Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 28s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Effect of Melia azedarach on the Crystal Formation of In Vitro Models of Urolithiasis

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Cite this paper as: Girish Chandra Tiwari, Manju Prajapati, (2025) Effect of Melia azedarach on the Crystal Formation of In Vitro Models of Urolithiasis. *Journal of Neonatal Surgery*, 14 (28s), 522-526.

ABSTRACT

Melia azedarach L, also known as Chinaberry tree, is a Southeast Asian plant with anti-inflammatory, antipyretic, analgesic, and insecticidal properties. It contains bioactive compounds like flavonoids, alkaloids, terpenoids, and saponins. Recent studies have shown that extracts from *Melia azedarach* L have anticancer properties and antimicrobial activity against various pathogens. Ayurveda, a traditional Indian medicinal system, has been practiced for thousands of years. *Melia azedarach* Linn, also known as mahanimba, is used for its anthelmintic, antilithic diuretic, emmenagogue, astringent, and stomachic properties. Research has demonstrated their ability to dissolve kidney stones, improve kidney function, and alleviate discomfort associated with urolithiasis. The current study aimed to assess the effectiveness of both plants in laboratory methods for urolithiasis by using a crystal nucleation assay at dosages of 0.1 mg/ml, 1 mg/ml, and 10 mg/ml. *Methanolic extract of Melia azedarach* exhibited a notable increase in the rate of crystal nucleation compared to *Aqueous extract of Melia azedarach*. This implies that *Methanolic extract of Melia azedarach* may have a stronger ability to promote crystal formation in a solution. The cause of this phenomenon can be ascribed to the chemical makeup of *Methanolic extract of Melia azedarach*, which perhaps includes substances that improve nucleation processes. A Fourier Transform Infrared (FT-IR) analysis was conducted on 10-50 mg of calcium oxalate (CaOx) crystals generated during the crystal nucleation experiment. The analysis confirmed the existence of oxalate ions and calcium ions within the crystal structure. The investigation revealed that *Methanolic extract of Melia azedarach* exhibited the highest level of crystal aggregation (56%) at a concentration of 1000 µg/ml, but Sample *Aqueous extract of Melia azedarach* demonstrated the most crystal aggregation (79%) at the same concentration. Aggregation had a positive correlation with the dosage administered. This study aimed to examine the impact of *Methanolic extract of Melia azedarach* Linn and *Aqueous extract of Melia azedarach* on crystal nucleation assay at concentrations of 0.1, 1, and 10 mg/ml. The experimental findings demonstrated a substantial increase in the rate of crystal aggregation when Gs were compared to As. The results indicated that Sample SS exhibited the highest level of Crystal Aggregation (56%) at a concentration of 1000 g/Ml compared to the standard (Cystone -75.19% at a concentration of 1mg/Ml). On the other hand, Sample AS demonstrated the highest level of Crystal Agglomeration (79%) at a concentration of 1000 Ml. Additionally, Sample AS successfully verified the existence of oxalate ions and calcium ions within the CaOx crystal lattice.

Keywords: *Methanolic extract of Melia azedarach, Aqueous extract of Melia azedarach, Crystal Aggregation and Nucleation Assay*

1. INTRODUCTION

Plants offer numerous health benefits through various means. They serve as the foundation of both medicine and sustenance. Cultures around the world have used different strategies to treat illness, depending on the resources available in their specific biocultural environments. Most developing countries rely on plants for their primary healthcare. A significant number of the current pharmaceuticals employed to treat various disorders are derived from plants or plant-based therapies [1]. Traditionally, nearly all drugs were derived from biological resources. They remain vital in the present day, as 60–70% of current pharmaceuticals are derived from natural sources [2]. According to the World Health Organisation (WHO), more than 80% of the global population depends on traditional medicines, with the majority of these treatments utilising plant extracts, as their main source of healthcare. India's traditional medical systems, including Unani, Ayurveda, Homoeopathy,

