01$) decrease in 24 h urine pH was observed in the lithiatic control group as compared to a normal control group. The curative treatment with EELCC (100, 200, and 400 mg/kg) significantly ($P < 0.01$) attenuated the decrease in urine pH in a dose dependent manner compared to a lithiatic control group (Table I). The EELCC at the dose of 400 mg/kg and Cystone (750 mg/kg) showed very similar significance ($P < 0.01$) in preventing the shift of pH from alkaline to acidic and restored it to near normal value. A significant ($P < 0.01$) increase in weight of both (left and right) kidneys' (g), day to day^{18,19} in a lithiatic control group as compared to a normal control group. These changes were significantly ($P < 0.01$) decreased in the EELCC (100, 200, and 400 mg/kg) and Cystone (750 mg/kg) curative treated groups as compared to a lithiatic control group (Table I). Furthermore, the EELCC (400 mg/kg) was more significant ($P < 0.01$) than Cystone (750 mg/kg) in the prevention of increase in weight of the left kidney. The same scenario was observed in the weight of the right kidney.

Table 1. Effect of EELCC on physiological parameters of ethylene glycol model

Groups	Body weight (g)		Urine volume (mL)	Urine pH	Kidney weight (g)	
	Before	After			Right	Left
Normal control	270±4.683	275±1.527	12.16±0.4772	7.90±0.0230	0.439±0.0017	0.420±0.0023
Lithiatic control	288±2.408 ^{###}	217±1.358 ^{###}	5.5±0.03651 ^{###}	6.47±0.0247 ^{###}	1.193±0.0043 ^{###}	1.537±0.0034 ^{###}
Cystone 750 mg/kg	269±4.781 ^{**}	248±1.621 ^{**}	10.33±0.4216 ^{**}	8.44±0.0142 ^{**}	0.520±0.0028 ^{**}	0.533±0.0017 ^{**}
EELCC 100 mg/kg	280±3.004 ^{ns}	257±1.673 ^{**}	8.53±0.0421 ^{**}	7.50±0.0210 ^{**}	0.431±0.0023 ^{**}	0.452±0.0017 ^{**}
EELCC 200 mg/kg	262±3.179 ^{**}	240±1.282 ^{**}	9.15±0.0341 ^{**}	7.61±0.0164 ^{**}	0.456±0.0034 ^{**}	0.462±0.0048 ^{**}
EELCC 400 mg/kg	284±3.177 ^{ns}	265±1.406 ^{**}	12.33±0.4216 ^{**}	8.41±0.0180 ^{**}	0.437±0.0034 ^{**}	0.449±0.0040 ^{**}

Values are expressed as Mean±SEM (n=6).

P values: ^{###}P<0.01 or ^{**}P<0.01 (Highly significant), ^{ns}P> 0.05 (Not significant).

4.2 Effect of EELCC on serum biochemical parameters

Kidney stone induction result in improper functioning of kidney and elevation of glomerular and tubular damaged markers in serum.²⁰ In the present study, ethylene glycol induced urolithiasis showed significantly (P< 0.01) elevation of various serum markers including Calcium, Oxalate, Creatinine, Uric acid, Urea, and Blood urea nitrogen (BUN) in lithiatic control group as compared to a normal control group. The treatment groups of EELCC (100, 200, and 400 mg/kg) were significantly (P< 0.01) reverted the alterations of serum markers in a dose dependent manner (Table 2). The curative treatment groups of the EELCC at the dose of 400 mg/kg and Cystone at the dose of 750 mg/kg showed almost similar significant (P< 0.01) in reverting the alterations of serum markers and restored them to near normal value.

Table 2. Effect of EELCC on serum biochemical parameters of ethylene glycol model

Groups	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)	BUN (mg/dL)	Calcium (mg/dL)	Oxalate (mg/dL)
Normal control	0.751±0.0268	2.22±0.0461	19.02±0.072	21.65±0.0403	5.23±0.0324	0.725±0.0152
Lithiatic control	2.135±0.0480 ^{###}	6.238±0.0454 ^{###}	32.50±0.2665 ^{###}	52.50±0.3225 ^{###}	8.78±0.0404 ^{###}	2.28±0.0893 ^{###}
Cystone 750 mg/kg	0.716±0.0125 ^{**}	2.20±0.0354 ^{**}	20.12±0.0351 ^{**}	26.12±0.7959 ^{**}	6.06±0.0204 ^{**}	0.621±0.0142 ^{**}
EELCC 100 mg/kg	1.291±0.0101 ^{**}	4.62±0.0580 ^{**}	23.08±0.054 ^{**}	35.30±0.4180 ^{**}	7.62±0.0412 ^{**}	0.876±0.0162 ^{**}
EELCC 200 mg/kg	0.985±0.0139 ^{**}	3.795±0.0604 ^{**}	22.35±0.0594 ^{**}	33.85±0.5739 ^{**}	7.15±0.0408 ^{**}	0.711±0.0087 ^{**}
EELCC 400 mg/kg	0.796±0.0269 ^{**}	3.361±0.0481 ^{**}	20.68±0.0573 ^{**}	31.04±0.2389 ^{**}	6.24±0.0409 ^{**}	0.643±0.0170 ^{**}

