Effect of Cystone on pediatric urolithiasis with special reference to urinary excretion of calculogenesis inhibitors

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ABSTRACT
The incidence of urolithiasis is high in developing countries; and the northern and north-western regions of India. These regions can be termed an endemic stone-forming belt, due to a dietary pattern rich in cereals and pulses. Urolithiasis is a consequence of complex physio-chemical processes and the major contributory factors are urinary super saturation, crystallization, calculogenesis and matrix formation.

This study was aimed to evaluate the efficacy and safety of Cystone in children with urolithiasis below 12 years of age, with special reference to urinary excretion of calculogenesis inhibitors. This study was a randomized, placebo-controlled, double blind clinical trial. Eighty-seven children below 12 years of age were included in this study. Children with complications, stones secondary to other developmental abnormalities, presenting with pyonephrosis, and those whose parents refused to give informed consent, were excluded from the study. All included patients were stratified by diagnosis and from each strata, patients were randomized to receive Cystone or placebo. All children were investigated for routine hemogram and blood urea, serum creatinine, sodium, potassium, calcium and phosphorus, and uric acid levels. For all children, routine and microscopic urine examination, were done, followed by urine culture for three consecutive days. Furthermore, all children were monitored for 24-hours urinary excretion of albumin, creatinine, calcium, phosphorus, citric acid and magnesium levels. All children underwent abdominal radio imaging and ultrasound examination. DTPA was done to rule out renal malfunction. Investigations were repeated at 4 months.

All patients received the same dosage of Cystone or placebo for 4-month period. The predefined primary outcome measures were the effect on urinary excretion of stone formation inhibitors, change in the number and size of stones, and spontaneous passage of stone. The predefined secondary outcome measures were symptomatic relief and incidence of recurrence. The relation of adverse events to study medication was predefined as “Unrelated”, “Possible”, and “Probable”. Non-compliance was not regarded as treatment failure, and reasons for non-compliance were noted.

A total of 87 patients were enrolled in the study, 4 patients were excluded from the study and 15 patients were lost to follow up. There was no statistical difference in the gender-wise distribution of patients in the drug and placebo groups. On starting Cystone, symptomatic relief was reported by 70.6% patients. The disappearance of stones was noted in 11 patients, as confirmed by X-ray KUB and ultrasound examination. In patients with “solitary stone”,
“multiple stones”, “calcium oxalate and magnesium-calcium phosphate stones” and “lower tract stones” there was no statistically significant difference in the 24-hour urinary excretion of calcium, phosphorus, citric acid and magnesium in the pre-treatment and post-treatment levels between the drug and placebo groups. In patients with “calcium oxalate and triple phosphate stones”, there was a statistically significant difference in the 24-hour urinary excretion of calcium and magnesium in pre-treatment and post-treatment levels between the drug and placebo groups. In patients with “upper tract stones”, there was a statistically significant difference in the 24-hour urinary excretion of phosphorus in pre-treatment and post-treatment levels between the drug and placebo groups. No recurrences were noted during the study period and no adverse events were reported during the study period.

This study indicates that in pediatric urolithiasis, Cystone appears to be an effective and safe treatment for long-term use. It also appears that Cystone has a favorable effect on inhibition of calculogenesis and it also seems to prevent recurrence in pediatric urolithiasis.

INTRODUCTION
Urolithiasis affects 1-5% of population in industrialized countries with a progressive decline in incidence in western countries. The incidence of urolithiasis is higher in developing countries (including India) than industrialized countries. It has been hypothesized that the main source of dietary proteins being cereals (unlike meat in Western countries), is an important etiological factor. The northern and north-western regions of India can be described as an endemic stone-forming belt, due to a dietary pattern, rich in cereals and pulses.

Urolithiasis is a consequence of complex physio-chemical processes and the major contributory factors are urinary supersaturation, crystallization, calculogenesis and matrix formation. Calculogenesis is influenced by interplay of critical factors, viz. stone inhibitors, complexing agents and stone promoters. The sequence of events in the formation of any urinary stone can be: urinary saturation → super saturation → nucleation → crystal growth → crystal aggregation → crystal retention → stone formation.

Kidney stones smaller than 5 mm in diameter are most likely to be flushed out in urine without any medical intervention, except occasional analgesics and antispasmodics that enable the patient to endure the episode, which may last several days. Kidney stones greater than 5 mm in diameter are less likely to be flushed out in urine on their own and these stones get larger in size over a period of time. If the kidney stone is larger than 10 mm in diameter, it has to be either removed by surgery or by lithotripsy.

