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Current Perspectives on Urolithiasis: Etiology, Clinical Presentation, Therapeutic Advances & Anti-Urolithiatic Evaluation Methods

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ABSTRACT
Urolithiasis is a prevalent urinary tract disorder marked by the formation of stones in the kidneys or other parts of the urinary system. Its incidence is rising worldwide due to lifestyle changes, dietary habits, climatic factors and metabolic abnormalities. Kidney stones are seen about twice as often in men compared to women. Stone formation is a multifactorial process involving urinary supersaturation, crystal nucleation, growth, aggregation and retention within renal tissues. This review provides a comprehensive overview of the etiology, epidemiology, types of stones and mechanism of stone formation, diagnosis and current alternative treatment approaches for Urolithiasis such as surgical interventions, pharmacotherapy and phytotherapy, *in-vivo* and *in-vitro* evaluation models of urolithiasis. Various dietary and lifestyle modifications have potential to prevent urolithiasis and recurrences. The diagnostic methods and preventive approaches along with complete removal of stones will improve the management of urolithiasis. *In vitr*o and *in vivo* evaluation methods for urolithiasis help researchers better understand stone formation and support the development of effective antiurolithiatic compounds.

INTRODUCTION

Urolithiasis is caused by uncontrolled pathological crystallization in the kidney, bladder or urethra. This condition occurs when the solvent becomes supersaturated and the individual's biological system fails to maintain calcium homeostasis, leading to the formation of precipitates known as kidney stones [1]. The main cause of stone formation is an imbalance between the solute and solvent in the urine.

Stones are made of crystals from the mineral's calcium oxalate, calcium phosphate, uric acid, magnesium ammonium phosphate, cysteine [2] and these crystals are held together by a matrix of organic compounds such as proteins, lipids and carbohydrates, which promote crystal nucleation, growth, and aggregation within the urinary tract. Factors such as dehydration, dietary habits, metabolic abnormalities, genetic predisposition, urinary tract infections and lifestyle changes contribute significantly to the development of Urolithiasis.

Etiology:

Approximately (80%) of urinary calculi are composed of calcium salts, mainly calcium oxalate and calcium phosphate. Other types of stones occur less frequently and include uric acid stones (9%), struvite stones (10%), and cysteine stones (1%). The development of these different stone types is influenced by several factors such as dietary habits, genetic predisposition, environmental exposure, use of certain medications and the individual's overall medical history [3].

Urolithiasis is a multifactorial disorder caused by an imbalance between stone-forming and stone-inhibiting factors in urine. The major etiological factors include:

1. Supersaturation of urine.
2. Metabolic factors like hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia, cystinuria.
3. Dietary factors - poor oral fluid intake, excess animal protein and salt consumption, high intake of oxalate rich foods (spinach, nuts, chocolates), low dietary calcium (paradoxically increases oxalate absorption).
4. Urinary tract infections - Certain bacteria (e.g.: *proteus*, *klebsiella*) produce urease, increasing urine pH and promoting struvite stone formation.
5. Urinary pH - Urine pH below 5.5 is usually low enough to encourage the formation and deposition of uric acid crystals. Uric acid stone formation is also linked to gout and some neoplastic disorders [4].
6. Medication-induced Urolithiasis accounts for 2% of all stones. Common medications include sulfadiazine and protease inhibitors like atazanavir and indinavir, which are used to treat HIV [5]. Patients receiving long-term therapy have been found to have an increased risk of stone formation when using ceftriaxone [6].
7. Environmental factors such as climate (hot and dry climates can lead to dehydration and concentrated urine), occupation (involving exposure to certain chemicals) and geographical location (areas with high incidence rates due to water composition)
8. Rarely, mutations in the SLC3AL and SLCA7A9 genes lead to the formation of cystine stones as a result of congenital disorder. These genetic defects impair the renal transport and reabsorption of cystine, causing cystinuria and subsequent stone formation. Cystine stones typically present during childhood or adolescence, although rare cases have been reported in

new born. In severe conditions, cystine stones may enlarge and develop into staghorn calculi [7].