Values are expressed as Mean±SEM (n=6).

P values: ^{###}P<0.01 or ^{**}P<0.01 (Highly significant).

4.3 Effect of EELCC on urine biochemical parameters

The urinary excretion of various urolithiatic promoters such as Calcium, Oxalate, Creatinine, Uric acid, Blood urea nitrogen (BUN), and Urea were measured.^{21,22} There was a significant (P< 0.01) increase in the urinary excretion of promoters in the lithiatic control group as compared to the normal control. However, the treatment groups of EELCC

(100, 200, and 400 mg/kg) displayed a significant (P< 0.01) reduction in urinary excretion of promoters in a dose dependent manner as compared to lithiatic control group (Table 3). Furthermore, curative treatment groups of the EELCC at the dose of 400 mg/kg and Cystone at the dose of 750 mg/kg showed almost similar significant (P< 0.01) reduction in urinary excretion of urolithiatic promoters and restored them to near normal value.

Table 3. Effect of EELCC on urine biochemical parameters of ethylene glycol model

Groups	Creatinine (mg/24 h)	Uric acid (mg/24 h)	Urea (mg/24 h)	BUN (mg/24 h)	Calcium (mg/24 h)	Oxalate (mg/24 h)
Normal control	0.354±0.0030	1.80±0.0248	60.95±0.0766	8.13±0.1816	3.496±0.0628	4.573±0.0979
Lithiatic control	1.306±0.0187 ^{###}	3.57±0.0328 ^{###}	117.66±0.3600 ^{###}	20.95±0.3993 ^{###}	6.736±0.1512 ^{###}	9.16±0.1895 ^{###}
Cystone 750 mg/kg	0.681±0.0050 ^{**}	1.88±0.0308 ^{**}	58.42±0.3441 ^{**}	11.49±0.2995 ^{**}	4.20±0.2393 ^{**}	5.21±0.0381 ^{**}
EELCC 100 mg/kg	0.729±0.0049 ^{**}	2.45±0.0237 ^{**}	89.13±0.1478 ^{**}	16.93±0.2230 ^{**}	4.515±0.1998 ^{**}	7.18±0.0904 ^{**}
EELCC 200 mg/kg	0.663±0.0048 ^{**}	2.19±0.0278 ^{**}	74.48±0.2896 ^{**}	14.87±0.1922 ^{**}	4.26±0.2397 ^{**}	6.60±0.0773 ^{**}
EELCC 400 mg/kg	0.606±0.0024 ^{**}	2.09±0.0267 ^{**}	63.86±0.1803 ^{**}	13.56±0.0645 ^{**}	4.078±0.1189 ^{**}	6.05±0.0626 ^{**}

Values are expressed as Mean±SEM (n=6).

P values: ^{###}P<0.01 or ^{**}P<0.01 (Highly significant).

4.4 Effect of EELCC on kidney homogenate parameters

The deposition of urolithiatic promoters in the renal tissues, namely Calcium, and Oxalate were recorded.^{23,24} However, those promoters were found to be significantly (P< 0.01) higher in the renal tissue of the lithiatic control group compared to the normal control group. The treatment groups of EELCC (100, 200, and 400 mg/kg) were significantly (P< 0.01) reduction in the deposition of urolithiatic promoters in a dose dependent manner when compared with the lithiatic control group (Table 4). Furthermore, curative treatment groups of the EELCC at the dose of 400 mg/kg and Cystone at the dose of 750 mg/kg showed almost similar significant (P< 0.01) in reduction of deposited urolithiatic promoters and restored them to near normal value. In the present study, ethylene glycol induced

urolithiasis showed significantly (P< 0.01) increase in the lipid peroxidation of kidney tissue by the involvement of oxidative stress (imbalance of free radicals) as indicated with higher MDA (Malonaldehyde) level (as concentration) and decrease level (concentration) of non-enzymatic antioxidant like GSH and antioxidant enzymes such as Catalase (CAT), and Superoxide dismutase (SOD) in a lithiatic control group as compared to a normal control group. The treatment groups of EELCC (100, 200, and 400 mg/kg) displayed a significant (P< 0.01) protection against the oxidative damage in a dose dependent manner as compared to a lithiatic control group (Table 4). Furthermore, curative treatment groups of the EELCC at the dose of 400 mg/kg and Cystone at the dose of 750 mg/kg showed almost similar significant (P< 0.01) decrease in level of lipid peroxidation (LPO) and increase in level of non-enzymatic like GSH and antioxidant enzymes such as CAT, SOD, and restored them to near normal value.

