An in vitro study of Hydrangea arborescens, homoeopathic preparation as an inhibitor of Calcium oxalate crystallisation

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Abstract

Background: Homoeopathic mother tincture Hydrangea arborescens (Hydrang.) is conventionally used for urinary complaints, such as urinary tract infection, renal stones and prostate hypertrophy. However, the mode of its action for annihilation of complaints remains uncertain. This study was designed to investigate in vitro effect of homoeopathic preparations of Hydrang. on urolithiasis (Calcium oxalate [CaOx] crystallisation). Objective: To analyse the role of Hydrang. a, homoeopathic preparation in an in-vitro CAOx crystallisation. Materials and Methods: Spectrophotometric crystallisation assay was carried out and the slopes of nucleation and aggregation phases were calculated using linear regression analysis, and the percentage inhibition exerted by the Hydrang. Q, 6C, 30C and 200C was calculated. Microscopic observations of crystals of CaOx were carried out in the presence and in the absence of Hydrang. Q, 6C, 30C and 200C to support the spectrophotometric crystallisation assay. Results: The crystallisation studies performed indicate Hydrang. to be a potent medicine against CaOx crystallisation both at the level of nucleation and aggregation. Hydrang. Q favours aggregation to a great extent by showing the inhibition of about -15%; while for 6C, 30C and 200C, the percentage inhibition was 13.70%, 42.30% and 14.90%, respectively. This shows inhibitory nature for aggregation, with maximum inhibition shown by Hydrang. 30C potency. Conclusion: The homoeopathic preparations of the Hydrang. inhibit the primary events of stone formation. The findings of the above experiment show the evidence to support the usefulness of Hydrang. in the cases of renal calculi.

Keywords: Calcium oxalate, Crystallisation, Homoeopathy, Hydrangea arborescens, Renal stone inhibitors, Urolithiasis

Introduction

Urolithiasis or urinary tract stones are among the most common and painful diseases of human beings. This is the third most common urinary tract disease that may lead to renal failure. Urolithiasis affects about 12% of the world population at some stage in their life time. It affects all ages, sex and races but occurs more frequently in men than in women, within the age group of 20–49 years.[1] In India, about 12% of the population is estimated to have urinary stones, and out of which, 50% may end up with renal failure. Calcium stones are predominant renal stones comprising about 80% of all urinary calculi.[2] The proportion of calcium stones may account for pure Calcium oxalate (CaOx), 50%, Calcium phosphate (termed as apatite, 5%) and a mixture of both (45%). Calcium oxalate monohydrate (COM) is the most thermodynamically stable form of stone. COM is more frequently observed than Calcium oxalate dihydrate in clinical stones.[3] Apart from conservative treatment, shock-wave lithotripsy and ureteroscopy are commonly practiced in conventional medicine in cases of calculi, but these interventions are expensive for the common people and may lead to complications.[4]

Besides the constitutional or individualized treatment, appropriate homoeopathic organ-specific medicines selected on the basis of the important particular symptoms can also be effective.[5] The method assumes that certain remedies have a specific affinity for certain organs; there are patients in whom it is desirable or necessary to treat specific organs or system in order that the whole person may be properly cured.[6] Organopathic prescriptions are made based on the Paracelsus principle that the given drugs affect given organs (parts) by self-elective action.

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preference. Many doctors have given in their experiences on the importance of selection of organopathic remedies.\(^7\) In the homoeopathic literature, Boericke mentions, 'Hydrang. causes burning in urethra frequent desire urine hard to start heavy deposits of mucus sharp pain in loins, especially left gravelly deposits. Profuse deposits white amorphous salts.'\(^8\) Hydrang. has profound action on the kidneys and ureteric stones; the case report has justified the fact. However, randomised control trials on action of Hydrang. in cases of urinary stones are suggestive.\(^9\) Hansen adds that it is particularly useful for profuse deposits of white amorphous salts in urine; and has arrested the tendency of formation of calculi, relieves distress from renal calculus.\(^9\) Its properties include cracking stone in small pieces or leaching stone to softer and smaller, increasing urinary outflow.\(^10\) Clarke mentions it as a ‘stone-breaking remedy having being used in calculus diseases.'\(^11^\)

**Materials and Methods**

**Drugs and chemicals**

Homoeopathic preparation of Hydrang. Q, 6C, 30C and 200C was procured from Dr Willmar Schwabe India Pvt Ltd., a GMP-certified pharmaceutical company. All other chemicals and reagents used were of analytical grade.

**Spectrophotometric crystallisation assay**

Spectrophotometric crystallisation assay was carried out by the method of Hess et al.\(^12\) CaOx crystals were synthesized by preparing the following solutions:

50% unsuccussed Ethanol was used as control in the present study because 90% Ethanol favours crystallization process, as samples of Hydrang. Q, 6C, 30C and 200C each have different percentage of alcohol.