Epidemiology:

The epidemiology of Urolithiasis varies widely across different geographical regions with respect to prevalence and incidence, age and sex distribution, stone composition and stone location. These regional variations are largely influenced by factors such as race, dietary habits and climate conditions. Furthermore, changing socio-economic status have significantly affected the prevalence and incidence of Urolithiasis, as well as its distribution according to age, sex, stone site and the physical and chemical composition of urinary caliculi [8]Around 11-16% of men and 7-8% of women will experience symptoms from Urolithiasis by age 70years old. Urolithiasis affects more than 70% of people between the ages of 20 and 50, and recurrence rate is close to 50% after 10 years. Men are twice as likely as women to develop stones, primarily as a result of environmental factors, food and other risk factors [9].

Approximately 12% of world's population is estimated to develop urinary stones and nearly half of these individuals may later experience impaired kidney functions [10]

Signs and symptoms:

Depending on whether a kidney stone is in bladder, ureter or kidney, different symptoms apply. A kidney stone usually causes symptoms until it moves around within the kidney or passes into one of the ureters. If a kidney stone gets stuck in the ureters, it may block the flow of urine and cause the kidney to swell and the ureter to spasm. Symptoms of Urolithiasis include:

- Severe flank pain
- Lower abdominal or groin pain
- Haematuria (presence of blood in urine)
- Dysuria (painful urination)
- Pyuria (presence of pus cells in the urine)
- Increased frequency and urgency of urination
- Nausea and vomiting
- Fever and chills if an infection is present
- Cloudy or foul-smelling urine [11][12].

Types of stones

Kidney stones are classified into different types based on their chemical composition. The nature of stone depends on urinary pH, metabolic abnormalities, dietary factors, infections and genetic predisposition. Understanding the different types of kidney stones is essential for diagnosis, effective treatment and prevention of recurrence. Types of kidney stones as follows:

1. Calcium stones
2. Uric acid stones
3. Straiten stones
4. Cystine stones
5. Drug-induced stones

Fig. 1: Types of stones

Calcium stones:

Calcium stones are the most common type and approximately 80% of kidney stones are calcareous stones [12]. The percentage of calcium stones can be attributed to pure calcium oxalate (CaOx) (50%), calcium phosphate (CaP, termed as apatite) (5%) and mixture of both (45%). Brushite, also known as hydroxyapatite or calcium hydrogen phosphate, is the primary component of calcium stones [13].

The pathophysiological mechanisms for calcium oxalate stone formation are complex and are caused by factors including hypercalciuria, hyperuricosuria, hyperoxaluria hypocitraturia, hypomagnesuria and hyper cystinuria. Mostly, urinary pH of 5.0 to 6.5 promotes calcium oxalate stones, whereas calcium phosphate stones usually develop when the urine becomes alkaline, especially at pH levels above 7.5. [12],[13]

Uric acid stones:

Uric acid stones accounts approximately for 5-10% of all stone types [15]. A diet rich in animal protein such as meat and fish, results in hyperuricosuria with low urinary volume and low urinary pH (pH<5.05) promotes uric acid stone formation, but solubility of stone increases at a pH >6.5. About 15-20% of individuals with uric acid stones have a history of gout and these stones are more common in men than in women. A diagnosis of uric acid calculi is radiolucent on plain radiographs but visible on ultrasonography or computerized tomography [14].

Struvite stones:

Struvite stones are composed of ammonium magnesium phosphate ($NH_4MgPO_4{\cdot}6H_2O$) having 'coffin lid' morphology by microscopy. About 10-15% 0f urinary calculi are struvite also known as "infectious stones", urease or triple-phosphate stones. The most common organisms responsible for stone formation are: *Proteus mirabilis*, *Proteus vulgaris*. Less common organism includes *Ureaplasma urealyticum*, and some species of *Providencia*, *Klebsiella*, *Serratia* and *Enterobacter*. These stones can grow rapidly and may form large staghorn calculi [15].

Cystine stones:

Cystine stones are rare and account for about 1-2% of kidney stones. These stones occur due to genetic disorder called cystinuria, characterized by impaired renal tubular reabsorption of amino acid called cystine, leading to its increased excretion in urine. Cystinuria is an autosomal recessive disorder caused by defect in the rBAT gene on chromosome. Cystine stones usually form in acidic urine and recurrent in nature [13].