Table 4. Effect of EELCC on kidney homogenate parameters of ethylene glycol model

Groups	Calcium (mg/100 mg kidney tissue)	Oxalate (mg/100 mg kidney tissue)	LPO (MDA) nmol/100 mg kidney tissue	GSH µM/100 mg kidney tissue	Catalase µmoles of H ₂ O ₂ utilized/mL/min./100 mg kidney tissue	SOD Units/100 mg kidney tissue
Normal control	2.78±0.056	1.42±0.036	0.26±0.01	86.10±0.06	16.69±0.01	199.1±0.27
Lithiatic control	5.05±0.060 ^{###}	6.44±0.137 ^{###}	6.32±0.01 ^{###}	41.90±0.23 ^{###}	8.96±0.04 ^{###}	98.45±0.48 ^{###}
Cystone 750 mg/kg	3.05±0.045 ^{**}	2.33±0.142 ^{**}	1.68±0.01 ^{**}	79.60±0.17 ^{**}	16.48±0.05 ^{**}	157.5±0.28 ^{**}
EELCC 100 mg/kg	3.62±0.0401 ^{**}	4.45±0.114 ^{**}	3.49±0.01 ^{**}	61.80±0.06 ^{**}	13.75±0.03 ^{**}	104.3±0.28 ^{**}
EELCC 200 mg/kg	3.43±0.0479 ^{**}	3.98±0.053 ^{**}	2.71±0.02 ^{**}	65.90±0.17 ^{**}	14.50±0.06 ^{**}	128.1±0.64 ^{**}
EELCC 400 mg/kg	3.26±0.0393 ^{**}	3.63±0.041 ^{**}	1.153±0.01 ^{**}	76.90±0.29 ^{**}	16.26±0.04 ^{**}	150.2±0.21 ^{**}

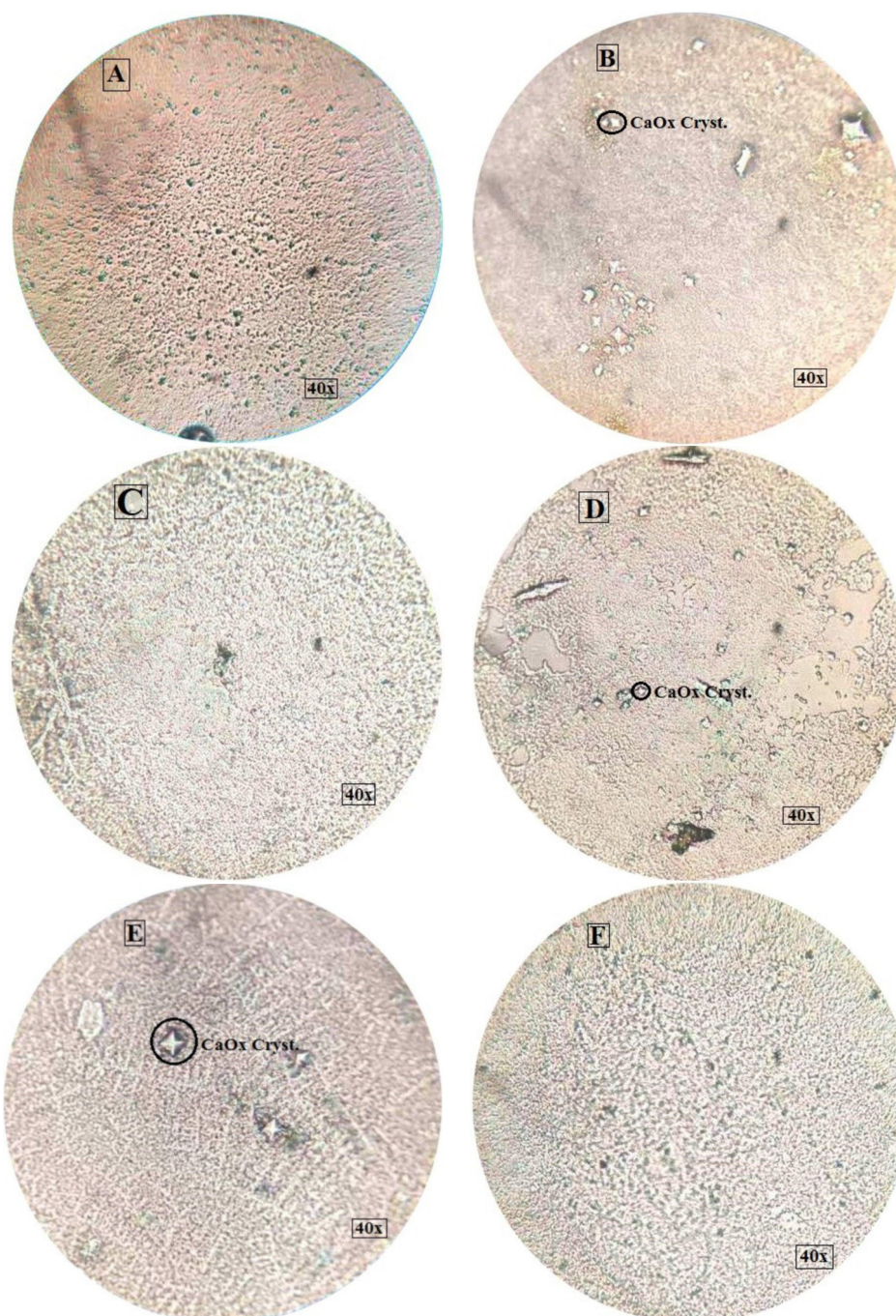
Values are expressed as Mean±SEM (n=6).

