In vitro anti-urolithic activity of Chandraprabavathi – A herbo-mineral formulation

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Abstract

Chandraprabhavati (CPV) is an Ayurvedic formulation clinically used in the management of urinary calculi. The present study was undertaken to investigate the in-vitro antiurolithic activity of CPV by artificial urine and nucleation assay. Cystone demonstrated better percentage inhibition of calcium oxalate crystals formation than CPV in nucleation assay, however CPV exhibited more inhibition of super saturation of calcium oxalate crystals in artificial urine assay than cystone. The percentage inhibition of calcium oxalate crystals formation increased in dose dependent manner for both the drugs. Thus our study demonstrates primary evidence for CPV possessing antiurolithiatic property in vitro.

Keywords: Chandraprabhavati; Anti-urolithic; Renal stones

1. Introduction

Urolithiasis is formation of urinary calculi at any level of urinary tract. The treatment of urolithiasis is involves the dissolution of existing stones and preventing the reoccurrence of stones. An alarming rise in the incidence and recurrence of urolithiasis coupled with adverse effects allopathic drugs necessitates exploration of traditional mode of treatment. [1] Chandraprabha vati (CPV) is an Ayurvedic formulation available in vati form and is clinically used in the management of urinary calculi. Though being clinically used in Ayurveda still no scientific evidence for antiurolithic activity of CPV has been established. The present study was undertaken to investigate the in-vitro antiurolithic activity of CPV by artificial urine assay and nucleation assay.

2. Material and methods

2.1. Chemicals

Cystone was procured from Himalaya Drug Company, Karnataka, India and CPV from Kottakkal Arya Vaidyasala, Kerala. Calcium chloride, sodium oxalate, Tris buffer, sodium chloride, sodium phosphate, sodium citrate, magnesium sulphate, sodium sulphate, potassium chloride, ammonium hydroxide, ammonium chloride, sulphuric acid, ammonia, hydrochloric acid, potassium permanganate were procured from Merck Specialities Private Limited, Mumbai.

2.2. Apparatus

UV Spectrophotometer- Thermos Fisher Scientific, UK

2.3. Nucleation assay

Solution of calcium chloride and sodium oxalate at concentrations of 5mmol/L and 7.5 mmol/L respectively was prepared in a buffer containing Tris 0.05 mmol/L and NaCl 0.15mol/L at pH 6.5. 950 μl of calcium chloride solution was mixed with of test drug and standard (cystone) at different concentrations. Crystallization was started by adding
950 μl of sodium oxalate and incubated for 30 min at 37º C and optical density of the solution was measured at 620 nm. The percentage inhibition of calcium oxalate crystal formation was calculated. [2]

2.4. Artificial urine (AU) assay

The artificial urine was prepared according to the method Burns and Finlayson, it consist of sodium chloride 105.5 mM, sodium phosphate 32.3 mM, sodium citrate 3.21 mM, magnesium sulfate 3.85 mM, sodium sulfate 16.95 mM, potassium chloride 63.7 mM, calcium chloride 4.5 mM, sodium oxalate 0.32 mM, ammonium hydroxide 17.9 mM, and ammonium chloride 0.0028 mM. The AU was prepared fresh each time and pH adjusted to 6.0. 1.0 ml of AU was added to 0.5 ml of distilled water and blank reading was taken. The 0.5 ml of 0.01 M sodium oxalate was added and the measurement is immediately started for a period of ten minutes. The standard and test substance were prepared in different concentration. A mixture of 1 ml of AU and 0.5 ml of test solution was taken as blank reading and then 0.5 ml of 0.01 M sodium oxalate solution was added and immediately the absorbance was measured for a period of ten minutes at 620 nm. The percentage of inhibition of calcium oxalate crystal formation was calculated. [3] All results are represented as mean ± SEM.

3. Results and discussion

Cystone exhibited better percentage inhibition than CPV (Fig 1) in nucleation assay and percentage inhibition increased in dose dependent manner for both the drugs. But CPV has more percentage inhibition of super saturation of calcium oxalate crystals (Fig 2) in artificial urine assay than cystone and percentage inhibition increased in dose dependent manner for both the drugs. Thus our study demonstrates that CPV exhibits good anti-urolithic activity comparable to that of the standard, cystone.

![Figure 1 Effect of CPV and cystone on calcium oxalate crystallisation for nucleation assay](image1)

![Figure 2 Effect of CPV and cystone on calcium oxalate crystallisation for artificial urine assay](image2)

CPV has phytoconstituents such as alkaloids, flavonoids, carbohydrates, sterols and triterpenoids, tanins and phenolic compounds. [4] Tannin might have contributed to the inhibition of calcium oxalate crystals formation. [5] Terpenoids has shown to inhibit the cytotoxicity induced by calcium oxalate, they are also know to normalize excretion of stone forming constituents. [6] Flavonoids possess urolithic dissolution potency and antioxidant activity. [5] Phenolic
constituents exhibit antioxidant activity and prevent crystal adhesion and subsequent formation of urinary stones.[7] Therefore anti-urolithic activity of CPV would have been an outcome of these phytoconstituents present in CPV.

4. Conclusion

This study has given primary evidence for CPV possessing antiurolithic property \textit{in vitro}. However, further study in animal models of urolithiasis is needed to evaluate its potential antiurolithic activity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest

References


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