

# Antilithogenic Potential of Green Tea, Oolong Tea, and Black Tea

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## ABSTRACT

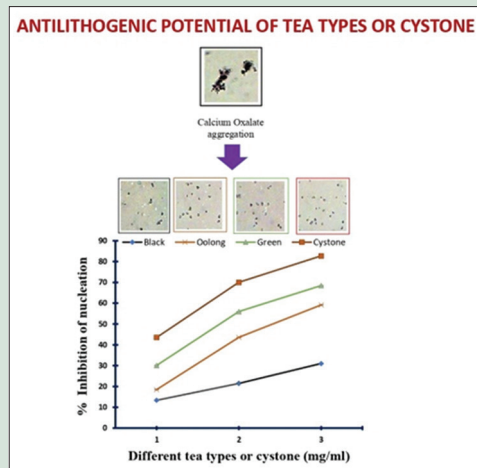
**Background:** Urolithiasis and nephrolithiasis are the commonly occurring painful diseases in most parts of the world. The number of new cases associated with lithiasis is on rise, although various therapies such as percutaneous nephrolithotomy, lithotripsy, and other surgical procedures can remove the stones, high recurrence rate is a major problem. **Objectives:** The present study was undertaken to evaluate the antilithogenic potential of green tea, oolong tea, and black tea employing chemical model. **Materials and Methods:** The antilithogenic action of different teas on calcium oxalate crystal formation was investigated on artificial urine spectroscopically, and the modulation of crystal size and density was recorded microscopically. **Results:** The different tea types showed significant inhibitory action on nucleation and crystal size morphology and density. The optical and spectroscopic techniques demonstrated that standard drug cystone exhibited highest inhibition followed by green tea, oolong tea, and black tea. **Conclusion:** Green tea illustrated maximum antilithogenic property as compared to other tea types and can be used as a potential dietary agent for the prevention of lithiasis.

**Key words:** Antilithogenic, black tea, cystone, green tea, nephrolithiasis, oolong tea, urolithiasis

## SUMMARY

- The new cases of urolithiasis and nephrolithiasis are on the rise in most parts of the world
- The surgical procedures can remove the stones, but high recurrence rate is a major problem
- *In vitro* antilithogenic action of green tea, oolong tea, and black tea on calcium oxalate crystal formation was investigated on artificial urine
- The different tea types showed significant inhibitory action on nucleation and crystal size morphology and density
- Green tea illustrated maximum antilithogenic property as compared to other

tea types and can be used as a potential dietary agent for the prevention of lithiasis.



**Abbreviations Used:** CaOx: Calcium oxalate; EC<sub>50</sub>: Effective concentration of drug causing 50% inhibition.

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## INTRODUCTION

Lithiasis or stone formation is one of the painful reoccurring pathogenic disorders known to human since ancient time.<sup>[1]</sup> The stone formation can occur in any region of urinary system which includes urinary bladder, ureter, gall bladder, and kidney. Urolithiasis is the formation of stone in any part of the urinary tract such as urinary bladder, ureter while nephrolithiasis particularly concern with kidney stone. Stone is an aggregation of calcium oxalate (CaOx), phosphate, and uric acid resulting from culmination of various physiochemical events which include supersaturation, nucleation, growth, and aggregation into crystals.<sup>[2]</sup> CaOx with or without calcium phosphate constitutes about 70%–80% of the urinary stones, while struvite, uric acid, and cystine accounts for 10%, 9% and 1% of renal stones.<sup>[3,4]</sup>

It is a multifactorial disease related to age, sex, geographical location, and dietary factors.<sup>[5]</sup> Despite the technological advancement in pathophysiology and treatment of urinary stones such as endoscopic stone removal and extracorporeal shock wave lithotripsy, there is an incomplete cure in many of the cases of clinical therapy.<sup>[6]</sup> The surgical procedures for removing urinary stones are costly, and recurrence rate is a common issue. Thus, to study dietary agents for preventing the formation of new and recurrent stones would be of great interest.

A meta-analysis report revealed increased consumption of water, tea, and alcohol may reduce the risk of kidney stone formation.<sup>[7]</sup> Tea is the second-most consumed beverage after water. Green, oolong, and black teas are obtained from the leaves of the same plant (*Camellia sinensis*) but differ in processing method used while manufacturing. Green tea is the least oxidized form, whereas oolong tea and black tea are partially and maximally oxidized, respectively. The oxidation step leads to variation in the polyphenols types and content that influences the flavor and biological properties of these tea types.<sup>[8]</sup> A recent study associated green tea consumption with lower incidence of stone formation.<sup>[9]</sup> In recent years, various medical studies have proved that tea prevents various diseases such as diabetes, skin damages, obesity, oral health, prevents hair loss, protects

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against cardiovascular diseases, and improves insulin sensitivity.<sup>[10]</sup> Although both *in vitro* and *in vivo* studies suggested role of green tea in the prevention of lithiasis, the studies about the effect of oolong and black teas are under-investigated. It is important to identify a dietary agent that can prevent both primary stone formation and its recurrence after therapy. Teas are commonly consumed beverages obtained from leaves of *C. sinensis*, depending on their processing they are categorized into minimally oxidized (green), partially oxidized (oolong), and maximally oxidized (black) form. To best of our knowledge, the present study is the first report on the comparative efficacy of different tea types on inhibition of CaOx crystal formation employing *in vitro* model.

