Assessment of potential antiurolithiatic property of *Carissa carandas* Linn. leaves on zinc disc insertion -incited urolithiasis in wistar male albino rats

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**ABSTRACT**

The goal of the research was to assess the antiurolithiatic property of *Carissa carandas* Linn. leaf extract in rats. The rats were segregated into 6 groups of 6 rats each. Calcium oxalate urolithiasis was surgically incited by insertions of pre-weighed and sterile zinc disc in bladders of animals. This was also followed by supplementing 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water *ad libitum* for 28 days. Upon postsurgical recovery period (3 days), Cystone (750 mg/kg) and three doses of EELCC (Ethanolic extract of leaves of *Carissa carandas* Linn.) namely 100, 200, and 400 mg/kg b.w., were given to zinc disc inserted animals for the duration of 28 days by oral route. Antiurolithiatic property was assessed by measuring the urinary volume, weight of the calculi, estimating the pH and analyzing the proportion of diverse biological markers in urine and serum specimens. An outstanding reduction in urine output and pH were noticed in zinc disc inserted rats, which were intercepted by the remedial extract. The extract also produced a significant enhancement in rate of glomerular filtration (GFR) and reduced the calculi deposition throughout the inserted zinc disc. The elevated levels of serum and urinary biochemical parameters like creatinine, urea, calcium, blood urea nitrogen (BUN), oxalate, and uric acid were also prevented by the extract. A significant (*P* < 0.01) potential antiurolithiatic property is observed at 400 mg/kg of EELCC.

**INTRODUCTION**

Urolithiasis is a typical urinary disorder that indicates calculi emanating in any place in the renal system, which are the kidneys’ and bladder. It is influencing almost 12% of the world population with a noticeable recurrence after an aciurgy dislodge (Lenin *et al.*, 2001) which demands an emergency requisite for proxy remedy. Surgical insertion of foreign substance such as zinc disc in the animal’s bladder, eventually causes acquisition of a calculus throughout the zinc disc. After insertion of a zinc disc, if ethane-1,2-diol (Ethylene glycol) is given orally to the rats with the crystals growing throughout the insert mostly constitute CaOx (Khan, 1997). Other than zinc discs, some of the other substances such as plastic discs, CaOx crystals, and non-absorbable surgical thread pieces can also be inserted in the bladder to incite the urolithiasis (Pawar and Vyawahare, 2016).
plant *Carissa carandas* (Family: Apocynaceae) is asserted to be beneficial for diverse indisposition; however, its anti-urolithiatic potential has not been validated scientifically. Hence, the current research was planned to assess the potential antiurolithiatic property of *Carissa carandas* Linn. leaves on zinc disc insertion- incited urolithiasis in wistar male albino rats.

**MATERIALS AND METHODS**

**Chemicals (AR Grade)**

Ethylene glycol (Ethane-1,2-diol) and all other chemicals, and miscellaneous biochemistry analyzing kits for assessment of serum and urinary biochemical parameters were acquired from Merck Life Science Pvt. Ltd., Nellore, India. Cystone (Himalaya Drug Company, Bangalore, India) was acquired from the Apollo Pharmacy, Nellore.

**Collection, authentication and extraction of plant material**

*Carissa carandas* Linn. leaves utilized in the current research were cumulated from the natural habitat around Nellore, Andhra Pradesh, India. The material of the plant was taxonomically recognized and validated 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). The dried *Carissa carandas* leaves were pulverized, loaded into thimble of soxhlet apparatus and extracted by using 99.9% v/v ethanol for 18 h at 60°C ([Hati et al., 2014](#)). The dried, crude concentrated extract (50 g, dark brown semi-solid, yield 12.5% w/w) was labeled as EELCC.

**Phytochemical testing**

The EELCC was treated to qualitative testing of the diverse phyto-constituents by grade procedures ([Rajaram et al., 2013](#)).

**Ethical clearance (Before the inception of the research)**

The protocol of research was endorsed by the IAEC (Institutional Animal Ethics Committee) with endorse no: IAEC/XIII/03/RIPER/2019 and following general rules of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) with reg. no: 878/PO/Re/S/05/CPCSEA), New Delhi, India.

**Experimental animals**

A total of 36 healthy (Seven to eight week-old) wistar male albino rats, weighing 200–250 g, were procured from Adita Biosys Private Limited, Bangalore (Reg. no: 1868/PO/Bt/S/16/CPCSEA) and permitted to get habituated for a week. The rats were housed in washed PP (Polypropylene) cages containing sterile paddy husk as bedding at room temperature (26 ± 2°C), humidity (45–55%), light intensity (325 lux) with 12 h dark–12 h light sequence throughout the research duration and were bestowed with quality chow and RO water *ad libitum*.

