Antiuroliathic activity of *Nardostachys jatamansi* DC on Modified Lithogenic Diet induced urolithiasis in rats

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**Abstract**

In an indigenous system of medicine, the rhizomes of *Nardostachys jatamansi* DC (family- Valerianaceae) to be useful in the treatment of urinary stone. Hence, in this present study the rhizome of *N. jatamansi* DC has been selected for its antiurolithic activity on experimentally induced urolithic rats. Antiurolithic activity of *N. jatamansi* DC was carried out on a modified lithogenic diet (30% lactose rich diet and 1% ethylene glycol) induced urolithiasis in rats. Treatment with hydroalcoholic extract (400mg/kg, p.o) of *N. jatamansi* DC significantly lowered ($P<0.001$) the increased levels of calcium, oxalate, phosphate, magnesium, urea & uric acid in serum & urine and significantly reduced ($P<0.001$) their retention in the kidneys. The histopathological study of the kidney also supported the above results. The results were comparable to that of the standard drugs (Cystone). The presented data indicate that administration of *N. jatamansi* DC rhizome extract in rats with experimentally-induced urolithiasis is reduced and also prevented the formation of urinary stones, supporting folk information regarding the antiurolithic activity of the plant. The reduction in the stone forming constituents in urine and renal tissue brought about *N. jatamansi* DC by could contribute to its antiurolithic property.

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**Key Words**
Lactose, Ethylene glycol, Urolithiasis
*Nardostachys jatamansi*, Rhizome,
INTRODUCTION

Urinary stones are one of the oldest and the most common afflictions in humans. This is the third most common condition of the urinary tract infection and pathologic condition of the prostate\(^{(1)}\). Urinary calculi are formed or located anywhere in the urinary system or the process of forming stones in the kidney, bladder, and ureters. The formation of these calculi involving several physiochemical aggregations and retention within the urinary tract\(^{(2)}\). Among the several types of kidney stones, the most common are calcium oxalate stones representing up to 80% of the analyzed stones\(^{(3)}\). Currently no allopathic medicines are available for urolithiasis. Surgery, lithotripsy, and local calculus disruption using a high power laser are used to treat calculi. However, these procedures are expensive and recurrence is quite common\(^{(4)}\).

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in traditional systems. *Nardostachys jatamansi* DC rhizome belonging to family Valerianaceae, has many therapeutic benefits such as its use in tribal area as antiarrhythmic, antispasmodic, diuretic, anti-inflammatory, urinary and vesicle calculi, analgesic agent and hair tonic\(^{(5)}\).

The present study is focused on the investigation of the antiurolithiatic activity of a hydro alcoholic extract of *N. jatamansi* rhizome.

MATERIAL AND METHODS

Animals

Adult male Wistar albino rats of (150-200 gm) were procured from Perundurai Medical College, Erode District, and Tamilnadu, India. Prior to the experiment the rats were housed in a clean polypropylene cage (6 rats/cages) for a period of 7 days under temperature (25-30°C) relative humidity (45-55%). They were fed with standard pellet diet and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee. (Approval No; PCOL/04/2013/IAEC/ECP).

Chemicals

Ethylene glycol was obtained from chemical laboratory, Erode, Tamilnadu.
Cystone (Himalaya Health Care, India) was procured from the local market. All other chemicals and reagents used were of analytical grade.

**Preparation of plant extract**

The rhizome of *Nardostachys jatamansi* DC was collected from Salem district, Tamilnadu and authenticated by Dr. A. Balasubramanian, Siddha research consultant, ABS Botanical garden, Salem, Tamilnadu. The air dried rhizomes (200g) were powdered and extracted with 50% ethanol in soxhlet apparatus for 72 hours. The extract was evaporated under reduced pressure to give solid residues, which was stored 0-4°C for subsequent experiments. The yield of the extract was 12.57 % w/w.

**Acute toxicity studies**

Acute toxicity study was performed as per the OECD guideline (no-425) using albino mice prior to the evaluation of antiurolithiatic activity. Four arbitrary doses of 200, 400, 800 and 1600mg/kg were selected for the study, as the extract was found safe even at doses more than 2000mg/kg without any sign of toxicity or mortality.

