

Evaluation of Antiurolithiatic Activity of Seed Extract of Cuminum Cyminum

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Abstract

PUrolithiasis or kidney stone disease is a urologic ailment that has a high prevalence rate worldwide and medicinal plants have been widely used for alternative therapy. A wide range of medicinal plants have been used conservatively for urolithiasis due to their fewer side effects and because they contain copious phytochemicals that show advantageous effects in urolithiasis. Cuminum cyminum traditionally used in the treatment of urolithiasis. Hyperoxaluria and hypercalciuria are among the major risk factors in pathogenesis of urinary stone formation. Evaluation of various medicinal plants are done mainly against calcium oxalate. In this present study, the antiurolithiatic potential in Cuminum cyminum was investigated through invitro assay. The work was performed by using invitro Anti-urolithiatic model for calculating percentage in comparison with standard drug cystone. The anti-urolithiatic properties were evaluated by titrimetric and turbidity assay. The result shows that for C. cyminum folds (55%) and for standard drug cystone (82%) for titrimetric assay. For turbidity assay. C. cyminum has significant properties ($47.9 \pm 19\%$). The findings of the nucleation assay indicate that phytoconstituents inhibited the crystallization of CaOx in solution. The size and the number of calcium oxalate crystals decreased with increasing concentration upto $400 \mu\text{g}$ of the phytoconstituents. The study concludes that the seed extracts of Cuminum cyminum have inhibitory effect on calcium oxalate for crystal nucleation. It also showed great efficacy in the dissolution of calcium oxalate crystals. Thus, this extract may be valuable resources for treatment of urolithiasis.

Acute kidney injury (AKI) is a severe problem for healthcare professionals due to its high mortality rate. The major causes of AKI are ischemia, hypoxia, and drug-induced nephrotoxicity. AKI is particularly related to an imbalance between oxygen and nutrients, which is caused by impaired circulation to the nephrons and increased energy requirement due to its oxidative stress. In vitro nephroprotective studies of Cuminum cyminum reveals promising result.

Cisplatin is a highly effective chemotherapeutic agent; its clinical use is severely limited by serious side effects as nephrotoxicity. The purpose of this study is to evaluate the nephroprotective activity of ethanolic extract of Cuminum cyminum. The nephroprotective activity was dose dependent.

Keywords: Cuminum Cyminum, Calcium Oxalate, Nephrotoxicity, Cisplatin

Introduction

Cuminum cyminum (Cumin) commonly known as 'Jeera' or 'Zeera' is an important spice used in Indian kitchens for flavoring various food preparations. It is very pungent and aromatic and is used whole and/or ground. Though Cumin is a native of Egypt, it is mostly produced in India. India is the largest producer of cumin in the world. Apart from India it is also grown in North Africa, China, and America. Cumin's aromatic, nutty-flavored seeds come in three colors: amber (the most widely available), white and black (both found in Asian markets). White cumin seed is interchangeable with amber, but the black seed

has a more complex, peppery flavor. Cumin is one of the main ingredients in curry powders, and the combination of cumin and coriander leaves gives characteristics smell to most Indian food. India produces 70% of the world supply and consumes 90% of that. Other producers are Syria (7%), Iran (6%), and Turkey (6%). The remaining 11% comes from other countries. In total, around 300,000 tons of cumin per year is produced worldwide.

Production Constraints

The scientific name of Cuminum cyminum L. (cumin) referred to as Cuminum odorum Salisb, Cuminum cyminum J.F. Gmel,

Cuminum his panicum Bunge, *Lingusticum Cuminum* (L.) Crantz and belonging to the Apiaceae family. The Apiaceae family is a collection of typically aromatic plants having hollow stems and the well-known members of this family are anise, asafetida, caraway, carrot, celery, coriander, cumin, dill, fennel, parsley, parsnip, and sea holly.

