**In vitro study of aqueous leaf extract of Chenopodium album for inhibition of calcium oxalate and brushite crystallization**

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**ABSTRACT**

The leaves of Chenopodium album Linn. are traditionally used for treatment of kidney diseases and urinary stones. The present work investigated the effect of aqueous extract of leaves of C. album (CAAE) on in-vitro crystallization of CaOx and brushite crystals. Crystallization was studied by using nucleation and aggregation assay of calcium oxalate (CaOx) crystals and growth assay of calcium oxalate monohydrate and brushite crystals. The effects of CAAE and cystone on slope of nucleation and aggregation as well as growth of calcium oxalate crystallization were evaluated spectrophotometrically. The densities of the formed crystals were compared under microscope. The effects of CAAE and citric acid on growth of brushite crystals were studied by using single diffusion gel growth technique, and the parameters evaluated were length, morphology and average size of the deposited crystals. CAAE significantly inhibited the slope of nucleation and aggregation of CaOx crystallization, and decreased the crystal density. It also inhibited the growth and caused the dissolution of brushite crystals. The standard drug cystone or citric acid also exhibited similar effects. The study reveals that the leaves of C. album were found effective in the prevention of the experimentally induced urinary stones and substantiate the traditional claim. It is concluded that the leaves of C. album have beneficial inhibitory effect on in-vitro crystallization of CaOx and CHPD (brushite) crystals.

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**Keywords:** Bathua, Urolithiasis, Cystone, Calcium oxalate, Brushite, Citric acid

**1. Introduction**

Urolithiasis is defined as the presence of one or more calculi in any location within the urinary tract [1]. It is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70–80% in male and 47–60% in female [2]. Majority of the stones are calcium-containing stones, especially calcium oxalate (80%) and others are 20% [3].
The medical management of urolithiasis involves drug treatment and extracorporeal shock wave lithotripsy (ESWL). The various therapies including thiazide and diuretic and alkali-citrate are used to prevent the recurrence of hypercalciuria and hyperoxaluria, which induce calcui formation, but evidence for their efficacy is less [4]. The surgical endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionized the treatment of urolithiasis but does not prevent the likelihood of new stone formation [5]. Besides imposing the high cost, shock waves in therapeutic doses may cause acute renal injury, decrease in renal function and an increase in stone recurrence. The recurrence of stone formation is also very high (50–80%). In addition, persistent residual stone fragments and the possibility of infection after ESWL represent a serious problem in the treatment of stones. Thus, medical management of urolithiasis is either costly or poses serious side effects.

The crystallization of the stone begins with increased urinary supersaturation, with the subsequent formation of the solid crystalline particles within the urinary tract. This is followed by nucleation, by which stone-forming salts in supersaturated urinary solution coalesce into clusters that then increase in size by the addition of new constituents [6]. These crystals then grow and aggregate with other crystals in solution, and are ultimately retained and accumulated in the kidney [7]. Therefore, if this progression of crystallization can be prevented, then lithiasis can also be prevented.

There is growing interest of public in herbal medicine, particularly in the treatment of urolithiasis partly because of limited choice in the pharmacotherapy. Data from in-vitro, in-vivo and clinical trials reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of urolithiasis. Many Indian plants are useful as antilithiatic agents [5,8–13]. Hence, the Indian medicinal plants are constantly being evaluated for possible antilithiatic effects.

Chenopodium album L. (family: Chenopodiaceae) is a herbaceous vegetable plant locally known as Bathua. It is cultivated as pot-herb and usually grown in gardens, but can be found in the corner of early grain fields in Bombay presidency and elsewhere in India (Kashmir and Sikkim). The medicinal property of this plant is mainly present in leaves and seeds. The leaves of C. album are used in ethno-medicinal practices for treatment of kidney diseases and urinary stones. Ethnobotanical studies of Aravalli region of Rajasthan (India) report the folk medicinal uses of cooked leaves of C. album in kidney stones and urinary tract troubles [14]. Cooked leaves of C. album are used as traditional medicine in the Shekhavati region of Rajasthan for treatment of urinary troubles and colic [15]. In Ladakh, leaves are also used traditionally for controlling painful urination [16]. C. album is an important medicinal weed of Moradabad useful in the treatment of urinary retention and kidney diseases [17].

In view of traditional and ethno-medicinal use of leaves of C. album in the treatment of kidney stones, the present work demonstrated the effect of aqueous extract of the leaves of C. album on in-vitro crystallization of CaOx and brushite crystals. As this plant is consumed as food substance by human beings and as weed fodder by cattle, its antilithiatic property would be good preventive option available.

2. Materials and methods

2.1. Plant material

The leaves of C. album were collected from the local market of Gwalior in December 2012 and identified by Dr. N.K. Pandey, Research Officer (Botany), National Research Institute for Ayurveda-Siddha Human Resource Development, Aamkho, Gwalior. A voucher specimen (Field Book No. 5-4/12–13/NRIASHRD/Tech/Survey/134) of the authenticated C. album has been deposited in the herbarium of the institute.

2.2. Drugs and chemicals

Cystone (Himalaya Drug Company) and citric acid 1-monohydrate (E. Merck (India) Ltd., Mumbai) were purchased from the local market. All remaining chemicals used in the experiment were of the highest grade commercially available.

2.3. Preparation of aqueous extract of the leaves of C. album (CAAE)

The leaves were separated from other extraneous matter and subjected to shade drying. The dried leaves were subjected to a coarse powder by using dry grinder. The powder (100g) was soaked (maceration) in 1 L purified water and kept in dark and dry place for 48 h at a temperature range of 20–26 °C. Chloroform was added in quantity of 1% total mixture to avoid microbial growth. After 48 h, solutions were filtered by Whatman Filter Paper No. 1. The filtered extracts were dried in a rotary evaporator to obtain a dark brown powdery extract (13.4% w/w).

2.4. Preliminary phytochemical screening and quantitative estimation of phytoconstituents

Preliminary phytochemical screening [18] of CAAE was carried out to detect the presence of sterols, alkaloids, saponins, terpenes, tannins, phenolic substances, carbohydrates, volatile oil and mucilage. The total phenolic content of the extracts was determined spectrometrically [19] and expressed as milligram of tannic acid equivalents (TAE) per gram of extract. Total flavonoid content was measured by aluminum chloride colorimetric assay [20] and expressed as milligram of quercetin equivalent per gram of extract. Total saponins were determined according to the previously described methods by Obadoni and Ochuko (2002) with little modification [21].

2.5. Effect of CAAE on in-vitro crystallization

2.5.1. In-vitro crystallization of calcium oxalate

2.5.1.1. Nucleation and aggregation assay. Nucleation and aggregation assay were performed as per method previously described by Hess et al. [2000] with minor modifications [22]. Briefly, freshly prepared solution of 10 mM calcium chloride dihydrate and 1.0 mM sodium oxalate, containing 200 mM NaCl and 10 mM sodium acetate trihydrate, was adjusted to pH 5.7. All experiments were performed at 37 °C, using a circulating...
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