In-vitro anti-lithiatic activity study of Meyna laxiflora seeds and Tectona grandis bark

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Abstract---This study involves the evaluation of Meyna laxiflora seeds and Tectona grandis bark for antiurolithic activity. Homogeneous precipitation method was used for in-vitro anti-lithiatic activity on generated calcium oxalate crystals. Extracts were compared with synthetic drug Spironolactone, polyherbal formulation Cystone, other extracts and furosemide. Extracts like aqueous extract, pet ether extract, chloroform extract, methanol extract and aqueous extract were used for study at different concentrations.

Keywords---Meyna laxiflora, Tectona grandis, Cystone, extraction.
1. **Introduction**

Urolithiasis is a disease known from ancient times, but still now the causes responsible for the formation of some of the different kinds of stones are unknown, and consequently efficient therapeutic treatments have not yet been developed (Ghaisas M.M., 2008). There are several types of stones, most commonly consisting of calcium phosphates and oxalates; others are composed of magnesium, ammonium phosphate (struvite), uric acid or cystine. Struvite stones are associated with urinary tract infections, often of urease-secreting bacteria that increase urinary ammonium concentration (Asif M., 2011). Calcium stones may form for a number of reasons, including hypercalciuria and hyperoxaluria. Hypercalciuria may arise from changes to calcium resorption from bone, or renal and gastrointestinal tract handling of calcium; these are often associated with changes to parathyroid hormone secretion. Hyperoxaluria can arise from increased production or increased gut absorption (Qazi Majaz, A., 2015). Other factors causing hypercalciuria include renal tubular acidosis or conditions that reduce the urinary concentrations of certain inhibitors, such as citrate and magnesium. Uric acid and cystine stones can form as a result of increased production or urinary concentration of the primary constituents (Siddiqui, W. A., 2018). The extracts of *Meyna laxiflora* and *Tectona grandis* bark have inhibitory effect on CaOx crystallization thus may be beneficial in the treatment of urolithiasis. But there is a need of detailed investigation in elaborated preclinical experimentation to establish the use of plant as antiurolithiatic agent.

2. **Experimental Work**-

**Model-1**

The experiment consisted of the following test tubes of 10 ml capacity and marked the tubes as control and tests into 8 groups, each group has 6 test tubes, in each tube 1ml of calcium chloride anhydrous and 1ml sodium oxalate were added to the tubes and 2 ml of Tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) adjusted at 7.4 pH which to the kidney pH and incubated at 36.7°C over night. The next day the test tubes were centrifuged for 10min to decant to remove top liquid layer. The calcium oxalate crystal formed in the test tube were checked using the compound microscope under 45x magnification, the crystal formed were resembling the prisms shape, to this 5ml (5mg/ml) equivalent to 25mg to each test tube of the different sequential extracts *Meyna laxiflora* seeds and *Tectona grandis* bark and were induced to the tubes and at the same quantity the synthetic drugs Spironolactone, Furosemide and the Poly herbal formulation Cystone were administered to the test tube, all the above treating agents was administered as aqueous suspension using Tween 60 as suspending agent and again it was incubated 36.7°C for 3 days on the fourth day all the test tubes were taken and checked for dissolution of the crystals under the microscope (Mishra, H., 2022).

**Groupings:**

Group- I - generated calcium oxalate crystals and referred as control,
Group- II - generated calcium oxalate crystals + 5ml Furosemide,
Group- III - generated calcium oxalate crystals + 5ml Spironolactone,
Group- IV - generated calcium oxalate crystals + 5ml Cystone,
Group- V - generated calcium oxalate crystals + 5ml Pet Ether Extract of *Meyna laxiflora* seeds,
Group- VI- generated calcium oxalate crystals + 5ml Pet Ether Extract of *Tectona grandis* bark
Group-VII - generated crystals + 5ml Chloroform Extract of *Meyna laxiflora* seeds,
Group-VIII- generated crystals + 5ml Chloroform Extract of *Tectona grandis* bark,
Group-IX - generated crystals + 5ml Alcohol Extract of *Meyna laxiflora* seeds,
Group-X- generated crystals + 5ml Alcohol Extract of *Tectona grandis* bark
Group-XI - generated crystals + 5ml Aqueous Extract of seeds of *Meyna laxiflora*.
Group-XII- generated crystals + 5ml Aqueous Extract of and *Tectona grandis* bark.

