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Research Article

## *In-Vitro* antiurolithiatic activity of aqueous extract *Pavonia lasiopetala*

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### ABSTRACT

**Background:** Upto date, the scientific documentation regarding *in-vitro* antiurolithiatic activity of has been reported, although it has not reported for *in-vitro* antiurolithiatic activity up till now.

**Objective:** To explore anti-urolithiatic activities of *Pavonia lasiopetala* leaves extract by utilizing different *in-vitro* models.

**Material and Methods:** Aqueous extract of *Pavonia lasiopetala* fresh leaves was prepared and arranged in the different concentrations. Homogenous precipitation method was used to prepare artificial stones such as calcium oxalate and calcium phosphate and semi-permeable membrane of eggs was used as dissolution bags. Dissolution models were incubated in 72 hrs and after that, the entire content in dissolution bags was estimated spectrophotometrically. The inhibitory activity of *Pavonia lasiopetala* leaves extract on the nucleation of calcium oxalate crystals and the rate of aggregation in calcium oxalate crystals was determined by spectrophotometric assay. Results: In dissolution models, the extract of *Pavonia lasiopetala* has greater capability to dissolve calcium oxalate while Cystone standard has shown better demineralization for calcium phosphate rather than extract of *Pavonia lasiopetala*. Cystone exhibited strongly inhibitory action in the nucleation assay rather than aggregation assay. The extract of *Pavonia lasiopetala* exhibited inhibitory action in both of nucleation and aggregation assays to significant level.

**Discussion:** Correlation between *in-vitro* and *in-vivo* studies may be helpful to understand the molecular mechanism of litholysis process and to reveal phytochemicals of the extract responsible for dissolving or disintegrating renal calculi.

**Conclusion:** *Pavonia lasiopetala* extract exhibited significant *in-vitro* anti-urolithiatic activity.

**Keywords:** *Pavonia lasiopetala*, spectrophotometrically, *in-vitro* antiurolithiatic.

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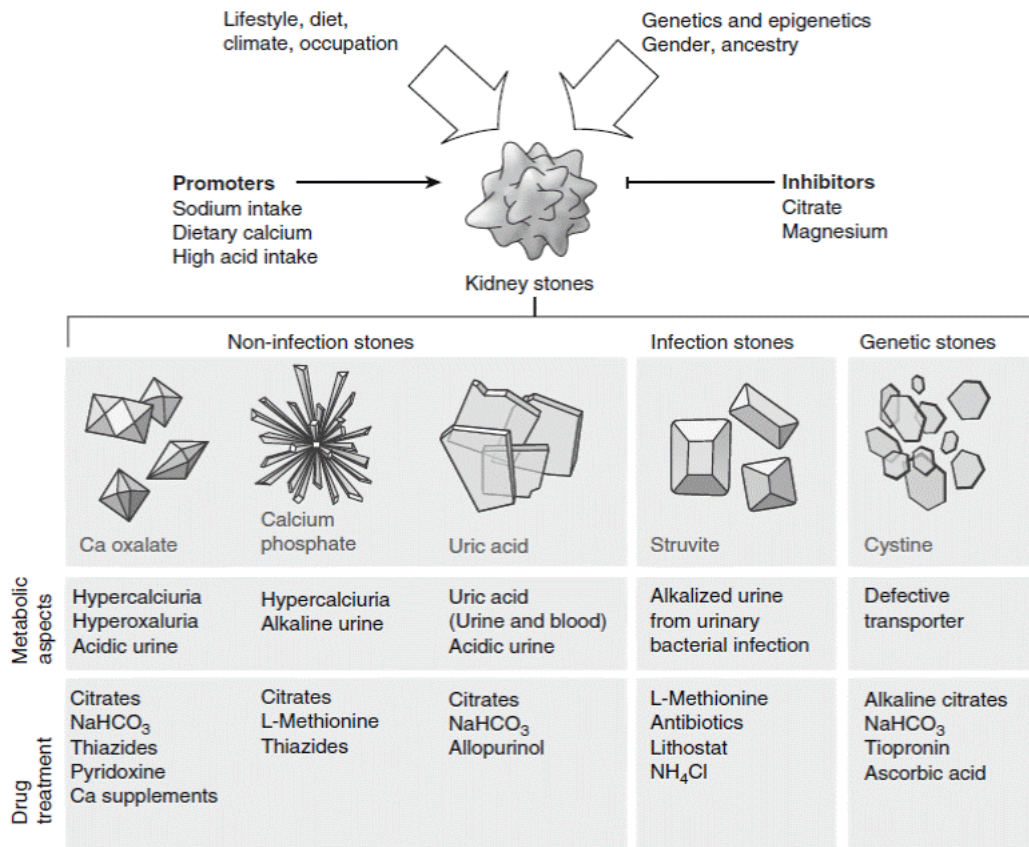
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### INTRODUCTION

Nowadays stone formation is the oldest and serious painful urologic disease with significant prevalence in the population due to change in lifestyle and dietary factors<sup>1,2,3</sup>. Stone formation or lithiasis is characterized by calculi formation. It has two main types such as nephrolithiasis and urolithiasis<sup>4,5,6</sup>. Calculi formation in urinary bladder, ureter or any part of urinary tract rather than kidney is known as urolithiasis while nephrolithiasis is characterized calculi formation in kidney<sup>7,8,9</sup>. Generally, calcification for the formation of bone and teeth takes place in controlled biological situations<sup>10,11</sup>. Uncontrolled pathological crystallization occurs when solvent becomes supersaturated

leading to the formation of precipitates in the body called as kidney stones<sup>12,13</sup>.