**Acute toxicity investigation**

No separate toxicity examination for the curative agent was done since the ethanolic extract was found to be safe as per OECD (Organization for Economic Co-operation and Development) guidelines 423 ([Shamim, 2014](#)).

**Selection of dose**

As the boundary dose did not exhibit signs of toxicity, at the doses of 100, 200 and 400 mg/kg, p.o., which were 1/20th, 1/10th and 1/5th of 2000 mg/kg respectively was taken up for the research. Cystone in the dose of 750 mg/kg was taken as standard drug.

**Cystone solution preparation**

Two tablets of Cystone were pulverized and 1 g of Cystone powder was dissolved in 5 mL of 3% (v/v) Tween 80 daily before administration [5 mL = 1000 mg of Cystone; 1 mL = 200 mg; 1 unit (1 mL syringe = 40 units) = 5 mg].

**Extract suspension preparation**

Precisely weighed quantity of plant extract was dissolved in Sodium carboxymethyl cellulose (NaCMC) to prepare required suspension before administration (Two gram of plant extract was dissolved in 14 mL of 0.5% (w/v) NaCMC; 14 mL = 2000 mg of extract; 1 mL = 143 mg; 1 unit = 3.6 mg).

**Zinc disc insertion incited urolithiasis in wistar male rats (Preventive regimen)**

Urinary CaOx calculi were incited by surgical insertions of pre-weighed and sterile zinc disc in the bladders of animals. This was also followed by supplementing 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water *ad libitum* for 28 days ([Revathy et al., 2016](#)). The treatment schedule was planned as follows,

**Normal control**

Saline solution (2 mL/kg b.w., p.o., q.d) for 28 days.

**Lithiatic control**

Surgical insertion of zinc disc + 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water for 28 days.

**Cystone 750 mg/kg**
Table 1: Consequence of EELCC on body weight, urinary volume and pH of zinc disc model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Urinary volume (mL)</th>
<th>Urinary pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>274± 3.095</td>
<td>279± 1.527</td>
<td>13.33± 0.4216</td>
</tr>
<tr>
<td>Lithiatic control</td>
<td>266± 1.701##</td>
<td>229± 1.390##</td>
<td>5.43± 0.0421##</td>
</tr>
<tr>
<td>Cystone 750 mg/kg</td>
<td>280± 2.577**</td>
<td>257± 1.607**</td>
<td>10.33± 0.4216</td>
</tr>
<tr>
<td>EELCC 100 mg/kg</td>
<td>276± 2.108*</td>
<td>245± 1.183**</td>
<td>5.93± 0.0421</td>
</tr>
<tr>
<td>EELCC 200 mg/kg</td>
<td>286± 1.869**</td>
<td>265± 1.335**</td>
<td>8.93± 0.0421</td>
</tr>
<tr>
<td>EELCC 400 mg/kg</td>
<td>280± 2.081**</td>
<td>261± 1.290**</td>
<td>10.33± 0.4216</td>
</tr>
</tbody>
</table>

Values are specified as Mean±SEM (n=6). P values: ##P<0.01 or ##P<0.01 (Highly significant), *P<0.05 (Significant).

Table 2: Consequence of EELCC on kidney, bladder and calculi weight of zinc disc model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney weight (g)</th>
<th>Bladder weight (mg)</th>
<th>Supposed bladder calculi weight (mg)</th>
<th>Actual bladder calculi weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>0.440± 0.0023</td>
<td>0.432± 0.0011</td>
<td>247±</td>
<td>-</td>
</tr>
<tr>
<td>Lithiatic control</td>
<td>0.709± 0.0028##</td>
<td>0.797± 0.0028##</td>
<td>498± 1.1547##</td>
<td>422± 2.3094##</td>
</tr>
<tr>
<td>Cystone 750 mg/kg</td>
<td>0.609± 0.0034**</td>
<td>0.653± 0.0017**</td>
<td>227± 4.0414**</td>
<td>45± 2.8867**</td>
</tr>
<tr>
<td>EELCC 100 mg/kg</td>
<td>0.661± 0.0005**</td>
<td>0.689± 0.0028**</td>
<td>255± 5.8972**</td>
<td>70± 2.8867**</td>
</tr>
<tr>
<td>EELCC 200 mg/kg</td>
<td>0.638± 0.0023**</td>
<td>0.689± 0.0023**</td>
<td>232± 2.3094**</td>
<td>50± 2.3094**</td>
</tr>
<tr>
<td>EELCC 400 mg/kg</td>
<td>0.352± 0.0017**</td>
<td>0.332± 0.0011**</td>
<td>223± 1.7320**</td>
<td>41± 2.3094**</td>
</tr>
</tbody>
</table>

Values are specified as Mean±SEM (n=6). P values: ##P<0.01 or ##P<0.01 (Highly significant). Note: Supposed bladder calculi weight = Weight of calculi along with zinc disc (20±2 mg); Actual bladder calculi weight = Weight of calculi without zinc disc.