The chance of having recurring stones are about 70-80% once a person suffers their first stone attack. After the first stone attack occurs, the person has a cumulative 10% chance per year of forming another stone, i.e. a 50% chance over a 5-year period of time, and genetic predisposition increases this risk. It has also been observed that about 60% of individuals who have experienced one stone will develop another within 7 years. The younger a person is when they have their first stone attack, the greater the risk of having additional attacks.

The present study was planned to evaluate the efficacy and safety of Cystone, a polyherbal formulation, in pediatric urolithiasis. Each Cystone tablet comprises of extracts of Didymocarpus pedicellata, Saxifraga ligulata, Rubia cordifolia, Cyperus scariosus, Achyranthes aspera, Onosma bracteatum, Vernonia cinerea and powders of purified Shilajeet and Hajrul yahood bhasma.
AIM OF THE STUDY
This study was aimed to evaluate the efficacy and safety of Cystone in children with urolithiasis, below 12 years of age, with special reference to urinary excretion of calculogenesis inhibitors.

MATERIALS AND METHODS
Study Design
This study was a randomized placebo-controlled double blind clinical trial approved by Ethics Committee of All India Institute of Medical Sciences, New Delhi.

Inclusion Criteria
Eighty-seven children, below 12 years of age, diagnosed with renal or bladder stones, with or without any demonstrable metabolic abnormality, attending the Children’s Surgical O.P.D. of the All India Institute of Medical Sciences, New Delhi, were included in this study. A written informed consent was obtained from parents of all patients.

Exclusion Criteria
Children who developed any complication like severe pain, hematuria or obstruction requiring immediate surgery with stones, secondary to other developmental abnormalities (e.g. posterior urethral valves, extrophy bladder, etc.), presenting with pyonephrosis, and those whose parents refused to give informed consent, were excluded from the study.

Study Procedures
All included patients were stratified by diagnoses (renal or bladder calculi) and from each strata, patients were randomized to receive Cystone or placebo. A computer generated random number allocation program did the randomization.

A baseline history was obtained in order to determine the child’s eligibility for enrolment in the trial, to compare the study groups and to describe the study population. The baseline assessment included personal data, a description of symptoms, and details of past medical history, after which all patients underwent a complete clinical examination.

All children were investigated for routine hemogram and blood urea, serum creatinine, sodium, potassium, calcium and phosphorus, and uric acid levels. In all children, routine and microscopic urine examinations were done, which was followed by urine culture for three consecutive days. Furthermore, all children were monitored for 24-hours urinary excretion of albumin, creatinine, calcium, phosphorus, citric acid and magnesium levels.

All children also underwent abdominal radio imaging and ultrasound examination. Dynamic renal scintigraphy with radiotracer imaging technique-using Tc - 99m DTPA (Technetium 99m diethylentriamine pentaacetic acid) was done to rule out renal malfunction. All these investigations were repeated at 120 days.

All patients received the same dosage of Cystone or placebo (2 tablets, twice daily) for a period of 4 months.

Primary And Secondary Outcome Measures
The predefined primary outcome measures were the effect on urinary excretion of stone formation inhibitors, change in the number and size of stones, and spontaneous passage of
stone. The predefined secondary outcome measures were symptomatic relief and incidence of recurrence.

Adverse Events
All adverse events reported or observed by patients were recorded with information about severity, date of onset, duration and action taken regarding the study drug. Relation of adverse events to study medication were predefined as “Unrelated” (a reaction that does not follow a reasonable temporal sequence from the administration of the drug), “Possible” (follows a known response pattern to the suspected drug, but could have been produced by the patient’s clinical state or other modes of therapy administered to the patient), and “Probable” (follows a known response pattern to the suspected drug that could not be reasonably explained by the known characteristics of the patient’s clinical state).

Patients were allowed to voluntarily withdraw from the study, if they had experienced serious discomfort during the study or sustained serious clinical events requiring specific treatment. For patients withdrawing from the study, efforts were made to ascertain the reason for dropout. Non-compliance (defined as failure to take less than 80% of the medication) was not regarded as treatment failure, and reasons for non-compliance were noted.

Statistical Analysis
Statistical analysis was done for the drug and placebo groups to compare baseline characteristics with regard to age, blood urea, serum creatinine, calcium and phosphorus levels by using the “unpaired t test”, both by “assuming and not assuming equal variances”. “Pearson Chi-square” and “Fisher’s Exact test” tested the gender-wise distribution of patients in the drug and placebo groups; and followed by evaluation for “Likelihood Ratio” and “Linear-by-linear Association”. Further, the data was also analyzed for significant difference between drug and placebo groups as per the type of the stone (solitary, multiple, calcium oxalate, triple phosphate and magnesium-calcium phosphate stones), location of stone (upper tract or lower tract stones) by using “paired t test”, both by “assuming and not assuming equal variances”.