Drug induced stones:

Drug induced stones are uncommon and accounts for about 1% of all kidney stones. Drugs such as guaifenesin, triamterene, atazanavir, sulpha drugs may precipitate under conditions of low urine volume or altered urinary pH, leading to stone formation. People taking protease inhibitor indinavir sulphate (a drug used to treat HIV infection), are at risk of developing stones. These stones are generally preventable with adequate hydration and dose adjustment [13].

Mechanism of Renal stone formation:

A biological process known as super saturation of urine and physiochemical changes lead to the formation of renal stones. Kidney stones develop when the amount of calcium oxalate (CaOx) in urine becomes much higher than its normal solubility. When CaOx levels raise 4 times the normal solubility and

nucleation starts when the concentration rises 7 to 11 times the normal level. Low urine volume, along with high calcium and oxalate, increases the supersaturation of CaOx. Citrate in urine normally forms a soluble complex with calcium, which helps prevent stone formation. When urinary citrate levels are low, CaOx stone formation is promoted. If the urine pH is greater than 6.5, the proportion of divalent and trivalent ions increases, favouring the formation of calcium phosphate (CaP) stones. Ultimately, the level of supersaturation of different solutes in urine determines the type of kidney stone formed [14].

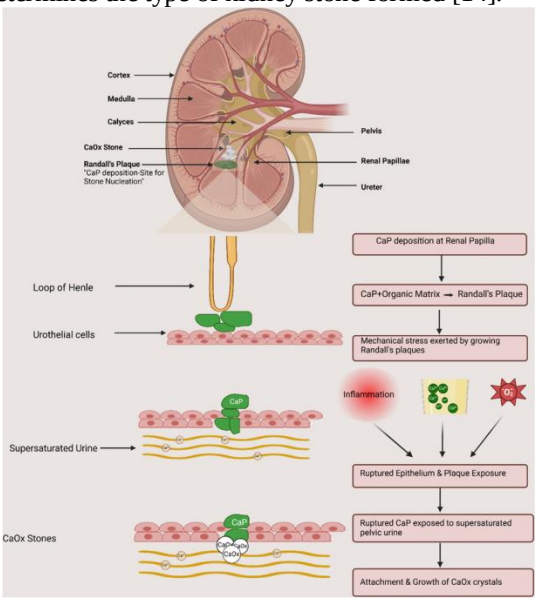

Fig 2: Mechanism of kidney stone formation

Diagnosis: The diagnostic methods include: [14], [16]

Ultrasonography

Ultrasonography (US) is widely available and safe as it involves no radiation exposure, reproducible and cost-effective, making it the preferred first-line imaging modality. It can detect calculi in the renal calices, pelvis, and at the pyeloureteric and vesicoureteric junctions. In addition, US provides information on upper urinary tract dilation, renal parenchymal thickness, echogenic patterns and abnormalities in kidney size, shape or position.

Non-contrast enhanced computed tomography of the urinary tract (NCCT-UT): NCCT-UT is the current standard for diagnosing acute urolithiasis. It is far more accurate than other imaging modalities and has largely replaced intravenous urography. NCCT-UT can accurately determine the size and density of urinary calculi and may also identify associated abnormalities or alternative causes of abdominal pain when stones are not present.

KUB Radiography

Kidney–ureter–bladder (KUB) radiography has a sensitivity of 44–77% and a specificity of 80–87% for detecting ureteric and renal stones, respectively. When stone density is accurately assessed using NCCT-UT, KUB radiography is generally not required. However, it remains useful for distinguishing radiopaque stones from radiolucent ones.

Metabolism-related-diagnosis

All emergency patients with calicular disease whether high- or low-risk should undergo a metabolic evaluation of urine and blood along with imaging studies.

Urine:

A dipstick test can be performed on a spot urine sample to check for red blood cells (RBCs), white blood cells (WBCs), nitrites, and to estimate urine pH. This can be followed by urine microscopy and/or culture for a more detailed analysis.