and Siddha, recommend around 95% of medicines derived from plants [3]. Urolithiasis, also known as urinary calculus, is the process of developing and keeping solid nonmetallic minerals (stones) in the urinary system. In the context of Ayurveda, it is referred to as Mutra-ashmari. This condition is prevalent in about 12% of the population, with a recurrence rate of 70-80% in males and 47-60% in females. The majority of the stones, specifically 80%, consist of calcium, particularly calcium oxalate. Urolithiasis, also referred to as urinary calculus, is the process of forming and collecting solid nonmetallic minerals (stones) in the urinary system. In Ayurvedic nomenclature, the condition is known as Mutra-ashmari. The male recurrence rate ranges from 70% to 80%, whereas the female recurrence rate ranges from 47% to 60%. Calcium is the main component of 80% of the stones, with calcium oxalate being the primary form [4]. Although there are multiple treatments available to prevent the recurrence of hypercalciuria and hyperoxaluria, their efficacy is diminishing [5]. The therapies consist of thiazide as a diuretic and alkali-citrate. While extracorporeal shock wave lithotripsy and surgical endoscopic stone removal have revolutionized the treatment of urolithiasis, they do not completely prevent the formation of new stones [6]. Melia azedarach L, also known as Chinaberry tree, is a Southeast Asian plant with anti-inflammatory, antipyretic, analgesic, and insecticidal properties. It contains bioactive compounds like flavonoids, alkaloids, terpenoids, and saponins. Recent studies have shown that extracts from Melia azedarach L have anticancer properties and antimicrobial activity against various pathogens. Ayurveda, a traditional Indian medicinal system, has been practiced for thousands of years. Melia azedarach Linn, also known as mahanimba, is used for its anthelmintic, antilithic diuretic, emmenagogue, astringent, and stomachic properties [7-9].

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Ethylene glycol was purchased from Qualigens Fine Chemicals, Mumbai, India. Cystone (Himalaya Drug Company) was purchased from local market. Urea, creatinine, uric acid, calcium, phosphorus estimation kits of ERBA diagnostics Mannheim GmbH, Germany were procured from Transasia Biomedicals Private Limited, Mumbai. All remaining chemicals such as Calcium chloride (50 mM), Sodium oxalate (50 mM), Tris buffer (100Mm, Ph 6.8), Sodium chloride (5M), used in the experiment were of the highest grade commercially available.

2.2 *In vitro* Evaluation of *Methanolic extract of Melia azedarach* Linn and *Aqueous extract of Melia azedarach* on Crystal Nucleation Assay

Calcium chloride and sodium oxalate solutions were made at final concentrations of 50 mM and 50 mM, respectively, in a pH 6.8 buffer containing 100 mM of Tris and 5 M of NaCl. A 0.22 µm filter was used to filter both solutions. A mixture of 33 ml of calcium chloride solution and 3.3 ml of either the 1 mg/ml cystone or 0.1 mg/ml, 1 mg/ml and 10 mg/ml of GS and AS was added. Addition of 33 millilitres of sodium oxalate solution initiated crystallisation. A stirring bar coated in PTFE was used to magnetically stir the final solution at 800 rpm. 37°C was the constant temperature. A UV-VIS spectrophotometer (Shimadzu UV 1800) was used to measure the absorbance of the solutions at 620 nm every 15 seconds for up to five minutes [10, 11].

3. RESULTS AND DISCUSSION

3.1 Effect of *Methanolic extract of Melia azedarach* Linn seeds (MEMA) and *Aqueous extract of Melia azedarach* seeds (AEMA) on Crystal Nucleation Assay

Crystal nucleation assay is a valuable tool in studying the crystallization process of various substances. In this study, the effect of *Methanolic extract of Melia azedarach* Linn seeds (MEMA) and *Aqueous extract of Melia azedarach* seeds (AEMA) on crystal nucleation assay at a concentration of 0.1 mg/ml, 1 mg/ml and 10 mg/ml was investigated. These seeds are known for their potential role in promoting crystal growth and have been used in traditional medicine for centuries. The results of the experiment showed that MEMA significantly increased the rate of crystal nucleation compared to AEMA. This suggests that MEMA may have a greater influence on promoting crystal formation in solution. The mechanism behind this effect could be attributed to the chemical composition of MEMA, which may contain compounds that enhance nucleation processes (Figure 1, 2 and 3).