RESULTS
Eighty-seven patients were enrolled in the study (69 males and 18 females), 4 patients were excluded from the study and 15 patients were lost to follow up. Both the drug and placebo groups were statistically comparable on the parameters of age, blood urea, and serum creatinine, calcium and phosphorus during enrolment for the study (Figure 1). There was no statistical difference in the gender-wise distribution of patients in the drug and placebo groups (Figure 2).

Figure 1: Baseline comparison for selected parameters of placebo and Cystone groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Group</th>
<th>Cystone Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Blood Urea</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Serum Calcium</td>
<td>0.474</td>
<td>0.464</td>
</tr>
<tr>
<td>Serum Phosphorus</td>
<td>0.626</td>
<td>0.627</td>
</tr>
</tbody>
</table>

Note:
1. Parameter, p value assuming equal variances and p value assuming unequal variances: Age: 0.263 and 0.263 (NS); Blood urea: 0.151 and 0.165 (NS); Serum creatinine: 0.455 and 0.465 (NS); Serum calcium: 0.474 and 0.466 (NS) and Serum phosphorus: 0.626 and 0.627 (NS). NS – Non significant and S - Significant
2. Independent samples test (Student’s ‘t’ test for equality of means)
On starting Cystone, symptomatic relief was reported by 60 of the 87 patients (70.6%). Disappearance of stones (dissolution or spontaneous passage) was noted in 11 patients and out of these 11 patients, 4 had a past history of stone passage. In the remaining 7 patients, the stone had disappeared at the end of the study period, as confirmed by X-ray KUB and ultrasound examination.

There was no statistically significant difference in the 24-hour urinary excretion of calcium, phosphorus, citric acid and magnesium in pre-treatment and post-treatment levels between drug and placebo groups (Figure 3).

In patients with “solitary stone”, there was no statistically significant difference in the 24-hour urinary excretion of calcium, phosphorus, citric acid and magnesium in pre-treatment and post-treatment levels between the drug and placebo groups (Figure 4).

In patients with “multiple stones”, there was no statistically significant difference in the 24-hour urinary excretion of calcium, phosphorus, citric acid and magnesium in pre-treatment and post-treatment levels between the drug and placebo groups (Figure 5).

In patients with “calcium oxalate and triple phosphate
stones”, there was a statistically significant difference in the 24-hour urinary excretion of calcium and magnesium in pre-treatment and post-treatment levels between the drug and placebo groups (Figure 6).

In patients with “calcium oxalate and magnesium-calcium phosphate stones”, there was no statistically significant difference in the 24-hour urinary excretion of calcium, phosphorus, citric acid and magnesium in pre-treatment and post-treatment levels between the drug and placebo groups (Figure 7).

In patients with “upper tract stones (renal, ureteric or both renal and vesicular stones)”, there was a statistically significant difference in the 24-hour urinary excretion of phosphorus in pre-treatment and post-treatment levels between the drug and placebo groups (Figure 8).

In patients with “lower tract stones (vesical and urethral stones)”, there was no statistically significant difference in the 24-hour urinary excretion of phosphorus between pre-treatment and post-treatment levels between the drug and placebo groups (Figure 9).

There were no recurrences and adverse events reported during the study period.

DISCUSSION
The most important etiological factor in the formation of urinary stones is saturation of urine; and, thermodynamic solubility product (Ksp) is the concentration at which saturation is reached and the process of crystallization in a solution initiated. Urine is a complex solution containing ions constantly interacting with calcium and phosphate. Formation product (Kf) of a particular salt is the concentration at which urine can hold no more salt in solution, at a particular temperature and pH. As the normal body temperature is more or less constant at 37°C, the variations in pH are clinically important in the process of crystallization. e.g. as the pH increases, more ionic phosphate is detected in urine, thereby reducing the solubility of
calcium-phosphate in urine. Supersaturation can be a continuous or intermittent process and urine may become supersaturated after meals, especially after consumption of large amounts of calcium and oxalate. This effect may be more influential in the process of crystallization after evening meals due to the lack of fluid consumption during sleep.

A process called homogenous nucleation forms crystal nuclei at the earliest in pure solutions. In urine, nuclei are usually formed on the existing surfaces (epithelial cells, urinary casts, red blood cells and other crystals), hence it is referred as heterogenous nucleation. The saturation needed for heterogenous nucleation is much less than for homogenous nucleation.