P values: ^{###}P<0.01 or ^{**}P<0.01 (Highly significant).

4.5 Effect of EELCC on microscopic studies of 24 h urine

In the present study, urine microscopy analysis revealed the frequency and size of calcium oxalate crystals is larger in the urine of the lithiatic control group when compared with normal control group.²⁵ The treatment groups of EELCC (100, 200, and 400 mg/kg) showed significant reduction in

frequency and size of calcium oxalate crystals compared with lithiatic control group in a dose dependent manner. The treatment groups of EELCC at the dose of 400 mg/kg showed no presence of calcium oxalate crystals compared with 100, and 200 mg/kg showed less, and very less frequency of CaOx crystals respectively (Figure 1). The curative treatment group of the Cystone (750 mg/kg) showed no presence of calcium oxalate crystals.



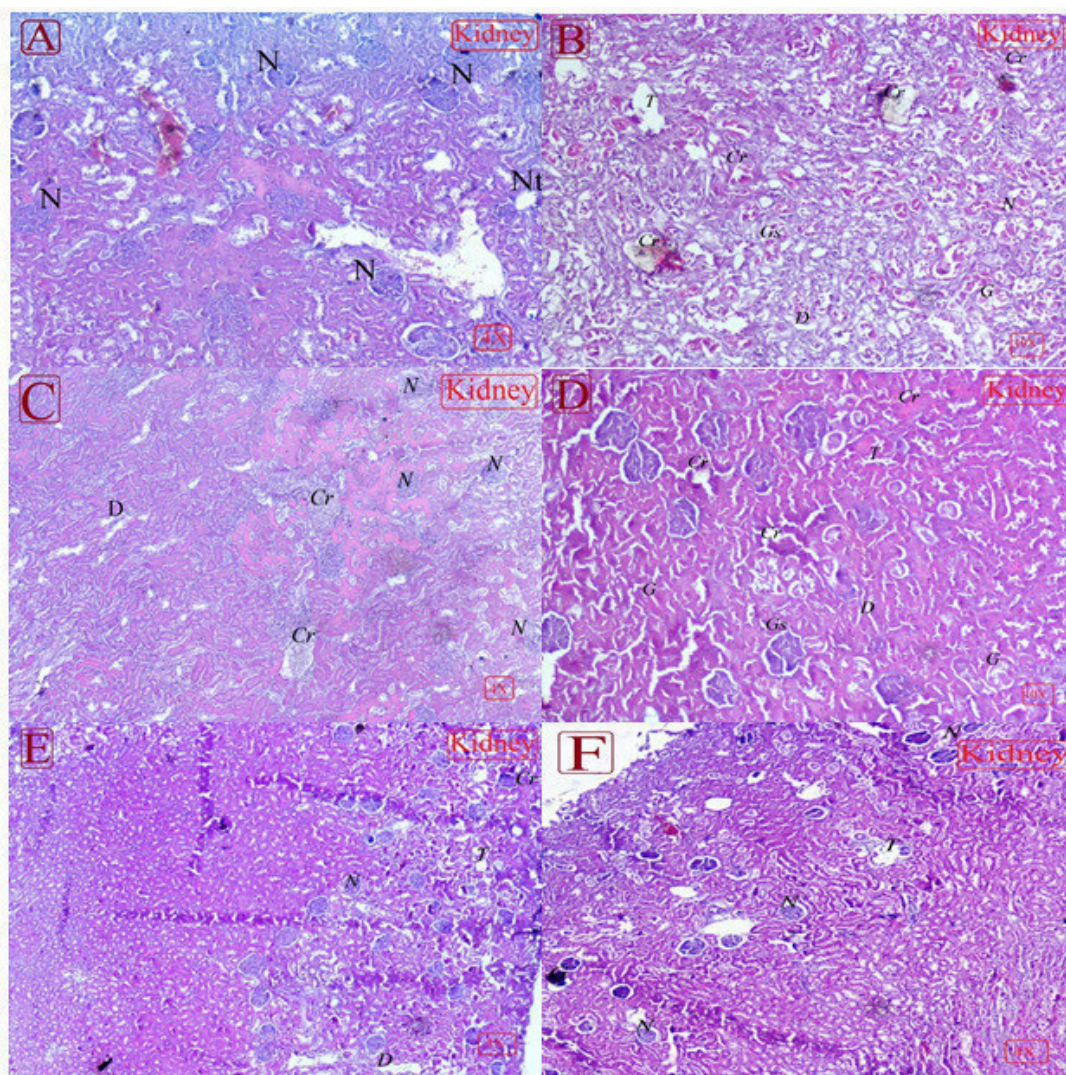
A = Normal Control: No CaOx crystals were seen; B = Lithiatic control: Numerous CaOx crystals were seen; C = Cystone 750 mg/kg, and F= EELCC 400 mg/kg: No CaOx crystals were seen; D = EELCC 100 mg/kg: Less CaOx crystals were seen; E = EELCC 200 mg/kg: Very less CaOx crystals were seen

Fig 1. Comparison of microscopic observation of CaOx crystals in 24 h urine of different groups in ethylene glycol model.

4.6 Histopathological results

The histopathological study of the kidneys from the rats in the normal control group, presented a normal appearance with normal glomeruli, proximal and distal convoluted tubules without any inflammatory changes, normal blood vessels, no membrane damage and no calcium oxalate crystal deposits or other abnormalities in the nephron segment.²⁶ In lithiatic control group, there was presence of severe dilatation of tubules (this might be attributed to oxalate formation), tubular necrosis, glomerular damage, glomerular sclerosis, infiltration of inflammatory cells into the interstitial