No mortality of animals was observed in the dose range and hence two different doses 200 and 400 mg/kg was taken for the study.

**Experimental design**

Modified lithogenic diet induced urolithiasis model was used to assess the antiurolithiatic activity in Wistar albino rats. Animals were divided into seven groups, each group consisting six of them. Group-I served as a control and received regular rat food and drinking water *ad libitum*. Modified lithogenic diet (MLD) (30% lactose rich diet and 1%EG) The 30% lactose rich lab diet contains 3.68%sucrose, 30%lactose, 23.4% protein, 10%fat, 5.3% crude fibre, 6.9% ash minerals (calcium 0.95%, phosphorus 0.67%, magnesium0. 21%), vitamin A 22 IU/g, vitamin D3 4.5IU/g, vitamin E 49 IU/G) and 1% EG in drinking water) was fed to groups II-VII for 28 days to induce formation of renal calculi. Group-III served as a standard antiurolithiatic drug cystone (750mg/kg). Groups IV and V served as curative regimen and received HNJ 200mg/kg and 400mg/kg body weight respectively from 15th to 28th day. Groups VI and VII received HNJ 200mg/kg and 400mg/kg body weight respectively from 1st day till the 28th day and served as a preventive
regimen. All extracts and standard were given once daily by oral route.

**Analysis of urine and serum**

Formation of crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. At the end of the experiment, all animals kept in individual metabolic cages and 24-hour urine samples were collected and measured on the 28th day. Animals had free access to drinking water during the urine collection period. The urine was analysed for calcium, magnesium, phosphate, urea, uric acid, oxalate and citrate.

On the 29th day, the animals were anesthetized and blood was collected from the retro-orbital sinus under mild anaesthesia. Serum was separated by centrifugation at 15,000 rpm for 20 minutes and analyzed for calcium, oxalate, magnesium, phosphate, urea, uric acid and creatinine(9).

**Histopathological studies**

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were rinsed in an ice-cold physiological solution, after the extraneous tissues are removed. The kidneys were fixed in 10% formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin for histopathological examination. The slides were examined under a light microscope to study the architecture of the kidney and calcium oxalate deposits(10).

**Statistical analysis**

Results were expressed as mean ± SEM and determine the significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test, using Instat v 2.02 software (GraphPad Software Inc.) Differences between groups (p value) were considered significant at P<0.05.

**RESULTS**

The acute toxicity studies showed no adverse effect or mortality in albino rats up to 2000mg/kg p.o. of hydroalcoholic extract of *N. jatamansi* DC. Hence the therapeutic dose was taken as 200mg/kg and 400mg/kg body weight of the hydroalcoholic extract.
Tab 1 depicts the physical parameter data that were obtained at the end of the experiment in each group. In the present study, HNJ significantly (p<0.001) increased the urine volume at doses of 200mg and 400mg/kg indicating its activity. Urine pH in normal rat was found between 6.0 and 7.0. On the induction of calcium oxalate stones pH reduced to (5.0-6.0) in preventive and curative control groups. After completion of the study, prevention (IV and V) and curative (VI-VII) groups treated with hydroalcoholic extract of N. jatamansi DC showed an increase in urinary pH (7.0-8.0) when compared to the respective control groups. Moreover administration of MLD caused an inflammatory condition and deposition of crystalline components in the kidney which, leads to an increase in the weight of the kidneys. Concurrent administration of hydroalcoholic extract of N. jatamansi DC reduced the kidney weight by decreasing inflammation and increasing excretion of crystalline components. Chronic administration of modified lithogenic diet to male albino Wistar rats resulted in hyperoxaluria. There was an increase in urinary calcium, oxalate, magnesium, phosphate, urea, and uric acid in calculi induced animals (Tab 2, group II). However, supplementation with HNJ (200 and 400mg/kg) significantly inhibited these changes in urinary calcium, oxalate, phosphate, uric acid, magnesium, urea excretion dose dependently in both curative and preventive regimens (Tab 2, group IV-V). Urinary citrate excretion was decreased after the administration of MLD in group II compared to the normal group. (Group-1). However supplementation with HNJ (200 and 400mg/kg) significantly increased (p<0.001) this parameter and restores the urinary citrate levels to near-normal value. The results were consistent with Cystone –treated animals. (Tab 3 group III).