Several medicinal plant extracts have been reported for *in vitro* anti-crystallization activities till date such as *Herniaria hirsuta*, *Tribulus terrestris*, *Bergenia ciliate*, *Piper nigrum*, *Dolichos biflorus*, *Bergenia ligulata*, *Plantago major*. *Pavonia Lasiopetala*. um is one of many medicinal plants as anti-urolitholytic known as traditionally folk medicine for *Pavonia Lasiopetala* is a scientific term used in the Ayurveda system of medicine describing the potency of medicinal plant for breaking up and disintegrating renal and urinary calculi i.e., kidney stones and diuretic capacity<sup>14,15</sup>.



**MATERIAL AND METHODS**

Calcium chloride dihydrate, sodium oxalate, p-phenylene diamine were purchased from Sigma-Aldrich Ltd. Potassium permanganate, sodium meta-bisulfite, and trisbuffer were purchased from Sigma-Aldrich. Extraction Fresh leaves of *Pavonia Lasiopetala* were chopped into small pieces by hand and put into a conical flask. 100ml of distilled water was added to the conical flask and boiled for a while in order to maximize the extraction<sup>16,17</sup>. After cooling it was filtered through Whatmann filter paper and as aqueous extract stock solution transferred to a suitable container.

Preparation of the semi-permeable membrane from eggs Apex of eggs was punctured by a glass rod in order to squeeze out the entire content. Empty eggs were washed thoroughly with distilled water and placed in a beaker consisting 4ml concentrated HCl in 200ml distilled water. It was kept for overnight which led to the complete decalcification of semi permeable membrane<sup>18</sup>. On the next

day, semi permeable membranes were removed carefully from egg shells; washed thoroughly with distilled water and placed it in ammonia solution for neutralization of acid traces, and then rinsed it with distilled water. It was stored in refrigerator at a pH of 7-7.4 in the moistened condition. 10mg of the calcium oxalate was suspended in 10ml of distilled water as negative control<sup>19</sup>. 5ml of hot aqueous extract of fresh leaves of *Pavonia Lasiopetala* was taken. 500mg tablet of Cystone was placed in absolute ethanol for removing colour coating and 400mg was obtained. Cystone tablet was crushed into powder form and dispersed into 100ml of distilled water and filtered. Filtrate of Cystone was used as positive control for in vitro anti-urolithiatic activity. Group I: 1ml of calcium oxalate (1mg/ml) + 1ml of distilled water, Group II: 1ml of calcium oxalate (1mg/ml) + 1ml of Cystone solution (400mg/ml), Group III: 1ml of calcium oxalate (1mg/ml) + 1ml of hot aqueous extract of *Pavonia Lasiopetala* (20mg/ml)<sup>20</sup>.

**RESULTS & DISCUSSION**

**Table 1: Dissolution of calcium oxalate**

Group	Mean ± SEM	Weight of calcium reduced	Dissolution Percentage
Group I	0.231 ± 0.0025		
Group II	0.083 ± 0.0037	0.087	77.32
Group III	0.129 ± 0.014	0.071	51.75

**Table 2: Dissolution of calcium phosphate**

Group	Mean ± SEM	Weight of calcium reduced	Dissolution Percentage
Group I	0.214 ± 0.0025		
Group II	0.094 ± 0.0037	0.027	33.19
Group III	0.123 ± 0.014	0.080	81.98

As in vitro crystallization study was performed, since nucleation is an important first step for the initiation of crystals, which then grow and form aggregates. Extract of *Pavonia Lasiopetala* inhibited the crystallization by inhibiting nucleation of calcium oxalate through disintegrating into smaller particles with increasing concentrations of the fraction. From the results of the nucleation assay confirmed that the extract contained nucleation-preventing agents. No pharmacologic intervention has definitively been shown to be effective for lithiasis. The present investigation will be supportive as additional information to the scientific evidences regarding in-vitro studies <sup>21</sup>.

## CONCLUSION

Since mechanism of anti-urolitholytic activity in the extract is exact unknown till date, correlation between *invitro* and *in-vivo* studies should be further investigated to reveal the phytochemicals of the extract are responsible for dissolving or disintegrating renal calculi and to know better understanding in the molecular mechanism of litholysis. *Pavonia Lasiopetala* extract exhibited significant *in-vitro* anti-urolithiatic activity.

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