Blood:

A blood sample can be tested for creatinine, uric acid, ionized calcium, and C-reactive protein (CRP). Coagulation parameters such as INR (international normalized ratio) and PTT (partial thromboplastin time) may also be assessed. Testing for sodium, potassium, CRP, and coagulation times can be skipped if no intervention is planned. A stone-specific metabolic evaluation is recommended for patients at high risk of recurrence, as it helps identify potential metabolic disorders based on the mineral composition of the stones.

Analysis of calculus composition:

All patients who form a stone for the first time should have their calculus analysed. In clinical practice, repeat analysis is indicated in cases of recurrence despite medical prophylaxis, early recurrence after ESWL, endoscopic or surgical complete stone removal and late recurrence following a long stone-free period. The most commonly used techniques for stone analysis are infrared spectroscopy (IRS) and X-ray diffraction (XRD), while polarization microscopy is reserved for specialized centres. Traditional chemical (wet) analysis is no longer in use.

Differential Diagnosis:

The use of an objective clinical predication rule for kidney stones in the form of STONE criteria is advisable. Patients with higher STONE scores are more likely to develop kidney stones, and vice versa. The following are some essential differentials to be considered in patients: [17] appendicitis, constipation, lower urinary tract infection, pyelonephritis, renal abscess, renal artery aneurysm, diverticulitis, mesenteric ischemia, pancreatitis, cholecystitis, small bowel obstruction, ovarian torsion, dysmenorrhea, ectopic pregnancy, spontaneous abortion, pelvic inflammatory disease (PID)

Treatment:

The treatment for Urolithiasis is based on the patient's acute presentation and includes both conservative medical therapies and surgical interventions.

Approximately 86% of urinary stones pass spontaneously within 30-40 days. The time required for stone passage and chance of spontaneous expulsion are strongly influenced by stone size. < or equal to 2mm stones, 8 days for mean passage and passage rate of 87%. 3mm stones, 12 days for mean passage and passage rate of 76%. Between 4-6mm, 22 days for mean passage and passage rate of 60%. 7mm stone with a passage rate of 48%. 8-9mm stone with a passage rate of 25% [17]

Surgical procedures

According to estimates, 25% of individuals having stones are surgically removed. The surgical procedures vary according to stone characteristics and patient-specific factors and include multiple modalities with differing indications, success rates and risk of complications. The common surgical procedures are as follows:

Extra-corporeal shock wave lithotripsy (ESWL)

Shock wave lithotripsy (SWL) remains one of the most commonly used treatments for kidney stones worldwide. It is effective for managing small to medium sized stones, but larger, more complex stones are located in the kidney's bottom pole are

not well managed with this technique.Potential contraindications for ESWL include pregnancy, uncorrected bleeding disorders, aortic aneurysms, severe skeletal deformities and morbid obesity. In such cases, technical limitations may impair shock wave penetration and accurate stone localization, making alternative modalities such as ureteroscopy (URS) or percutaneous nephrolithotomy [18-19].

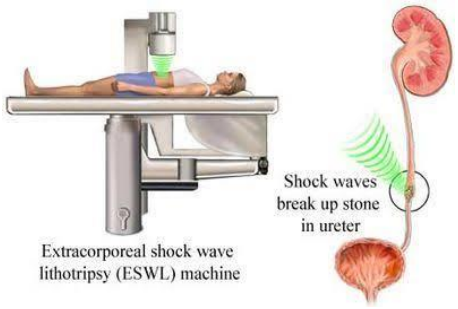

Fig 3: Extra-corporeal shock wave lithotripsy (ESWL)

Ureteroscopy (URS)

Although SWL can be used to manage small kidney stones or stones in the proximal ureter, most stones located in the intermediate and distal ureter are still primarily treated using ureteroscopy. Holmium or thulium laser lithotripsy is the preferred technique during URS for fragmenting kidney or ureteral stones. Thulium laser lithotripsy offers faster ablation rates and reduced retropulsion, making it particularly advantageous in complex cases. In cases involving large or complex ureteral or renal calculi, a second or third URS treatment also known as staged URS may be required for complete treatment.