The Available and Useful Parts of Cumin Plants Are:

Leaves: The leaves of cumin are multi-fid, with long filiform segments

- **Flowers:** The flowers, small, white, or pink are overtopped by the bracts, which, after flowering, are reflexes. The umbels, both partial and general, consist of about 5 rays, with the involucre consisting of 2 or 3 filiform, 1 sided bract.
- **Fruit:** The fruits of cumin are ovate or fusiform, of a light brown or grayish color. The fruit resembles caraway, but is larger and about 2 lines in length, much longer than the pedicels, nearly tapering, but little contracted at the sides, fusiform, crowned by the short teeth of the calyx, densely upon the ridges, which are paler, filiform, and a little raised; The odor and taste of cumin fruit is like caraway, but it's so warmer and so agreeable.
- **Seeds:** The cumin seed is yellow to brownish gray in color and is elongated in shape with nine protuberances that possesses numerous medicinal properties. The seeds of cumin are carminative, aromatic, stomachic, stimulant, astringent, and cooling and synergistic in effect. Cumin seed oil is used as multifunctional Luminescent paints or in tropical clothing ointment.

The essential oil of cumin seeds has shown a significant antibacterial activity against *K. pneumoniae* in vitro.



Figure: 1.

Stone formation in the kidney is one of the oldest and most widespread disease known to man. Urinary stone disease is a common disorder estimated to occur in approximately 12% of the world population, with occurrence rate of 70-81% in males and 47-60% in females.

Urolithiasis

'Urolithiasis' is a problem world over, affecting people from thousands of years. It is also called as 'Nephrolithiasis', kidney stones or renal calculi. It is a condition where in crystal formation occurs in the urinary tract eventually leading to stone structures. There are multiple factors contributing to the formation of these calculi/stone related to lifestyle or dietary habits of an

individual. Even congenital tendencies or geographical impact cannot be denied in certain cases. Calculi are made up of deposits of polycrystalline aggregates. These aggregates are made up of varying amounts of crystalloid and organic may be found in any part of the entire urinary tract, along with the surgical and other conventional treatment management, Ayurveda treatment option has been explored over the past few years. Numerous Ayurvedic medicinal herbs as single drugs or combined formulations have become exposed to potential research and studies. These medicines are being used for management of urinary disorders since thousands of years as the history of Ayurveda dates to. These drugs are known to have lithiolytic (disintegration of stones) and litho-preventive (non-formation of stones) properties. *T. procumbens* is a medicinal herb used since a very long time in Ayurveda and later in Unani, folklore, or tribal traditional medicinal practices. Nephrolithiasis is one of the most prevalent urologic diseases in Asia. The worldwide prevalence, incidence and composition of calculi varies and have changed in the last several decades, with prevalence ranging from 7% to 13% in North America, 5 to 9% in Europe, and 1% to 5% in Asia.

Kidney stone is an increasing urological disorder of human health, affecting about 12% of the world population at some stage in their lifetime. It affects all ages, sexes, and races it has been associated with an increased risk of end-stage of renal failure. Currently, there is no satisfactory drug to cure and / or prevent kidney stone recurrences. Medicinal plants are considered as a rich source of therapeutic agents due to the belief and observations regarding traditionally used medicinal plants for the preventions of various ailments. Although medicinal plants produce slow recovery, these are affordable and less expensive, evidence based traditionally proven dissolution or elimination of kidney stones, less relapse of urolithiasis, their successful prophylactic use, less side effects, not only revealing their therapeutic potential but encourages patient's belief and increasing their interest in traditional practices to find an herbal cure for kidney stones. A wide range of medicinal plants have been used in different countries and cultures as a prophylactic and curative agent for urolithiasis. Kidney stone disease is a multi-factorial disorder resulting from the combined influences of epidemiological, biochemical, and genetic risk factors. Urolithiasis is considered as the third most common affliction of the urinary tract. It is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidney. The formation of kidney stone involves several phytochemical events beginning with crystal nucleation, aggregation, and end with retention within the urinary tract. Stone disease is 2-3 times more common in males, than in females. Despite substantial progress in pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Kidney dialysis, endoscopic stone removal and extra corporeal shock wave lithotripsy are prohibitively costly, and reoccurrence is quite common with these procedures. (8). Urinary supersaturation is the driving force behind crystal formation in the kidneys. The inner diameter of the various segments of the renal tubules ranges from 15 to 60 μm . CaOx crystals, growing at the rate of 1-2 $\mu\text{m}/\text{min}$.