The solution A was prepared in deionized water containing 200 mmol/L Sodium chloride, 10 mmol/L Sodium acetate and 1.5 mmol/L of Potassium oxalate (pH 5.7).

The solution B was prepared in deionized water containing 200 mmol/L Sodium chloride, 10 mmol/L Sodium acetate and 8.5 mmol/L of Calcium chloride (pH 5.7).

In a quartz cuvette containing 1 ml solution A, 1 ml of solution B was added to give a final concentration of calcium 4.25 mmol/L and oxalate 0.75 mmol/L. The time course of the optical density (OD) at 620 nm was measured automatically using a UVIKON 930 spectrophotometer (UV 1800, Shimadzu Corporation, Japan). The values were also measured in the 50 μl, and 100 μl of Hydrang. Q, 6C, 30C and 200C OD at 620 nm increases initially during nucleation phase and decreases during the aggregation phase.

Slopes of the nucleation (till the maximum) and aggregation (after the peak) phases were calculated at 620 nm using linear regression analysis, and the percentage inhibition exerted by the samples was calculated using the formula: Percentage inhibition = ((S0 – S1)/S0) × 100, where S0 is the slope of the control and S1 is the slope of test samples.\(^11^\)

**Light microscopic studies**

CaOx crystals for light microscopic studies were prepared according to the method of Nakai et al. CaOx crystals were formed as follows.

The solution A was prepared in deionized water containing 200 mmol/L Sodium chloride, 10 mmol/L Sodium acetate and 0.1 ml of 20 mmol/L Potassium oxalate (pH 6.5).

The solution B was prepared in deionized water containing 200 mmol/L Sodium chloride, 10 mmol/L Sodium acetate and 0.2 ml of 20 mmol/L Calcium chloride (pH 6.5).
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**Results**

**Nucleation and aggregation assay**

The maximum OD was obtained at 620 nm of these solutions, viz. control Ethanol 50% (50 µl), Hydrang. Q (50 µl), Hydrang. 6C (50 µl), Hydrang. 30C (50 µl) and Hydrang. 200C (50 µl). Graph of OD and different time intervals is plotted to show nucleation and aggregation. The OD increased linearly initially, which indicated the nucleation process and then decreased linearly indicating the aggregation process. Hydrang. 6C, 30C and 200C has inhibited both the rate of nucleation and the rate of aggregation. The maximum OD of the solutions, viz. control Ethanol 50% (50 µl) was 0.189, Hydrang. Q (50 µl) was 0.191, Hydrang. 6C (50 µl) was 0.389, Hydrang. 30C (50 µl) was 0.402 and Hydrang. 200C (50 µl) was 0.524. The nucleation and aggregation of CaOx crystals [Figure 1] [Table 1]. The photographs of the CaOx crystals, in solutions of control Ethanol 50%, Hydrang. Q (50 µl) shows aggregation, Hydrang. 6C (50 µl), Hydrang. 30C (50 µl) and Hydrang. 200C (50 µl) [Figure 2]. Mother tincture, i.e. Hydrang. Q, favours aggregation to greater extent by showing the inhibition of about −15% for aggregation hence favours crystal formation, and 6C was 13.70%, 30C was 42.30% and 200C was 14.90%, showing inhibitory nature for aggregation, with maximum inhibition shown by Hydrang 30C [Table 2].

**Discussion**

As CaOx is the most common renal stone found in the urine; the inhibitors of CaOx crystallisation are used as a prophylactic agent to prevent them.

We used a classical model of sample supersaturated with Calcium chloride and Potassium oxalate to determine the growth and aggregation of CaOx crystals. The normal human urine is not a static solution, as new solutes are constantly being added and subtracted from the solution. However, it is difficult to mimic the urinary tract *in vitro*, but the growth of crystals in synthetic urine in a static environment can be useful to some extent for explaining the formation of urinary calculi.\(^{[14]}\) As homoeopathic mother tincture is not potentised, it shows crystal formation and favours crystallisation, while the homoeopathic dilutions of 6C, 30C and 200C potency are potentised and have a dynamic action, so they inhibit crystal formation so can be used to prevent the formation of the urinary CaOx calculi.