Renal stone induction caused impairment of renal functions of the untreated rats as evident from the makers of Glomerular and tubular damage. Elevated serum creatinine, uric acid and urea .These markers were significant (p<0.001) reduced in the animals which were treated with HNJ in a dose dependent manner. The serum calcium, inorganic phosphate and magnesium were significantly increased in calculi induced animals compared to group 1 (Tab 3, group II) indicating marked renal damage. However,
treatment with HNJ (200 and 400mg/kg) significantly (p<0.001) lowered the elevated serum level of calcium, inorganic phosphate, and magnesium in both curative and preventive regimens (Tab 3 group IV-VII).

Histopathological analysis revealed no calcium oxalate deposits or other abnormalities in the nephron segment of the vehicle treatment group (Fig 1a). on the other hand, several calcium oxalate deposits inside the tubules and dilation of the proximal tubules along with interstitial inflammation were observed in the renal tissues of urolithiatic rats (Fig 1 b) the number of calcium oxalate deposits in the tubules of HNJ treated rats (group IV-VII) and cystone treated rats (group-II) were less than group II (Fig 1c-e).

Tab 1. Effect of hydroalcoholic extract of rhizomes of N.Jatamansi DC on physical parameters of control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight in g</th>
<th>kidney wt in</th>
<th>urine volume in ml</th>
<th>pH of the urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-1</td>
<td>173.8±1.10</td>
<td>193.3±0.49</td>
<td>1.63±0.03</td>
<td>19.25±0.06</td>
</tr>
<tr>
<td>Group-2</td>
<td>167.3±0.3</td>
<td>166.0±0.93</td>
<td>2.25±0.05</td>
<td>10.30±0.05</td>
</tr>
<tr>
<td>Group-3</td>
<td>160.6±0.76</td>
<td>173.5±0.42</td>
<td>1.66±0.04***</td>
<td>17.76±0.14***</td>
</tr>
<tr>
<td>Group-4</td>
<td>157.5±0.42</td>
<td>164.8±1.16</td>
<td>1.98±0.10</td>
<td>8.65±0.07***</td>
</tr>
<tr>
<td>Group-5</td>
<td>183.1±0.60</td>
<td>187.1±1.81</td>
<td>1.83±0.07**</td>
<td>9.41±0.12***</td>
</tr>
<tr>
<td>Group-6</td>
<td>180.57±0.7</td>
<td>185.3±0.30</td>
<td>1.76±0.08**</td>
<td>12.45±0.13***</td>
</tr>
<tr>
<td>Group-7</td>
<td>178.3±2.43</td>
<td>196.6±2.41</td>
<td>1.66±0.08*</td>
<td>14.18±0.08***</td>
</tr>
</tbody>
</table>

Values are given in Mean±SEM.*p<0.05, **p<0.01, ***p<0.001. For n=6. Toxic group compared with normal group and all other groups were compared with toxic group.