Crystal Nucleation

The initial step in the transformation from a liquid to a solid phase in a supersaturated solution is called nucleation. This process begins with the coalescence of stone salts in solution into

loose clusters that may increase in size by addition of new components or clusters. In vitro cell degradation following renal tubular cell injury produces numerous membrane vesicles, which have been shown to be good nucleators of calcium crystals.

Crystal Growth

Crystal growth is one of the prerequisites for particle formation and thus for stone formation.

crystal growth and aggregation have important functions in each step of stone formation.

Crystal Aggregation

The process whereby crystals in solution stick together to form larger particles is called aggregation.

Nephrotoxicity

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. Several therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis, and nephritic syndrome because there is an increasing number of potent therapeutic drugs like aminoglycoside antibiotics, NSAID's, chemotherapeutic agents have been added to the therapeutic arsenal in recent years. Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium, and arsenic also induces nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only necessary therapy. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood. The renal failure is fundamentally categorized into acute and chronic renal failure.

Literature Review

Cumin Seeds

cannot grow larger than a few microns and are therefore excreted with urine without causing stone development. In tubular fluid and urine, crystallization processes are largely dependent on solution composition. A variety of urinary constituents may affect solution supersaturation because of their activity as chelators. For instance, by forming soluble complexes with calcium and oxalate, respectively, citrate and magnesium reduce free ion activity and the relative supersaturation of calcium oxalate.

The Previous Studies on *C. cyminum*

According to literature, the quality and quantity of the compounds commonly identified in cumin vary in the various parts of the plant, such as the leaves, shoots, roots, and flowers. Though both the shoots and flowers have relatively similar terpene compounds, their concentrations are higher in the flowers. Furthermore, alpha-pinene and beta-pinene were not found in the roots, alpha-phellandrene was notably the only detected terpenoid compound in the leaves while the flowers had the highest concentration of alpha-pinene. Cumin fruits mainly contain cellulose, fixed oil content (about 10%), mineral elements, proteins, sugar, and volatile oils (1.5%), as well as appreciable amounts of phenolic compounds. Formulated *C. cyminum* essential oil-in-water nano emulsions have demonstration successful incorporation of lipophilic bioactive agents into functional food gels. Natural deep

eutectic solvents have been used to significantly enhance cumin essential oil extraction with a higher yield and premium quality, as an eco-friendly and economical extraction technique.

Ethnopharmacology of Cumin

The common ethnomedicinal uses of cumin are summarized. Traditionally, cumin is commonly used as a remedy against gastrointestinal, inflammatory, and neurological disorders, as well as toothaches. In Iranian traditional medicine, cumin fruits are also used as a medication for colic, diarrhea, dyspepsia, and flatulence, and for stimulation of breast milk production. It is used in Morocco for the flavoring of foods and soft dates. It is also commonly used in Tunisia as aromatic herbs and culinary spices, as well as in Italy for various gastrointestinal and neurological diseases.

prevention of various ailments. According to WHO 75% people rely on traditional medicines for the prevention and cure of different ailments. Although medicinal plants produce slow recovery, these are affordable and less expensive, evidence based traditionally proven dissolution or elimination of kidney stones, less relapse of urolithiasis, their successful prophylactic use, less side effects, not only revealing their therapeutic potential but encourages patient's belief and increasing their interest in traditional practices to find an herbal cure for kidney stones. The belief and observations regarding traditionally used medicinal plants, increasing the interest of people to use natural medicine for their primary health care needs. A wide range of medicinal plants have been used in different countries and cultures as a prophylactic and curative agent for urolithiasis.

Many medicines and remedies have been during the past many years to treat urinary stones. Generally, in the traditional system of medicine, most of the remedies are based on plants, and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies expect for some composite herbal drugs and plants.