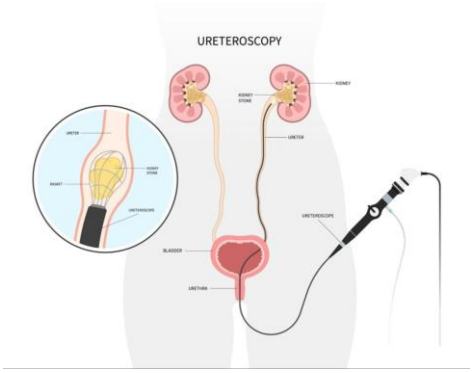

Fig 4: Ureteroscopy (URS)

Percutaneous nephrolithotomy (PNL)

For most kidney stones, percutaneous nephrolithotomy (PNL) is generally considered more effective than SWL or URS. Percutaneous nephrolithotomy is typically reserved for large or complex stones or used as a secondary option when SWL or URS fails. Patients with staghorn calculi or stones larger than 20mm are usually advised to consider PNL. [18] PNL is associated with certain risks, with bleeding and infection being the most common complications and in rare cases injury to adjacent organs. The procedure is contraindicated in patients with active urinary tract infections, severe cardiopulmonary comorbidities, or uncorrected coagulation disorders. Recent advancements, including suction-assisted devices and laser-based technologies, have improved clinical outcomes while reducing complication rates. Consequently, percutaneous nephrolithotomy remains a dependable treatment option for patients with large or complex stone burdens [19].

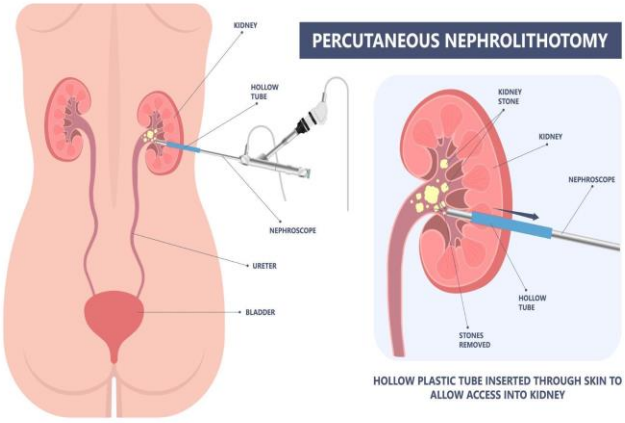

Fig 5: Percutaneous nephrolithotomy (PNL)

Pharmacotherapy

Agents that promote spontaneous stone passage while reducing associated symptoms are needed. Various drugs including hormones, corticosteroids, nonsteroidal anti-inflammatory drugs, calcium-channel blockers and beta-adrenergic blockers have been evaluated for this purpose. Among these 'calcium-channel blockers' have emerged as the most promising pharmacological option.

Table 1: Drugs used in Pharmacotherapy [18], [19]

s.no.	Drug (class)	Mechanism of action	Dose	Side effects
1.	Hydrochlorothiazide (thiazide)	Increases calcium reabsorption in tubules	12.5-50 mg/day	Hypokalaemia, hyponatraemia, hyperglycaemia, hypocitraturia, headache, dizziness, nausea, muscle weakness, confusion, fatigue, constipation, gastrointestinal upset
2.	Chlorthalidone (thiazide like diuretic)	Increases calcium reabsorption in tubules	25-50 mg/day	Hypokalaemia, hyponatraemia, hyperglycaemia, hypocitraturia, dizziness, fatigue, orthostatic hypotension, gastrointestinal upset
3.	Indapamide (thiazide like diuretic)	Reduces urinary calcium excretion and promotes calcium reabsorption	1.25-2.5 mg/day	Hypokalaemia, hyponatraemia, hyperglycaemia, hypocitraturia, dizziness, headache, photosensitivity, orthostatic hypotension