Tab 2. Effect of hydroalcoholic extract of rhizomes of *N.Jatamansi* DC on urinary parameters of control and experimental animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calcium (mg/dl)</th>
<th>Phosphate (mg/dl)</th>
<th>Mg (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Citrate (mg/dl)</th>
<th>Oxalate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>3.7±0.14</td>
<td>3.78±0.12</td>
<td>10.34±0.14</td>
<td>10.84±0.19</td>
<td>10.03±0.08</td>
<td>3.66±0.04</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>Group-2</td>
<td>5.05±0.31***</td>
<td>9.58±0.17</td>
<td>19.48±0.14***</td>
<td>18.6±0.31</td>
<td>25.73±0.08</td>
<td>2.63±0.07</td>
<td>3.23±0.14</td>
</tr>
<tr>
<td>Group-3</td>
<td>4.13±0.06**</td>
<td>4.1±0.14***</td>
<td>12.08±0.18***</td>
<td>12.83±0.18***</td>
<td>12.25±0.07***</td>
<td>3.80±0.05***</td>
<td>1.26±0.06**</td>
</tr>
<tr>
<td>Group-4</td>
<td>4.96±0.19</td>
<td>8.18±0.30*</td>
<td>17.5±0.12*</td>
<td>15.38±0.17*</td>
<td>23.30±0.11</td>
<td>2.80±0.03***</td>
<td>1.65±0.12</td>
</tr>
<tr>
<td>Group-5</td>
<td>4.10±0.16*</td>
<td>7.03±0.36***</td>
<td>16.48±0.06***</td>
<td>14.55±0.17***</td>
<td>20.51±0.60*</td>
<td>2.71±0.07***</td>
<td>1.61±0.10</td>
</tr>
<tr>
<td>Group-6</td>
<td>4.85±0.21</td>
<td>7.56±0.13***</td>
<td>17.16±0.04</td>
<td>15.93±0.35*</td>
<td>22.53±0.07**</td>
<td>2.70±0.07</td>
<td>1.85±0.09*</td>
</tr>
<tr>
<td>Group-7</td>
<td>4.0±0.15*</td>
<td>7.16±0.44***</td>
<td>16.25±0.07**</td>
<td>14.91±0.09**</td>
<td>19.85±0.07**</td>
<td>2.91±0.05</td>
<td>1.65±0.07**</td>
</tr>
</tbody>
</table>

*Values are given in Mean±SEM. *p<0.05, **p<0.01, ***p<0.001. For n=6. Toxic group compared with normal group and all other groups were compared with toxic group*
Tab 3. Effect of hydroalcoholic extract of rhizomes of *N. Jatamansi* DC on serum parameters of control and experimental animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calcium (mg/dl)</th>
<th>Phosphate (mg/dl)</th>
<th>Mg (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>8.65±0.22</td>
<td>4.36±0.16</td>
<td>2.33±0.16</td>
<td>33.18±0.80</td>
<td>2.5±0.12</td>
<td>0.62±0.06</td>
</tr>
<tr>
<td>Group-2</td>
<td>13.26±0.23</td>
<td>10.5±0.25</td>
<td>4.08±0.10</td>
<td>55.62±0.29</td>
<td>4.20±0.10</td>
<td>1.36±0.08</td>
</tr>
<tr>
<td>Group-3</td>
<td>8.75±0.10***</td>
<td>5.46±0.16***</td>
<td>2.45±0.13***</td>
<td>35.83±0.49**</td>
<td>2.83±0.07**</td>
<td>0.73±0.08**</td>
</tr>
<tr>
<td>Group-4</td>
<td>12.26±0.15**</td>
<td>10.06±0.14</td>
<td>3.71±0.17</td>
<td>48.43±0.31**</td>
<td>4.13±0.13*</td>
<td>1.56±0.08</td>
</tr>
<tr>
<td>Group-5</td>
<td>11.38±0.14***</td>
<td>8.43±0.19**</td>
<td>2.78±0.17***</td>
<td>42.11±0.51**</td>
<td>3.63±0.05***</td>
<td>0.91±0.11</td>
</tr>
<tr>
<td>Group-6</td>
<td>12.41±0.14*</td>
<td>6.16±0.23**</td>
<td>3.03±0.13*</td>
<td>40.33±0.52</td>
<td>2.85±0.07</td>
<td>1.30±0.09**</td>
</tr>
<tr>
<td>Group-7</td>
<td>11.66±0.12***</td>
<td>6.45±0.18***</td>
<td>2.93±0.20***</td>
<td>39.45±0.43**</td>
<td>3.46±0.04***</td>
<td>1.11±0.10</td>
</tr>
</tbody>
</table>