Chemical Constituents

Cumin seeds are nutritionally rich: they provide high amounts of fat, proteins, and dietary fiber. Vitamins B and E and several dietary minerals, especially iron, are also considerable in cumin seeds. Cumin aldehyde, cymene, and terpenoids are the major volatile components of cumin. Cumin has a distinctive strong flavor. Its warm aroma is due to its essential oil content. Its main constituent of aroma compounds is cumin aldehyde and cuminal alcohol. Other important aroma compounds of roasted cumin are the substituted pyrazins, 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-sec-butylpyrazine, and 2-methoxy-3-methylpyrazine. Other components include gamma-terpinene, safranal, p-cymene, and beta-pinene.

Antidiabetic Effects

The antidiabetic effect of cumin seeds has been reported in human diabetics. In this study, 80 patients with non-insulin dependent diabetes mellitus were orally administered for 24 weeks with an Ayurvedic formulation containing *C. cyminum*. Fasting and post-prandial blood sugar at 6-week intervals was significantly reduced in all the patients. Methanolic extract of *C. cyminum* has been investigated in streptozotocin-diabetic rats on diabetes, oxidative stress, and formation of advanced glycosylated end products (AGE) in comparison with glibenclamide. In vi-

tro studies indicated that cumin inhibited free radicals and AGE formation.

Anti-inflammatory Effects

Cumin essential oil was investigated for the anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells and the underlying mechanisms. Volatile constituents were identified in essential oil using Gas Chromatography-Mass Spectrometry (GC-MS), the most abundant constituent being cumin aldehyde (48.8%).

Antimicrobial Activity

By using the microdilution method, ethanol extract of *Cuminum cyminum* seed were tested in vitro for antimicrobial activity. Biofilms were resistant to an ethanol extract of the seed. *E. coli*. Using agar diffusion and serial dilution techniques, all essential oils, and cuminic aldehyde were evaluated against Gram-positive and Gram-negative bacteria obtained from various dietary sources. (Pork fillet, minced meat, and sausages) and clinical isolates, as well as three different *Candida albicans* isolates.

Antioxidant Activity

The significant amount of antioxidant compounds with high antioxidant activity also showed by the oil of *C. cyminum* and good inhibition properties are also shown by its nonvolatile extracts against the free radicals. It is found that there is better antioxidant action in methanol extracts as compared to the n-hexane extracts. Antioxidant activities and the total phenolic content also found a good correlation among their nonvolatile extracts. So, it concludes that there is good antioxidant potential in *C. cyminum*.

Anti-fungal Activity

The essential oil extracted from *C. cyminum* express antifungal activity against the isolated fungus that also belongs to the occurrence of some compounds in its chemical composition such as Cumin aldehyde, Pinene, and P-cymene. Because these and other phenolic compounds of *C. cyminum* oil can inhibit some significant fungal enzymes like pectinase which is used to hydrolyze the fruit cell wall and invade the host cell by the fungi.

Hepatoprotective Activity

Cumin seed powder demonstrated strong hepatoprotective activity upon CCl₄ induced hepatic damage in rats.

Anti-helminthic Activity

It has been demonstrated that some synthetic phenolic anthelmintic and polyphenolic substances with anti-helminthic action prevent helminthic parasites from producing energy by decoupling oxidative phosphorylation.

Nephroprotective Activity

As drugs, natural products, industrial chemicals, environment pollutants that because nephrotoxicity has been increased. These nephrotoxics can produce a variety of clinical syndromes such as acute renal failure, chronic renal failure, nephritic syndrome, hypertension, and renal failure etc.

Materials and Methods

Sample Collection and Preparation of Seed Extract

The Cumin seeds were purchased from a local shop in Kalaburagi. The seeds of *Cuminum cyminum* were washed thoroughly

with distilled water and dried in open air at room temperature. The seeds were powdered mechanically. 25g of powder was macerated with 200ml of ethanol for 24 hours at room temperature. The extract was filtered through the Whatman filter paper, and the obtained filtrate was allowed to air dried for 5 days. The solid extracts were scraped before complete drying.