The *In-vitro* lithiatic activity which has been carried, where the calcium oxalate crystals were generated by the sodium oxalate and calcium chloride on incubation at 36.7°C with tris phosphate buffer at 7.4 pH. The generated crystals were treated with the different agents mentioned above in experimental part into twelve groups and group-I kept as control and others groups as treated, the estimation of calcium and oxalate were carried out, on comparison, the group-I with other groups found that the alcoholic extract of plants *Meyna laxiflora* and *Tectona grandis*, has shown significant and better action in dissolving the crystals, the cystone and spironolactone which has shown restrained activity, where as other extract and furosemide were much equivalent to the control. On detail *In-vitro* study, it was found that the alcoholic extract of the plants *Meyna laxiflora* and *Tectona grandis* has shown significant anti-lithiatic activity in dissolution of regenerated calcium oxalate crystals.

**Model-2**

Experimentally kidney stones were prepared by homogenous precipitation method. Semi-permeable membrane was removed chemically by placing the eggs in 2M HCl for overnight, which caused complete decalcification. Then the membranes were washed thoroughly with distilled water, stored in refrigerator at a pH of 7-7.4 (Rai, K., 2022).

**Preparation of Standard Solution**

A poly herbal formulation such as Cystone was selected and tablets were placed in absolute ethanol for removing colour coating and were crushed into powder form. The powder was dispersed into 100ml of distilled water and filtered. Filtrate was used as positive control.
**Figure 1:** Eggs kept for calcification  
**Figure 2:** Decalcified Eggs

*In vitro* experimental model setup to evaluate antiurolithiatic activity—Estimation of CaOx and CaPO_{4} by Titrimetry—

Comparison of Antilithiatic activity by using standard Cystone tablets were carried out by taking control, Standard, Test groups.

MLAE: *Meyna laxiflora* alcoholic extract, TGAE: *Tectona grandis* alcoholic extract, CaOx: Calcium Oxalate

**Table 1:** *In-vitro* Antilithiatic activity of CaOx Dissolution model

<table>
<thead>
<tr>
<th>Control</th>
<th>Standard</th>
<th><em>Meyna laxiflora</em></th>
<th><em>Tectona grandis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1ml (1mg/1ml)</td>
<td>1ml CaOx +1ml (100mg/ml) MLAE</td>
<td>1ml CaOx +1ml (100mg/ml) TGAE</td>
<td></td>
</tr>
</tbody>
</table>
Calcium Oxalate

All the models were prepared by packing in semi permeable membrane as mentioned above. The membranes were sutured and were allowed to suspend in 100ml of 0.1M Tris buffer. All the flasks were subjected to incubation, preheated to 37°C for 7hrs for 3days. After 3 days content of each membrane was collected in different test tubes. 2ml of 1N Sulphuric acid was added to each test tube and titrated with 0.9494N KMnO₄ till the colour disappears. The amount of undissolved Calcium oxalate is subtracted from the total quantity used in the experiment in the beginning; to know much quantity of Calcium oxalate actually test substances could dissolve.

All the models were prepared by packing in semi permeable membrane as mentioned above. The membranes were sutured and were allowed to suspend in 100ml of 0.1M Tris buffer. All the flasks were subjected to incubation, preheated to 37°C for 7hrs for 3days. 4ml of 1N H₂SO₄ and 3ml of molybdate-sulphuric acid reagent, 1ml of reducing solution were added and kept aside for 2hrs. Change in colour intensity was measured against 620nm spectrophotometrically. The amount of undissolved Calcium Phosphate is subtracted from the total quantity used in the experiment in the beginning; to know much quantity of Calcium phosphate actually test substances could dissolve.

Table 2: In-vitro Antilithiatic activity of CaPo₄ Dissolution models

<table>
<thead>
<tr>
<th>Control</th>
<th>Standard</th>
<th>Meyna laxiflora</th>
<th>Tectona grandis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1ml (1mg/1ml) CaPo₄ +1ml water</td>
<td>1ml CaPo₄ +1ml (400 mg/ml) Cystone</td>
<td>1ml CaPo₄ +1ml MLAE 100mg/ml</td>
<td>1ml CaPo₄ +1ml TGAЕ 100mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml CaPo₄ +1ml MLAE 200mg/ml</td>
<td>1ml CaPo₄ +1ml TGAЕ 200mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml CaPo₄ +1ml MLAE 300mg/ml</td>
<td>1ml CaPo₄ +1ml TGAЕ 300mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml CaPo₄ +1ml MLAЕ 400mg/ml</td>
<td>1ml CaPo₄ +1ml TGAЕ 400mg/ml</td>
</tr>
</tbody>
</table>

3. Results

Model 1- Model 1- On detail In-vitro study, it was found that the alcoholic extract of the plants Meyna laxiflora and Tectona grandis has shown significant anti-lithiatic activity in dissolution of regenerated calcium oxalate crystals.
Fig: 4) Generated Calcium oxalate

Group I: (Fig: 4) The huge number of calcium oxalate crystals creation were seen

Group II: (Fig: 5) The huge numbers of calcium oxalate crystals creation were not dissolved by the drug.