Surgical insertion of zinc disc + 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water for 28 days + Cystone 750 mg/kg for 28 days.

**EELCC 100 mg/kg**

Surgical insertion of zinc disc + 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water for 28 days + EELCC 100 mg/kg for 28 days.

**EELCC 200 mg/kg**

Surgical insertion of zinc disc + 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water for 28 days + EELCC 200 mg/kg for 28 days.

**EELCC 400 mg/kg**

Surgical insertion of zinc disc + 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water for 28 days + EELCC 400 mg/kg for 28 days.

**Body weight**

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

**Urine collection and examination**

For this purpose the rats were retained on fasting for...
Figure 1: Consequence of EELCC on the accumulates throughout the inserted zinc discs in the bladder could be envisioned in radiographs from 28 days post-insertion.
24 h, on 29th day of the research, rats were placed in separate diuresis cages, hydration with 5 mL (Every 6 h) of RO water and urine of 24 h was collected. Urinary volume was noted, and urinary pH was determined (Kumar et al., 2016). Urine was investigated for Creatinine, Calcium, Urea, Oxalate, Uric acid, and Blood urea nitrogen (BUN) content.

**Urinary volume**

On 29th day of the experiment, rats were accommodated in individual diuresis cages for 24 hours and complete volume of urine was calculated by using the graduated cylinder and described in millilitres (Mariappan et al., 2016).

**Urinary pH**

The pH of animal’s urine was analysed on 29th day of the experiment using the pH meter (Rabie and Abdel-Halim, 2005).

**Microscopic studies**

On 29th day of the experiment, urine microscopy of all the animals were done. Microscopic examination should be performed on centrifuged sample (Kaur et al., 2009).

**Serum collection and examination**

On the 29th day of research, 1 mL of blood was drained from retro-orbital by anaesthetic conditions (Chinnala et al., 2013). Serum was segregated by centrifugation process at 15000 rpm for 20 minutes and investigated for Creatinine, Calcium, Oxalate, Blood urea nitrogen (BUN), Urea, and Uric acid content.

**Radiographical examination**

Radiography must be utilised to easily notice the

### Table 3: Consequence of EELCC on serum biochemical parameters of zinc disc model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>Calcium (mg/dL)</th>
<th>Oxalate (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.75±</td>
<td>2.27±</td>
<td>19.02±</td>
<td>21.60±</td>
<td>5.23±</td>
<td>0.72±</td>
</tr>
<tr>
<td>Lithiatic control</td>
<td>2.44±</td>
<td>7.55±</td>
<td>36.20±</td>
<td>52.50±</td>
<td>8.79±</td>
<td>2.39±</td>
</tr>
<tr>
<td>Lithiatic control</td>
<td>0.1626##</td>
<td>0.1320##</td>
<td>0.2206##</td>
<td>0.3170##</td>
<td>0.0556##</td>
<td>0.0226##</td>
</tr>
<tr>
<td>Cystone 750 mg/kg</td>
<td>0.82±</td>
<td>3.61±</td>
<td>22.68±</td>
<td>30.89±</td>
<td>6.64±</td>
<td>0.73±</td>
</tr>
<tr>
<td>EELCC 100 mg/kg</td>
<td>1.33±</td>
<td>4.65±</td>
<td>24.92±</td>
<td>35.61±</td>
<td>7.60±</td>
<td>0.91±</td>
</tr>
<tr>
<td>EELCC 200 mg/kg</td>
<td>0.95±</td>
<td>3.87±</td>
<td>23.18±</td>
<td>34.29±</td>
<td>7.20±</td>
<td>0.81±</td>
</tr>
<tr>
<td>EELCC 400 mg/kg</td>
<td>0.80±</td>
<td>3.39±</td>
<td>20.92±</td>
<td>29.44±</td>
<td>6.30±</td>
<td>0.67±</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n=6). P values: ##P<0.01 or ##P<0.01 (Highly significant).