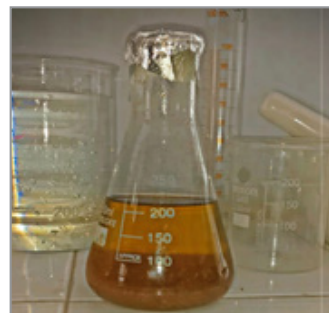


Figure 2: Cuminum cyminum extraction

Chemicals used

The chemicals and reagents used were Cystone tablet (The Himalaya, India), potassium citrate, calcium chloride dihydrate (CaCl₂), sodium oxalate (Na₂C₂O₄), Tris-buffer, ammonia solution, sulphuric acid (H₂SO₄), potassium permanganate (KMnO₄), hydrochloric acid (HCl), barium chloride (BaCl₂).

Invitro Calcium Oxalate Aggregation

Step 1: Preparation of Experimental Kidney Stones (Calcium Oxalate Stones) by Homogenous Precipitation 3.5gm of calcium chloride dihydrate was dissolved in 100ml distilled water and 3.10gm of sodium oxalate was dissolved in 100ml of 2N H₂SO₄. Both solutions were mixed equally in a beaker to precipitate out calcium oxalate with stirring. The resulting precipitate was calcium oxalate which was freed from traces of sulphuric acid by treating with ammonia solution. Finally, it was washed with distilled water and dried at a room temperature until all the water was evaporated.

Step 2: Preparation of the Semi Permeable Membrane from Eggs The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin and yolk. The preparation includes the following steps: Apex of eggs was punctured by a glass rod to squeezed out the entire content. Empty eggs were washed thoroughly with distilled water and placed in a beaker consisting of 2M HCl for an overnight, which caused complete decalcification. Then, the semi-permeable membranes were washed with distilled water, placed in ammonia solution for neutralization of acid traces in the moistened condition for a while and was then rinsed with distilled water. Finally, the egg membranes were stored in ammonia until used.



Figure 3 and Figure 4.

Anti-urolithiatic Assay

Titrimetric (Calcium Oxalate Dissolution) Assay

The dissolution percentage of calcium oxalate was evaluated by weighing exactly 10mg of experimental calcium oxalate and 10mg of the seed extract, packed it together in the semi-permeable membrane. The semipermeable membrane was previously weighed. This was allowed to suspend in a conical flask containing 100ml of 0.1 M Tris buffer as shown in Figure (3). The first group was served as blank containing only 10mg of calcium oxalate. The second group was served as a positive control containing 10mg of calcium oxalate and along with the 10mg of standard drug which are cystone. The third group along with 10mg of calcium oxalate were contained ethanol extract of cumin seed. The conical flasks of all groups were kept in an incubator for 7-8 hours. The contents of semi-permeable membranes from each group were removed into separate test tubes. Approximately 2 mL of 1N sulphuric acid was added to each test tube and titrated with 0.9494 N KMnO₄ till a light pink color endpoint was obtained. % Dissolved of calcium = $[(C-T)/C] \times 100$ Where, C= precipitate of CaOx remained in control (mg) and T= precipitate of CaOx remained when the test solution was used (mg).



Figure: 5.

Egg Membrane along with the Contents Suspended into the 0.1m Tris Buffer

Nucleation Assay

In vitro antiurolithiatic assay was conducted by the procedure as mentioned in literature. The assay was carried out in the absence (control) and presence of inhibitor (standard/extract/fraction). About 0.2mM and 0.12mM calcium chloride and sodium oxalate solutions were made in a buffer composed of 0.3mM Tris and 0.45mM sodium chloride at 6.5 pH. Stock solutions of stan-

dard (Cystone) and samples were prepared at a concentration of 10mg/ml. about 1ml of calcium chloride was added in both the control and sample sets. Additionally, 1 ml of distilled water was added in the control set. On the contrary, 1 ml of various dilutions of sample (200, 400, 600, 800, 1000µg/ml) were added in the sample set instead O.D distilled water. The onset of crystallization was achieved by the addition of 1 ml of sodium oxalate solution to all the sets. The tubes were incubated at 37°C for 30 min after which the absorbance was read at 620 nm using Perkin Elmer Lambda 25 UV/V is spectrophotometer.