The tiny nuclei grow in size by precipitation of additional salt on the lattice net. This being a slow process, it requires many years to form a small stone in the urinary tract. The earliest site of stone formation is usually the papillary duct or the collecting duct tubule. Once nuclei are formed, they become kinetically active, bounce apart from each other, float freely and are usually washed away by urine flow. Under certain circumstances, however, these nuclei come together due to electrochemical bonding forces, and the process is called crystal aggregation. The crystal aggregates attach to the renal epithelium and over a period of time develop into stone. If the crystals are retained in the kidney for a long time, stone growth occurs whenever there is urinary supersaturation or crystal aggregation.

Normally in urine, the concentration of calcium-oxalate is four times more than its solubility, and precipitation occurs only when supersaturation is 7-11 times its solubility. Individuals who have never formed stone often pass small crystals, which is possible because of presence of calcium oxalate crystallization modifiers in the urine.

Figure 7: Patients with calcium oxalate and magnesium – significance of difference in 24 hour excretion of calcium, phosphorus, citric acid and magnesium between pre- and post-treatment between placebo and Cystone groups

Note:
1. Parameter, \( p \) value assuming equal variances and \( p \) value assuming unequal variances: Calcium: 0.089 and 0.078 (NS); Phosphorus: 0.939 and 0.939 (NS); Citric acid: 0.245 and 0.275 (NS) and Magnesium: 0.413 and 0.389 (NS).
2. Independent samples test (Student’s ‘t’ test for equality of means)

Figure 8: Patients with upper tract stones - significance of difference in 24 hour excretion of calcium, phosphorus, citric acid and magnesium between pre- and post-treatment between placebo and Cystone groups

Note:
1. Parameter, \( p \) value assuming equal variances and \( p \) value assuming unequal variances: Calcium: 0.055 and 0.035 (NS); Phosphorus: 0.018 and 0.017 (S); Citric acid: 0.323 and 0.388 (NS) and Magnesium: 0.606 and 0.563 (NS).
2. Independent samples test (Student’s ‘t’ test for equality of means)
significantly more calcium and oxalate than normal subjects and non-calcium stone-formers. But, not all individuals who excrete more calcium and oxalate in urine form calcium stones, which is due to presence of inhibitors in the urine. Stone formation is dependent on the balance between saturation and inhibitors in urine. Robertson et al. derived a highly specific “saturation inhibition ratio” to differentiate stone formers from normal subjects.

Organic and inorganic inhibitors have been identified for calcium-phosphate and calcium-oxalate systems, but not for the urate system. It has been shown that patients with calcium-oxalate stones have intrinsically abnormal acidic glycoproteins. The glycoproteins in healthy persons contain gamma carboxyglutamic acid, which is a strong inhibitor of calcium oxalate growth but glycoproteins from stone-formers do not have gamma carboxyglutamic acid.

Magnesium, citrate, pyrophosphate and nephrocalcin (specific glycoprotein inhibitor) are the urinary inhibitors for calcium-phosphate crystal systems, while citrate, pyrophosphate, nephrocalcin, glycosaminoglycans and RNA fragments are calcium-oxalate crystal system inhibitors. These inhibitors get adsorbed to the crystal growth sites, retarding crystal growth and aggregation. RNA fragments increase nucleation but retard crystal growth and aggregation, while glycosaminoglycans (e.g. chondroitin sulphate) decrease crystal aggregation but are less effective in retarding crystal growth. Nephrocalcin and Tamm-Horsfall protein are urinary glycoproteins that are potent inhibitors of calcium-oxalate monohydrate crystal aggregation. Nephrocalcin from stone-formers decreases calcium-oxalate monohydrate crystal aggregation ten fold less than the nephrocalcin from non-stone-formers. Tamm-Horsfall protein inhibits crystal aggregation (but not crystal growth) and is the most potent inhibitor identified (ten times more potent then nephrocalcin). Although no quantitative difference appears in the urinary excretion of Tamm-Horsfall protein between stone-formers and non-stone-formers, Tamm-Horsfall protein exists in self-aggregated form in stone-formers, reducing its effectiveness as an aggregation inhibitor.

Complexing agents are substances that form complexes with lattice ions for specific crystals (e.g. calcium-oxalate) and these agents decrease free ionic activity reducing the saturation level of the stone forming substance. Citrate is a potent complexing agent for calcium exerting its maximum effect at pH 6.5 and magnesium a divalent cation, is a complexing agent for oxalate.