### Table 4: Consequence of EELCC on urinary biochemical parameters of zinc disc model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>Calcium (mg/dL)</th>
<th>Oxalate (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.354±</td>
<td>1.80±</td>
<td>60.95±</td>
<td>8.13±</td>
<td>3.49±</td>
<td>4.57±</td>
</tr>
<tr>
<td>Lithiatic control</td>
<td>1.31±</td>
<td>3.91±</td>
<td>119.10±</td>
<td>21.23±</td>
<td>6.89±</td>
<td>9.66±</td>
</tr>
<tr>
<td>Cystone 750 mg/kg</td>
<td>0.58±</td>
<td>2.19±</td>
<td>71.72±</td>
<td>14.36±</td>
<td>4.49±</td>
<td>6.47±</td>
</tr>
<tr>
<td>EELCC 100 mg/kg</td>
<td>0.70±</td>
<td>2.45±</td>
<td>102.2±</td>
<td>16.95±</td>
<td>4.81±</td>
<td>7.29±</td>
</tr>
<tr>
<td>EELCC 200 mg/kg</td>
<td>0.70±</td>
<td>2.45±</td>
<td>102.2±</td>
<td>16.95±</td>
<td>4.81±</td>
<td>7.29±</td>
</tr>
<tr>
<td>EELCC 400 mg/kg</td>
<td>0.55±</td>
<td>2.09±</td>
<td>69.82±</td>
<td>12.71±</td>
<td>4.48±</td>
<td>6.41±</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n=6). P values: ##P<0.01 or ##P<0.01 (Highly significant).
Figure 2: Comparison of microscopic observation of CaOx crystals in 24 h urine of different groups in zinc disc model.

A = Normal control and F = EELCC 400 mg/kg: No CaOx crystals were seen; B = Lithiatic control: Numerous CaOx crystals were seen; C = Cystone 750 mg/kg: Very less CaOx crystals were seen; D = EELCC 100 mg/kg: Moderate CaOx crystals were seen; E = EELCC 200 mg/kg: Less CaOx crystals were seen.
augmentation of deposition throughout the inserted zinc disc in the bladder. Radiographical investigation was done prior to immolating the rats to prove the development of calculi by digital X-ray instrument (Vargas et al., 1999).

**Kidney and bladder weight**

After urine and blood collection, all rats were immolated by the euthanasia practice of cervical dislocation; the stomach was cut unravel to carefully excise the couple kidneys and bladder, then weighed.

**Weight of bladder calculi**

After urine collection period, the weight of bladder calculi reconciled by immolating the rats by cervical dislocation. The bladders were enacted and zinc disc along with the cohered crystals were dislodged and packed in individual polyethylene bags.

**Statistical study**

All the data were specified as mean ± SEM of 6 rats (n=6). Data investigation was carried out by using software of GraphPad Prism (Version 8.0). The data were investigated by using one-way ANOVA proceeded by Dunnett’s test (Multiple comparison) by using GraphPad Instat (Version 3.0) and P<0.05 value is contemplated as statistical significance.

**RESULTS AND DISCUSSION**

Phytochemical testing revealed the presence of tannins, flavonoids, saponins, triterpenes, phytosterols and phenols in the ethanolic extract of Carissa carandas L. leaves. In the oral acute toxicity investigation, EELCC was found to be safe as it did not cause any mortality or lethality or toxic reactions up to 2000 mg/kg.

**Consequence of EELCC on body weight, urinary volume and pH**
Figure 4: Photomicrographs of bladder sections at 4X, 10X and 20X magnifications where A, B, C, D, E and F corresponds to treated groups.

A = Normal control; B = Lithiatic control; C = Cystone 750 mg/kg; D-F = EELCC 100, 200, and 400 mg/kg, respectively.
D = Damaged cells; H = Hyperplasia; Ht = Healing tissue; I = Inflammation; MI = Multilayered (damaged); R = Regenerative changes; SI = Single layered (healthy).
The surgical implantation of zinc disc along with 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water, induced urolithiasis caused a significant (P< 0.01) day to day (Fan et al., 1999) reduction in weight of body (g), decrease in 24 h urinary volume (mL) and pH (Khan et al., 1982) in lithiatic control when distinguished from normal control. The above changes were significance (P< 0.01) prevented in the EELCC at the doses of 100, 200, and 400 mg/kg of preventive treated groups in a dose wise mode when distinguished from lithiatic control (Table 1). Furthermore, the EELCC at the dose of 400 mg/kg displayed a greater significance (P< 0.01) in preventing the body weight reduction, increase in urinary volume and preventing the shift of pH from alkaline to acidic than Cystone (750 mg/kg).