MTT Assay Protocol

The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in parts by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

Result

Extraction Yield

Extraction process is the step prior to analyzing phytochemical compounds and anti-urolithiatic properties of samples. Hence, the effect of solvents and extraction method was studied in terms of extraction yield. Extraction with ethanol yields (55%). This result reported methanol to be the best extraction solvent for the Seed of Cuminum cyminum.

Dissolution of Calcium Oxalate

The anti-urolithiatic activity of the ethanol extract of Cuminum cyminum with the standard drug cystone. Seed extract shows a considerable amount of anti-urolithiatic activity as the standard cystone drug.

The dissolution percentage by the extract of Cuminum cyminum at 10 mg concentration was 55%. Dissolution percentage by the standard drug cystone at 10 mg was 82%. The research finding revealed that even though cystone polyherbal drug has high dissolution ability, ethanolic extracts of Cuminum cyminum seeds also have considerable anti-urolithiatic activity.

Table 1: Titration readings

Burette readings	Control	C. cyminum Sample	Cystone
Initial level	0	1.5	5.4
Final level	1.5	5.4	9.3

Table 2: Anti-Urolithiatic Activity of C.Cyminum

Anti-urolithiatic assay	Standard drugs (%)	Plant samples (%)
	Cystone	C. cyminum
Titrimetric assay	82%	55%
Turbidity	91.4%	41% ±19%

Depicts the effect of different concentration of the ethanol extract of Cuminum cyminum on the nucleation of calcium oxalate crystals. The percent inhibition regarding the control was found

to be higher in range of 41.5%. The percentage inhibition was constantly increased with increase in concentration of the seed extract up to 400µg.

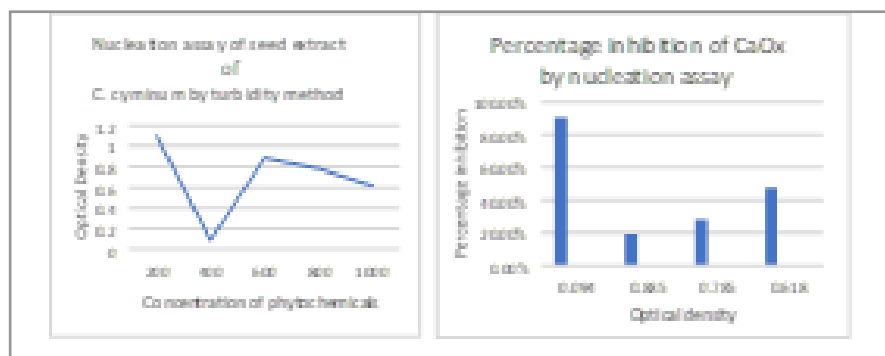


Figure 6: Effect of c. CYMINUM Ethanol Extract on Nucleation of CAOx

MTT Assay

The IC₅₀ values of the test compounds for HEK-293 Cell-line for 24-hour treatment were found to be:

Table 3: Table depicting IC₅₀ value.

C. cyminum	HEK-293 cell line IC ₅₀ (in µg/ml) 24hr
Sample	54.83µg/ml

Table 4: Depicting Percentage of Cell Viability after MTT Assay

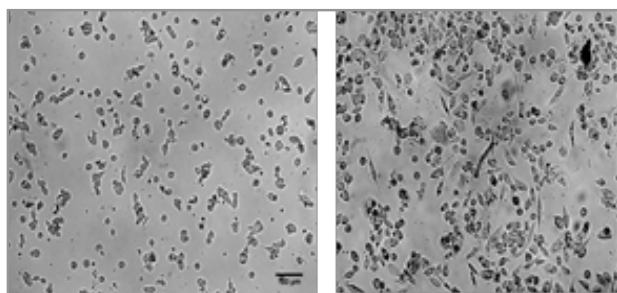
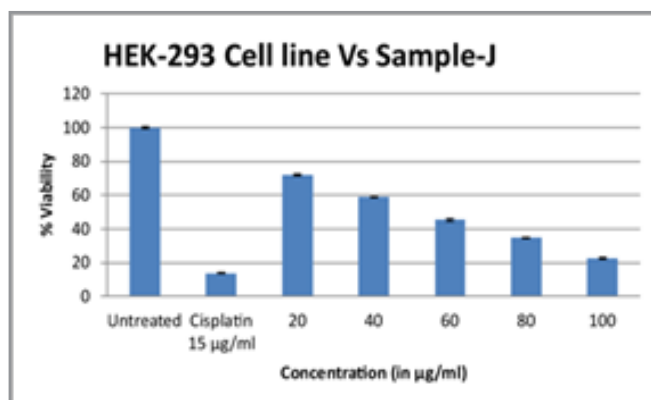