**Table 1:** Optical density values and time taken to induce the formation of detectable crystals, nucleation phase attained by saturated sample, *Hydrangea arborescens* Q, 6C, 30C and 200C and aggregation phase attained by saturated sample and *Hydrangea arborescens* Q

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time in seconds</th>
<th>OD values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated sample of Calcium chloride and Potassium oxalate</td>
<td>14</td>
<td>0.024</td>
</tr>
<tr>
<td>Mother tincture in saturated sample</td>
<td>14</td>
<td>0.181</td>
</tr>
<tr>
<td>6C in saturated sample</td>
<td>12</td>
<td>0.386</td>
</tr>
<tr>
<td>30C in saturated sample</td>
<td>14</td>
<td>0.401</td>
</tr>
<tr>
<td>200C in saturated sample</td>
<td>14</td>
<td>0.523</td>
</tr>
</tbody>
</table>

OD: Optical density
Several *in vitro* and *in vivo* studies of medicinal substances have proved homoeopathic medicines play an important role in delaying and preventing the crystallisation process, thereby confirming these as suitable treatment for urolithiasis.

In Figure 1, the time required to form the CaOx crystals, at OD 620 nm, increase in slope indicates nucleation and reflects an increase in the size of particle, while decrease in slope indicates aggregation of CaOx crystals from a supersaturated solution. Measured parameters were induction time (Ti), i.e., time to induce formation of detectable particles, where Sn denotes slope of increase mainly due to crystal nucleation and Sa denotes slope of decrease due to crystal aggregation.

Figure 3 represents the *in vitro* CaOx crystallisation that was carried out with 50% Ethanol, unsuccessed which served as a control in the present study. The Ethanol was found to influence the crystalluria and its resultant injury. *Hydrang.* has a relative effect on the solubility of CaOx which has been determined in simple salt solutions; the exact combination of the factors that are responsible for variations in CaOx solubility in urine is yet insufficiently known. Hence, further studies are a must to conclude its modus operandi in *in vivo* system.

**Conclusion**

The above study of *in vitro* crystallisation assays and microscopic observation demonstrates the possibility to dissolve CaOx calculi in human beings, thereby inhibiting the crystalluria and its resultant injury. *Hydrang.* has a relative effect on the solubility of CaOx which has been determined in simple salt solutions; the exact combination of the factors that are responsible for variations in CaOx solubility in urine is yet insufficiently known. Hence, further studies are a must to conclude its modus operandi in *in vivo* system.

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Drugs and chemicals: Homoeopathic preparation of *Hydrangea Arborescens* (Q, 6C, 30C and 200C) was procured from Dr Willmar Schwabe India Pvt Ltd. All other chemicals and reagents used were of analytical grade.

**Conflicts of interest**

None declared.

**References**

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**Context:**

**Résultats:** Les études de cristallisation effectuées indiquent que l’Hydrang. est un puissant médicament contre la cristallisation de l’oxalate de calcium, tant au niveau de la nucléation que de l’agrégation. Hydrang. Q favorise l’agrégation dans une large mesure en montrant une inhibition d’environ 15% ; alors que pour 6C, 30C et 200C, le pourcentage d’inhibition était de 13,70%, 42,30% et 14,90% respectivement. Cela montre la nature inhibitrice de l’agrégation, l’inhibition maximale étant démontrée par Hydrang. 30C. **Le Conclusion:** Les préparations homéopathiques de l’Hydrang. inhibent les événements primaires de la formation de la pierre. Les résultats de l’expérience ci-dessus montrent les preuves de l’utilité de l’Hydrang. dans les cas de calculs rénaux.

**Antecedente:**

La tinctura madre homoeopática Hydrangea arborescens (Hydrang.), es tradicionalmente utilizada para las quejas urinarias como las infecciones de los riñones, los cálculos renales, la hipertrofia de la próstata, etc. sin embargo, el modo de su acción para la aniquilación de las quejas sigue siendo incierto. Este estudio fue diseñado para investigar el efecto in vitro de los preparados homeopáticos de Hydrang. sobre la urolitiasis (cristalización de oxalato de calcio).

**Conclusion:**

En los casos de cálculo renal, Hydrang. Q, 6C, 30C y 200C inhiben los eventos primarios de la formación de piedras. Los hallazgos del experimento anterior muestran la evidencia para apoyar la utilidad de Hydrang. En los casos de cálculo renal.
Eine In-Vitro-Studie von Hortensien arborescens, homöopathische Zubereitung als Inhibitor der Calciumoxalatkristallisation.


Ergebnisse: Die durchgeführten Kristallisationstudien deuten auf Hydrang. als ein wirksames Medikament gegen Calciumoxalatkristallisation sowohl auf der Ebene der Keimbildung als auch der Aggregation zu sein. Hydrang. Q begünstigt die Aggregation in hohem Maße, indem es die Hemmung von etwa 15% zeigt; während für 6C, 30C und 200C die prozentuale Hemmung 13,70%, 42,30% bzw. 14,90% zeigt. Dies zeigt eine hemmende Natur für die Aggregation, wobei die maximale Hemmung durch Hydrang gezeigt wird. 30C Potenz.