Consequence of EELCC on kidney, bladder and calculi weight

The surgical implantation of zinc disc along with 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water, induced urolithiasis caused a significant (P< 0.01) day to day, increase in (Eldin et al., 2008; Saha and Verma, 2015) weight of both (left and right) kidneys (g), weight of bladder (mg), and enormous (Perez-Hernandez et al., 2018) amount of stone (mg) formation hence increase content in the bladder in a lithiatic control when distinguished from normal control. The above changes were significantly (P< 0.01) prevented in the EELCC at the doses of 100, 200, and 400 mg/kg of preventive treated groups in a dose wise mode when distinguished from lithiatic control (Table 2 and Figure 3). Furthermore, the EELCC at the dose of 400 mg/kg displayed a greater significance (P< 0.01) in the prevention of increase in weight of kidneys and bladders, and decreased the building of accumulates throughout the zinc disc, hence decrease content in the bladder than Cystone (750 mg/kg).

Consequence of EELCC on serum and urinary biochemical parameters

The surgical implantation of zinc disc along with 0.75% v/v ethane-1,2-diol (Ethylene glycol) in quaffing water, incited urolithiasis produced a significant (P< 0.01) upraise of diverse serum and urinary markers namely Creatinine, Oxalate, Uric acid, Calcium, Urea, and Blood urea nitrogen (BUN) in lithiatic control when distinguished from normal control (Dinnimuth et al., 2017). The treatment groups of EELCC at the doses of 100, 200, and 400 mg/kg significance (P< 0.01) reverted the alterations of serum and urinary markers in a dose wise mode when distinguished from lithiatic control (Tables 3 and 4). The preventive treatment groups of the EELCC at the dose of 400 mg/kg displayed a greater significant (P< 0.01) in reverting the alterations of serum and urinary markers and restored them to near normal value than Cystone (750 mg/kg).

Consequence of EELCC on microscopic studies of 24 h urine

Urine microscopy analysis of 24 h urine revealed the number of CaOx crystals, which is greater in the urine of lithiatic control as distinguished from normal control (Ragini and Padala, 2014). The treatment groups of EELCC at the doses of 100, 200, and 400 mg/kg showed significant (P< 0.01) reduction in number of CaOx crystals in a dose wise mode when distinguished from lithiatic control (Figure 2). Furthermore, the EELCC in a dose of 400 mg/kg showed no presence of calcium oxalate crystals when compared with Cystone (750 mg/kg).

Consequence of EELCC on stone formation by radiographs

The digital radiographs of animal’s divulged appearance of CaOx crystals accumulates throughout the inserted zinc disc within 28 days, thereafter insertion in the lithiatic control (Vyas and Argal, 2013). The treatment groups of EELCC at the doses of 100, 200, and 400 mg/kg significantly (P< 0.01) decreased the formation of such deposits around the implanted disc when compared with lithiatic control group in a dose wise mode (Figure 1). Furthermore, the EELCC in a dose of 400 mg/kg significantly (P< 0.01) reduced the formation of CaOx crystal deposits around the implanted disc than Cystone (750 mg/kg). These findings were proved in actual calculi weights of the bladder (Table 2).

Consequence of EELCC on microscopic examination of bladder tissue

Histopathology of normal control rats by section study of the bladder microscopy showed normal morphology. There were marked histological changes such as structural modification in the epithelial cells, tissue damage, inflammation and hyperplasia in lithiatic rats (Malipeddi and Das, 2016). However, bladder section of rats treated with EELCC at the doses of 100, 200, and 400 mg/kg showed improvement of the above noticed histological changes when distinguished from lithiatic control (Figure 4). Whereas, the EELCC treated rats (Preventive: 400 mg/kg) apparently showed a retained normal morphology and healing tissue, regenerative changes and healthy epithelial cells as similar to normal control than Cystone (750 mg/kg).
CONCLUSIONS

The feasible mechanisms underlying in this property is communicated jointly by antioxidant, diuretic, free-radical scavenging, and anti-inflammatory properties of Carissa carandas. These findings can be applied to modern system of the medicine by incorporating it into a dosage form which will make it more acceptable for patients. This research projects the therapeutic potential of the ethanolic extract of leaves of Carissa carandas Linn. that may be developed as an alternative polyherbal anti-urolithiatic drug like Cystone.

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Ethical approval

Endorsed by the IAEC (Institutional Animal Ethics Committee).

Conflicts of interest

Nil.

REFERENCES