Figure-7: Sample with Cisplatin

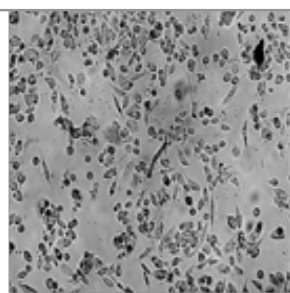


Figure 8: Untreated sample

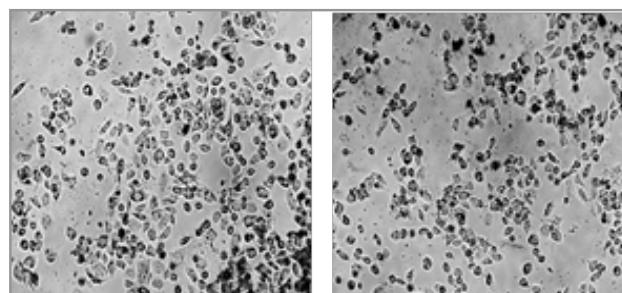
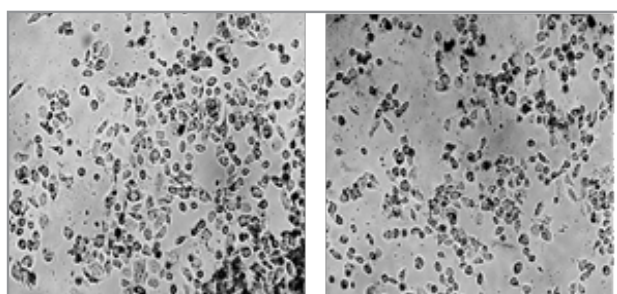
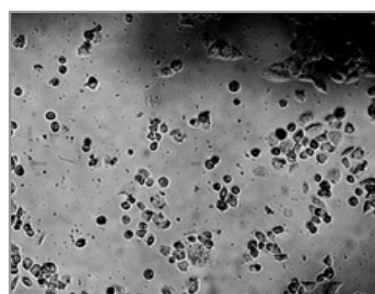


Figure 9: (a)-HEK 293 cell with 20µg sample (b) HEK 293 cell with 40µg sample



(c) HEK 293 cell with 60µg sample (d) HEK 293 cell with 80µg sample



(e) HEK cells with 100 µg sample

The Nephroprotective activity of Seed Extract was tested on HEK 293 cells. The result observed are depicted in the table.4. The nephroprotective activity was dose dependent. As the concentration of drug is increasing cell viability decreases which indicates that at lower concentration the current drug is showing nephroprotection [1-38].

Conclusion

The ethanolic extract of Seed extract of *Cuminum cyminum* was obtained by maceration method that has been established in folk remedies for urolithiasis. Invitro Anti- urolithiatic activity has been performed on the selected plant *Cuminum cyminum* seed extract by using the standard drug cystone by titrimetric method. The work was performed by using invitro Anti-urolithiatic model for calculating percentage dissolution of Calcium oxalate stone. The seed extract of *Cuminum cyminum* showed the higher dissolution than the standard drug cystone. The 10mg/ml seed extract of *Cuminum cyminum* was used for nucleation purposes. The percent inhibition regarding the standard drug cystone was found to be in higher range of 41.5%. the percentage inhibition was constantly increased with increase in concentration of the seed extract up to 400µg. This study has been primary evidence for *Cuminum cyminum* as the plant which possess Anti-urolithiatic property.

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