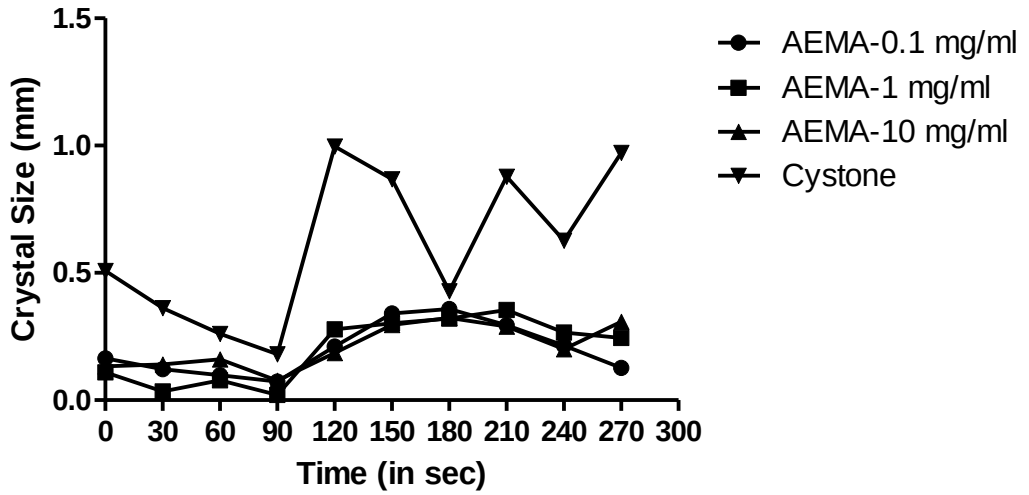

Figure 1: Effect of *Aqueous extract of Melia azedarach* seeds (AEMA) on Crystal Nucleation Assay

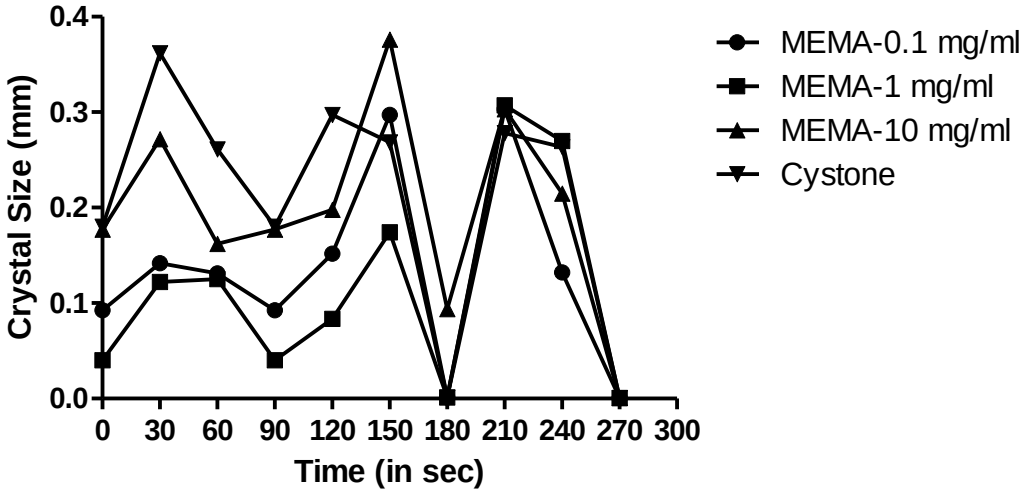

Figure 2: Effect of *Methanolic extract of Melia azedarach* Linn seeds (MEMA) on Crystal Nucleation Assay