## MATERIALS AND METHODS

### Chemicals

The chemicals used in this study were of analytical grade, procured from HiMedia Lab. Pvt. Ltd., Mumbai, India. Cystone (Himalaya Drug Company, Bengaluru) a polyherbal drug was purchased from a local chemist shop.

### Preparation of tea

The green tea (Batch No. 6011), oolong tea (Batch No. 5091), and black tea (Batch No. 6011) were purchased in the form of 50 g packets containing loose leaf tea from Bud White Teas Pvt. Ltd., Delhi, India. Tea extract was prepared by soaking 2 g of tea in 100 ml of hot water maintained at 90–95°C on heating mantle for 5 min.<sup>[11]</sup> The aqueous extracts were filtered with Whatman filter paper, allowed to cool to room temperature and are stored at 4°C.

### *In vitro* nucleation assay

*In vitro* CaOx was prepared to assess the nucleation inhibition capacity of the plant extract.<sup>[12]</sup> A solution of calcium chloride (5 mM) and sodium oxalate (7.5 mM) was prepared in Tris (0.05 M) and NaCl (0.15 M). The solution was kept at 37°C, and pH 6.5 was maintained. 0.95 ml of calcium chloride was taken in several test tubes in which varying concentration of tea types were added. Cystone was used as standard drug with same concentration as that of teas. The addition of 0.95 ml of sodium oxalate-initiated nucleation that was measured spectrophotometrically at 620 nm after 30 min of incubation period using the formula mentioned below

% inhibition of nucleation

$$= \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Where control is without any inhibitor and test includes inhibitor.

The size and morphology of CaOx crystals formed after 24 h were observed microscopically in the absence or presence of various tea types and cystone at  $\times 40$ .

### Statistical analysis

Experiment results were expressed as mean  $\pm$  standard deviation in triplicates. The data were analyzed by one-way analysis of variance followed by Tukey's honestly significant difference *post hoc* test using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The statistical significance was set at  $P < 0.05$ .

## RESULTS

*In vitro* antilithiatic potential of different tea types (green tea, oolong tea, and black tea) and a polyherbal drug (cystone) prescribed for the treatment of urolithiasis was compared. The extent of crystals formation

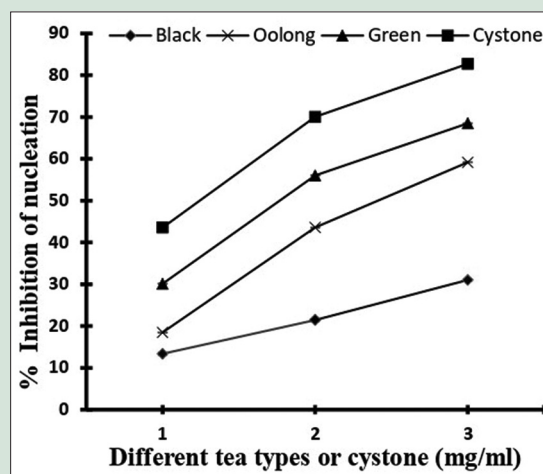
was assessed based on the turbidity of the solution and decrease in absorbance was observed with increase in the concentration of different tea types and cystone in all the treated groups. The percentage inhibition of nucleation at 1–3 mg/ml was observed highest for cystone (43.55%–82.72%) followed by green tea (30.12%–68.49%), oolong tea (18.49%–59.17%), and least in black tea (13.36%–31.02%). The EC<sub>50</sub> of cystone, green tea, oolong tea, and black tea was 1.18 mg/ml, 1.76 mg/ml, 2.38 mg/ml, and 7.3 mg/mL ( $P < 0.05$ ), respectively [Figure 1]. The results obtained were further supported by photomicrographs of the control (without tea/cystone) showing bigger clusters of CaOx crystallization whereas the reduction in the size of crystal formation was visualized with increase in concentration of cystone, green tea, oolong tea, and black tea from 1 to 3 mg/ml [Figure 2].