In the calcium-phosphate system, citrate and magnesium act as complexing agents and inhibitors. In the calcium-oxalate system, citrate acts as complexing agent and inhibitor, whereas magnesium acts as a complexing agent only.
Pure promoters of calculogenesis are rare and glycosaminoglycans promote crystal nucleation, but inhibit crystal aggregation and growth. Tamm-Horsfall protein, depending on its state of aggregation, may act as a promoter or an inhibitor of crystal formation.\(^\text{16}\)

Anton Von Heyde first described the presence of the non-crystalline organic matrix in urolithiasis in 1784. The matrix of most urinary calculi is 3% by weight, while cystine stones have a 10% matrix. Rarely, the matrix may constitute up to 65% in stones in the presence of urea-splitting organisms and such stones have a soft putty-like consistency. Chemical analysis of matrix has revealed that it contains 65% hexosamine and 10% bond water. Whether matrix is a cause or consequence of urinary stone is undecided, but some studies suggest that the matrix may be a ground substance for stone formation. Du Toit et al. have suggested that an alteration in the excretion of enzymes (urokinase, sialidase) might be an etiological factor of urolithiasis and decreased urokinase level with increased sialidase level in urine results in formation of stone matrix. Later it was reported that \textit{P. mirabilis} and \textit{E. coli} decrease urokinase and increase sialidase levels.\(^\text{17}\) The urine is usually supersaturated with crystals. Some crystalloids like calcium, oxalic acid, and uric acid have a tendency to precipitate in the urine conduit to form stones. On the other hand, substances like phosphates, magnesium, sodium, potassium, and many others help to hold the stone-forming crystalloids in solution.

In the present study, in pediatric patients with calcium-oxalate and triple phosphate stones, there was a statistically significant difference in the 24-hour urinary excretion of calcium and magnesium in pre-treatment and post-treatment levels between the drug and placebo groups, which indicates the beneficial role of Cystone on inhibitors of calculogenesis.

Similarly, there was a statistically significant difference in the 24-hour urinary excretion of phosphorus in pre-treatment and post-treatment levels between the drug and placebo groups, in patients with upper tract stones, suggesting the favourable effect of Cystone on calculogenesis inhibitors.

Symptomatic relief was reported at the initial stage by a majority of patients on Cystone, which is critically important in the management of pediatric urolithiasis and the symptomatic control might be due to the synergistic antispasmodic and anti-inflammatory activities of the ingredients.\(^\text{18-20}\)

Didymocarpene is the chief constituent of the leaves of \textit{Didymocarpus pedicellata} and two important polyterpenes didymocarpol and didymocarpol have been isolated from the essential oil, which have been reported to be beneficial in the management of urolithiasis.\(^\text{21,22}\) The rhizome, \textit{Saxifraga ligulata}, contains an active principle, bergenin (0.6%), which is a known diuretic and helpful in dissolving kidney stones.\(^\text{23,24}\) The principal constituents of \textit{Rubia cordifolia}, purpurin, munjistin, purpuroxanthin and pseudopurpurin and the oil of \textit{Cyperus scariosus} roots was found to have potent anti-inflammatory activity comparable with phenylbutazone.\(^\text{25}\) The principal alkaloids of \textit{Achyranthes aspera} (betaine and achyranthine) from the whole plant and Achyranthes saponin A with its ester (Achyranthes saponin B) from the seeds have potent diuretic activity.\(^\text{26}\) \textit{Onosma bracteatum} has diuretic and spasmolytic properties. \textit{Shilajeet} exhibits anti-inflammatory properties, while \textit{Hajrul yahood bhasma} is a diuretic and lithotriptic agent. Triterpenes are the major constituent of \textit{Vernonia cinerea} and have been found to be effective in urolithiasis.\(^\text{27}\)
Cystone probably prevents supersaturation of lithogenic substances and corrects the crystalloid-colloid imbalance. It appears that Cystone inhibits calculogenesis by reducing oxalic acid and calcium hydroxyproline, and causes their expulsion, probably by micro-pulverization. Some ingredients of Cystone appear to disintegrate the calculi and the crystals. In the present study, though the study duration was short a total absence of recurrence was observed, which is an important feature in long-term management of pediatric urolithiasis. In the present study, no adverse events were reported, which suggests the long-term safety of Cystone in management of pediatric urolithiasis.

**CONCLUSION**

Surgery or lithotripsy is the available option in pediatric urolithiasis and recurrence is the core issue in the clinical management of pediatric urolithiasis. A drug, which will inhibit calculogenesis, will be preferred for long-term management of pediatric urolithiasis.

This study indicates that in pediatric urolithiasis, Cystone appears to be an effective and safe treatment for long-term use. It also appears that Cystone has a favorable effect on inhibition of calculogenesis and seems to prevent recurrence in pediatric urolithiasis.

**REFERENCES**