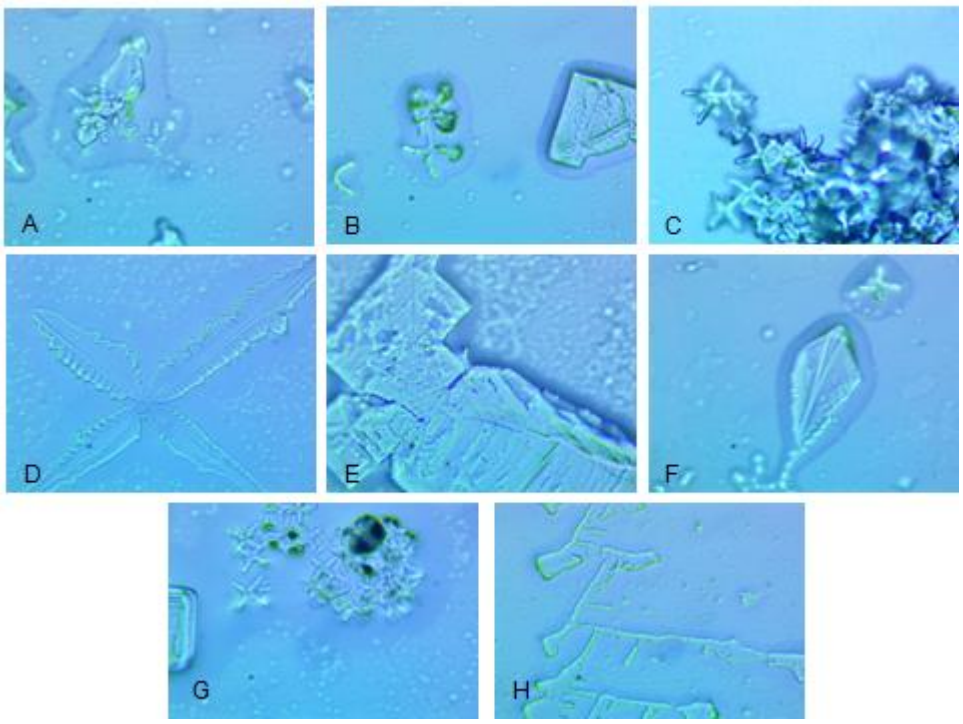

Figure 3: Effect of *Methanolic extract of Melia azedarach* Linn seeds (MEMA), *Aqueous extract of Melia azedarach* seeds (AEMA) and Cystone on Crystal Nucleation Assay (A: Blank, B: AEMA 0.1mg/ml. C: AEMA 1.0 mg/ml; D: AEMA 10 mg/ml; E: MEMA 0.1 mg/ml; F: MEMA 1.0 mg/ml; G: MEMA: 10 mg/ml AND H: Cystone 1 mg/ml)

4. CONCLUSIONS

The study examined the impact of *Methanolic extract of Melia azedarach* Linn seeds (MEMA) and *Aqueous extract of Melia azedarach* seeds (AEMA) on crystal nucleation assay at concentrations of 0.1 mg/ml, 1 mg/ml, and 10 mg/ml. MEMA exhibited a substantial enhancement in the rate of crystal nucleation when compared to AEMA. This indicates that MEMA might exert a more pronounced effect in facilitating the development of crystals in a solution. The cause of this phenomenon can be ascribed to the chemical makeup of MEMA, which may include substances that improve the formation of nuclei. The study also investigated the impact of MEMA and AEMA on the crystal aggregation assay. Sample SS exhibited a crystal aggregation of 56% at a concentration of 1000 µg/ml, whereas Sample AEMA demonstrated the highest crystal aggregation of 79% at the same concentration. Aggregation was shown to escalate in a manner that is dependent on the dosage. FT-IR spectroscopy was employed to ascertain the presence of functional groups and detect alterations in the molecular structure of CaOx crystals. The study also examined the impact of *Methanolic extract of Melia azedarach* Linn seeds (MEMA) and *Aqueous extract of Melia azedarach* seeds (AEMA) on the crystal aggregation assay. The results indicated that Sample SS exhibited the most crystal aggregation (56%) at a concentration of 1000 µg/ml, whereas Sample AEMA had the maximum aggregation (79%) at the same concentration. Furthermore, the level of aggregation was shown to rise proportionally with the dosage.

5. CONFLICT OF INTEREST

None

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