4.	Tiopronin	Binds to cystine to enhance its solubility urine	Adults: 600-900 mg/day; Children: 15mg/kg/day	Nausea, vomiting, diarrhoea, proteinuria, rash, oral ulcer, drug induced lupus
5.	Potassium citrate	Alkalinises urine and dissolves stones	10-20 g/day Or 30-60 mEq/dose	Epigastric pain, heart burn, nausea, vomiting, diarrhoea
6.	Allopurinol (xanthine oxidase inhibitor)	Reduces uric acid levels and prevents hyperuricosuria; effective only in uric acid stones associated with hyperuricosuria	100-300 mg/day	Skin rash, nausea, diarrhoea, muscle pain, hypersensitivity, alopecia, hepatotoxicity, nephritis
7.	Febuxostat (xanthine oxidase inhibitor)	Reduces uric acid levels and prevents hyperuricosuria; effective only in uric acid stones associated with hyperuricosuria	80 mg/day	Diarrhoea, headaches, skin rashes, nausea, abnormal liver test results, fluid retention (usually in lower legs and ankles)
8.	Calcium supplements	Binds to oxalate in the gut to reduce absorption	Not available	Not available
9.	Captopril (angiotensin-converting enzyme inhibitor)	Forms complexes with cystine to reduce levels	50-150 mg/day	Rash, hypotension
10.	Pyridoxine (vitamin B6)	Reduces oxalate synthesis	5-10 mg/kg/day	Diarrhoea
11.	Lumasiran (small interfering RNA)	Inhibits hepatic oxalate production	3 mg/kg monthly (loading), then every 3 months	Injection site reaction, headache, rhinitis, upper respiratory infection
12.	Tamsulosin (alpha blocker)	Facilitates stone passage (medical expulsive therapy)	0.4 mg/day	Orthostatic hypotension, priapism, iris syndrome
13.	Bisphosphonates	Reduces hypercalciuria by decreasing bone decalcification	Not available	Hypocalcaemia, oesophageal ulcers, joint pain, difficult dental implant
14.	D-Penicillamine	Chelates cystine to enhance its solubility in urine.	500-1500 mg/day	Pancytopenia, proteinuria, nausea, impaired taste, rash, hepatotoxicity

Phytotherapy

Herbs and herbal medicines have gained popular attention because of their proven scientific benefits. Nearly 75% of the world's population, particularly in developing countries, depends on herbal remedies for their primary healthcare needs. About 25% of modern pharmacopeial drugs are directly derived from plant sources, while another 25% consist of chemically modified compounds originally discovered in natural products. When it comes to Urolithiasis, several plant species are documented in the pharmacopoeias of many countries as effective therapeutic agents. These herbs exhibit diverse pharmacological properties including diuretic, antilithic, alkalizing, antispasmodic and anti-inflammatory effects, which helps in preventing stone formation, promote stone dissolution, relieve associated symptoms and support overall renal health.

Table 2: Herbal drugs used in Phytotherapy [18], [20], [21]

s.no.	Plant name (family)	Common name	Parts used	Active constituents	plants' mechanism of action
1.	*Achyranthes aspera L.* (Amaranthaceae)	Chaff flower, devil's horsewhip	leaves	Proteins, phenolic substances, alkaloids, flavonoids, glycosides, saponins and tannins. Tetracontanol-2 (C33H760) and 4-methoxyheptatriacont-1-en-10(C4H82O)	Identifying the causes of inflammation and removing reactive oxygen species that damage renal endothelial cells and promote stone formation.
2.	*Aerva lanata* (Amaranthaceae)	Mountain knotgrass	Whole plant	Alkaloids, flavonoids, lupeol	Inhibiting NF-kb, increasing Nrf2 and removing reactive oxygen species to decrease inflammation.