space, blood vessel proliferation, several CaOx crystal deposits inside the lumen of tubules, and degeneration of epithelial cells were observed in the renal tissue. However, the kidney section of rats treated with EELCC (100, 200, and 400 mg/kg) and Cystone (750 mg/kg) showed improvement of the above symptoms and reduced crystal deposition when compared with lithiatic control group (Figure 2). Whereas, the EELCC treated rats (400 mg/kg) showed no presence of CaOx crystal deposits and apparently retained normal morphology, tubular epithelial cells and glomeruli as in normal control group when compared with Cystone (750 mg/kg).



A= Normal Control, B=Lithiatic control; C=Cystone 750 mg/kg; D=EELCC 100 mg/kg; E=EELCC 200 mg/kg; and F=EELCC 400 mg/kg.

Cr – CaOx crystals; D –Dilatation of tubules; G –Glomerular damage; Gs –Glomerular sclerosis; N –Normal Glomeruli; Nt – Normal tubules; T –Tubular necrosis.

Fig 2. Photomicrographs of kidney sections at 4X and 10X magnifications where A, B, C, D, E, and F corresponds to treated groups.

5. CONCLUSION

From the present study it is concluded that urinary stones could be dissolved with ethanolic extract of *Carissa carandas* Linn., and without the aid of surgical intervention. Consequently, the present study provides scientific credence for the traditional claim of *Carissa carandas* Linn., as anti-urolithiatic.

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7. AUTHORS CONTRIBUTION STATEMENT

Madathala Sreekanth, designed and performed the experiments, derived the models and analysed the data. Veerasamy Haribaskar, Nunna BheemaLingeswara Prasad, were involved in planning and supervised the work processed the experimental data, drafted the manuscript and supervised the findings of this work.

8. CONFLICTS OF INTEREST

Conflict of interest declared none.

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