## DISCUSSION

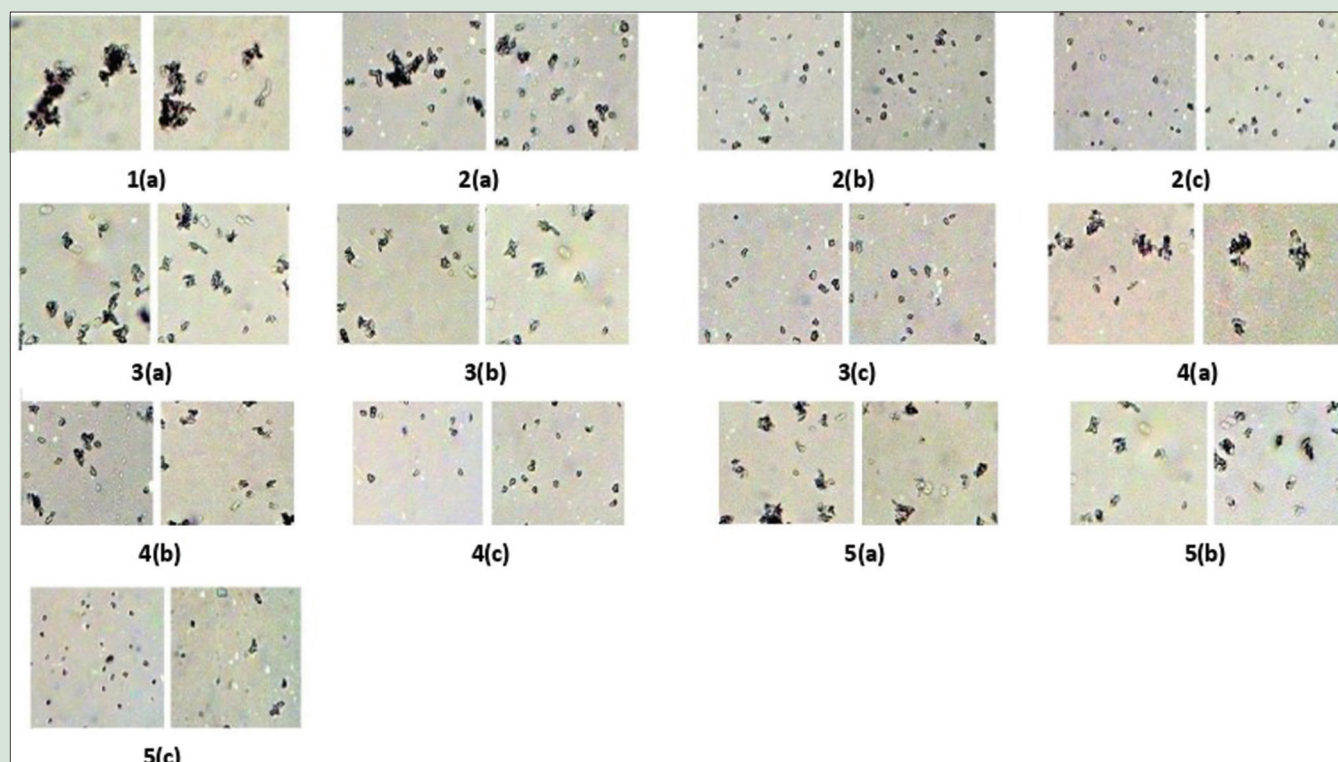
The key steps involved in stone formation include nucleation and aggregation, the inhibition of these events *in vitro*, provide screening methods for the development of antilithiatic agents.<sup>[13]</sup> Although cystone was found to be more effective as compared to other tea types, it is mainly used as a therapeutic herbal drug. The earlier study also reported cystone higher efficacy as compared to other herbs in preventing CaOx crystal formation.<sup>[14]</sup> Green tea exhibited highest anti-nucleation property, and maximum reduction in density and size of CaOx followed by oolong and black tea [Figures 1 and 2]. Tea extracts are rich in caffeine and polyphenols that act as both capping and reducing agents for the generation of metal nanoparticles.<sup>[15]</sup> The high phenolic content of green tea in comparison to oolong and black tea was cited as major contributor for its higher efficacy in synthesis of Fe nanoparticles.<sup>[16]</sup> These tea types may impede the formation of CaOx crystals by discouraging free ions present in the solution to associate and form microscopic particles due to their capping and reducing properties mainly attributed to phenolic compounds. Furthermore, an *in vitro* study demonstrated that green tea polyphenols hydrogen bond donating ability is responsible for morphological alteration and inhibition of CaOx crystal formation.<sup>[17]</sup>

## CONCLUSION

Among the studied tea types, green tea demonstrated most promising antilithiatic activity and very well fit into the category of dietary agent that can be incorporated in routine life of an individual which may



**Figure 1:** Percentage inhibition of nucleation by cystone, green tea, oolong tea, and black tea. Data are shown as mean  $\pm$  standard deviation for three independent experiments (each with triplicates for each test point)



**Figure 2:** Photomicrographic evaluation of size and density of calcium oxalate crystals at  $\times 40$ : Control without tea extract or cysteine (1a); cysteine (2a-c); green tea (3a-c); oolong tea (4a-c); and black tea (5a-c) at 1–3 mg/ml

prove to be beneficial for prevention and recurrence of stone formation. However, *in vivo* and clinical studies are required to confirm the obtained results.

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## Conflicts of interest

There are no conflicts of interest.

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