Fig: 6) Spironolactone (5mg/ml)

Group III: (Fig: 6) The number of calcium oxalate crystals dissolutions was less by the drug.

Group IV: (Fig: 7) The number of calcium oxalate crystals dissolution was more by the polyherbal formulation.

Fig: 8) Pet ether extract of *Meyna laxiflora* (5mg/ml)

Group V: (Fig: 8) The huge number of calcium oxalate crystals creation found undissolved by the extract.

Fig: 9) Pet ether extract of *Tectona grandis* (5mg/ml)
Group VI: (Fig: 9) The number of calcium oxalate crystals creation found undissolved by the extract.

Group VII: (Fig: 10) The number of calcium oxalate crystals was dissolved maximum by the extract.
Group VIII: (Fig: 11) The number of calcium oxalate crystals was dissolved less by the extract.

Group IX: (Fig: 12) The number of calcium oxalate crystals was dissolved maximum by the extract.
Group X: (Fig: 13) The number of calcium oxalate crystals was dissolved maximum by the extract.

Group XI: (Fig: 14) The number of calcium oxalate crystals creation found undissolved by the extract.
Group XII: (Fig: 15) The number of calcium oxalate crystals creation found undissolved by the extract - Model-2
Table 3: Percentage inhibition of CaOx mineralization in dissolution models

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
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<th>Tectona grandis</th>
</tr>
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<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>90.55±1.27%</td>
<td>83.45±1.27%</td>
<td>81.76±1.28%</td>
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<tr>
<td></td>
<td>85.94±1.26%</td>
<td>83.13±1.27%</td>
<td>86.72±1.29%</td>
<td>84.94±1.26%</td>
</tr>
<tr>
<td></td>
<td>88.47±1.32%</td>
<td>85.47±1.29%</td>
<td>86.72±1.29%</td>
<td>84.94±1.26%</td>
</tr>
</tbody>
</table>

Table 4: Percentage inhibition of CaPO₄ mineralization in dissolution models

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Standard</th>
<th>Meyna laxiflora</th>
<th>Tectona grandis</th>
</tr>
</thead>
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<tr>
<td>0.0</td>
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<td>90.55±1.27%</td>
<td>83.57±1.27%</td>
<td>80.57±1.32%</td>
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<td>84.93±1.28%</td>
<td>81.74±1.32%</td>
<td>86.65±1.28%</td>
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</tr>
<tr>
<td></td>
<td>87.38±1.29%</td>
<td>83.53±1.34%</td>
<td>86.65±1.28%</td>
<td>82.68±1.33%</td>
</tr>
</tbody>
</table>

4. Discussion

In the present study the plants Meyna laxiflora and Tectona grandis were selected for the antiurolithiatic activity. The successive extractions of plants were carried out and the extracts were evaluated for in-vitro anti-urolithiatic activity (Salem, P. P., 2020). Extraction is the first step to separate the desired natural constituents from the crude material (Wang, R., 2020). Extraction process involves solvent penetration into the crude drug, this solvent then diffuses into crude drug and all the constituents are then diffused into the extract. Similarly, extraction of Meyna laxiflora seeds powder and Tectona grandis bark were carried out using soxhlet apparatus by using four solvent systems, Methanol, water, chloroform and petroleum ether extracts (Vyas, P., 2022). The different solvent extracts of Meyna laxiflora seeds and Tectona grandis bark were executed on generated calcium oxalate crystals by homogenous precipitation method for in-vitro anti-lithiatic activity. Cystone a prescribed medicine for renal calculi showed highest inhibition of both CaOx and CaPO₄ mineralization (Bagali, R. S., 2020). MLAE showed almost similar inhibition whereas TGAE showed considerably less inhibition when compared with the standard Cystone at different concentrations. The Methanolic extracts of Meyna laxiflora seeds and Tectona grandis bark has shown significant activity on comparison to the synthetic drugs Spironolactone, polyherbal formulation Cystone and to the other extracts of the drug, the furosemide was found to non-active on formed crystals (Mosquera, D. M. G., 2020).

5. Acknowledgement

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