Values are given in Mean±SEM. *p<0.05, **p<0.01, ***p<0.001. For n=6. Toxic group compared with normal group and all other groups were compared with toxic group.
A number of renal pathological diseases, including calcium oxalate kidney stones, have resulted due to the oxalate-induced damage to the renal cells. Elevated level of oxalate is responsible for the toxic effects on the renal epithelial cells (11-13). In the present study, we used modified lithogenic diet (combining 30% lactose rich diet and 1% ethylene glycol in drinking water for 28 days) for inducing urolithiasis in albino Wistar rats. A renal calculus due to ethylene glycol in the drinking water has been widely used for inducing urolithiasis. Ethylene glycol is metabolized by alcohol dehydrogenase to glycolaldehyde, which is then oxidized to glycolic acid and finally to oxalic acid. Oxalic acid binds with calcium to form calcium oxalate crystals. The ingestion of 30% lactose rich diet increased urinary calcium excretion without changing urinary oxalate excretion and showed stable and marked crystalluria (14). Therefore an effective animal model, producing stable crystal deposition and non-nephrotoxic model for urolithiasis research.

The present study showed an increase in urine output of HNJ treated animals which dilute the concentration of urinary electrolytes. As a result, calcium and phosphates are flushed out via urine and there is a lesser chance of precipitation, decreased formation, as well as the growth of urinary stone. Most calculi in the urinary system arise from a common of urine such as calcium oxalate and
hypercalciuria representing up to 80% of analyzed stones. Increased urinary calcium is a favouring the nucleation and precipitation of calcium oxalate or apatite. However, HNJ lowered levels of oxalate as well as calcium excretion, which is beneficial in preventing calculi formation.

Increase urinary inorganic phosphate excretion observed on modified lithogenic diet which, is eventually induce the calcium phosphate stones. Treatment with HNJ restores inorganic phosphate level, thus reducing the risk of stone formation. Magnesium is a potent inhibitor of calcium oxalate crystallization and binds to oxalate to form a soluble complex, consequently reducing the concentration available for calcium oxalate precipitation. Magnesium deficiency accelerates the deposition of renal tubular calcium oxalate in rats. Experiments in animal models have shown protection against calcium oxalate deposition in kidneys by magnesium but clinical studies have not shown any beneficial effects in impeding the formation of calcium oxalate kidney stones. However supplementation with HNJ significantly reduced the level of magnesium in urine and serum and significant elevation in MLD control animals.

Citrate is an important urolithiasis inhibitor, which forms a soluble complex with calcium and inhibits precipitation and aggregation of calcium oxalate & phosphate. In our study HNJ and cystone treatment led to increase in citrate concentration which might have reduced crystallization of calcium oxalate. The glomerular filtration rate decreases in urolithiasis due to the obstruction to the outflow of urine by stones in the urinary system and waste products such as urea and uric acid get accumulated in blood. This indicates marked demage of kidney. The uric acid crystals absorb glutamic acid and other organic compounds and promote calcium oxalate crystal growth. The result showed a significant increase in uric acid level in serum as well as in urine in the MLD control group compared to normal group. The uric acid levels decreased after treatment with HNJ and cystone, thereby hastening the process of dissolving the performed stones and prevention of new stone formation in the urinary system\textsuperscript{(15-19)}. 

\textsuperscript{15-19}
Histopathological changes also support the above results. Microscopic examinations of the kidney sections derived from MLD induced urolithiatic rats showed polymorphic irregular crystal deposits inside the tubules, which cause dilation of the proximal tubules along with interstitial inflammation and severe glomeruli damage. Administration of HNJ decreased the number and size of calcium oxalate deposits in different parts of the renal tissue and prevented damages caused due to MLD.

CONCLUSION

In conclusion, the results indicate that administration of rhizome extract *Nardostachys jatamansi* DC (400mg/kg bw) reduced and prevented the growth of urinary stones. The underlying mechanism could be due to its diuretic activity, nephroprotective effect and lowering the concentration of urinary stone forming constituents. Further experimental and clinical studies are required to elucidate the chemical constituents of the extract with potent antiurolithiatic activity.

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