3.	*Alcea rosea L* (Amaranthaceae)	Hollyhock	roots	Flavonoids	Prevents reactive oxygen species from damaging the renal epithelial cells, leads to halting the formation of calcium oxalate stones.
4.	*Asparagus offincinalis* (Liliaceae)	Garden Asparagus	Root	Sterols, aspargamine A, polysaccharide and flavonoids	Lowering reactive stress and avoiding crystallization of the stone.
5.	*Azadirachta indica A. Juss.* (Meliaceae)	Neem	leaves	Zadirachtin, salannin, quercetin, nimbolinin, nimbin, nimbidin and nimbidol. (quercetin and –sitosterol) flavonoids	Prevents renal endothelium injury by suppressing p53, pten, NF-B, P13K/Akt, Bcl-2 and VEGF as well as renal endothelial cell death.
6.	*Basella alba L* (Saxifragaceae)	Malabar spinach, Indian spinach	Whole plant	Bergenin, tannic acid, gallic acid and coumarin	Lowering oxidative stress and avoiding renal stone formation.
7.	*Bergenia ligulata* (Saxifragaceae)	Pashanbheda, rockfoil, stone-breaker	Rhizome	Coumarin, bergenin, tannic acid and gallic acid	Lowering oxidative stress and avoiding renal stone formation.
8.	*Berberis vulgaris L.* (Berberidaceae)	Barberry	Roots	Flavonoids (quercetin, chrysanthamine, hyperoside, dolphinidin-3-O-beta-Dglucoside, pelargonin, petunidin3-O-beta-D-glucoside, alpha tocopherol and beta-caroten). Phenolic compounds (including anthocyanin and carotenoid pigments)	Reduced ROS and suppression of the pro-stone-forming inflammatory cytokines (IL-1, TNF and IFN-) that harm renal endothelial cells.
9.	*Boerhaavia diffusa Linn.* (Nyctaginaceace)	Purnava	Root	Rotenoids, flavonoids, derivatives of flavonoids, xanthones, purine nucleoside, ligans, ecdysteroids and steroids	Antioxidant activity of BDE greatly mitigates renal cell damage and hyperoxaluric oxidative stress in Urolithiasis.
10.	*Bryophyllum pinnatum Lam* (Crassulaceae)	Life plant, Miracle leaf	Leaves	Alkaloids (such as berberine), alkanes, triterpines, sterols, flavonoids, bufadienolides, glycosides and lipids	Stopping stone from crystallising by inhibiting oxidative stress.
11.	*Cassia fistula L* (fabaceae)	Golden shower tree, Indian laburnum	Stem bark	Triterpines, lupeol, polyphenols and sterols (-sisosterol, stigmasterol)	Lowering oxidative stress and avoiding renal stone formation.
12.	*Daucus carota L.* (Apiaceae)	Wild carrot	Seed	Carotenoids, polyacetylenes and phenols	Reduces oxidative stress induced during the crystallization phase, thereby protecting renal endothelial cells and promotes stone formation.
13.	*Pedalium murex* (Zygophyllaceae)	Large caltrops, Bara Gokhru, Crow thorn	Whole plant	Flavonoids, alkaloids, steroids, resins, saponins, protein, dinatoin glycosides. Herman, phytosterols, glucoside, rubusic acid.	Decreased production and agglomeration of stones.
14.	*Herniaria hirsute L.* (Caryophyllaceae)	Hairy rupturewort	Whole plant	Triterpene saponins, including phenolic acids, tannins, gypsogen, medicagen and 16-hydroxy-medicagen aglycones as well as flavonoids and hydroxycoumarins.	Limiting reactive stress and preventing renal stone formation.

Methods for evaluation of Anti-Urolithiasis Activity:

Different screening models for Urolithiasis and potential antiurolithiatic agents are broadly classified into *in vivo* and *in vitro* evaluation methods [22], [23]. This study assists researchers in selecting appropriate experimental models for the identification and development of effective antiurolithiatic compounds.

***In-vivo* animal models**

1. **Ethylene glycol induced Urolithiasis in rats**

   The toxicity of ethylene glycol is primarily attributed to its metabolite, oxalic acid, which crystallizes as calcium oxalate in body tissues. When ethylene glycol is administered in drinking water at 2.5% to 10% concentrations, it induces oxalate stone formation in

the urinary tract of animals. Stone formation mainly results from hyperoxaluria, leading to increased renal retention and excretion of oxalate. Alterations in urinary oxalate levels play a more critical role in stone formation than changes in calcium levels. However, elevated urinary calcium favours the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine, followed by crystal growth. Increased urinary phosphate excretion, together with oxalate stress, creates a favourable environment for stone formation by promoting the formation of calcium phosphate crystals, which can epitaxially induce calcium oxalate deposition. Urolithiasis reduces glomerular filtration rate due to urinary outflow obstruction by stones. This results in accumulation of nitrogenous wastes such as urea, creatinine and uric acid in the blood. Magnesium is a well-known inhibitor of urinary crystallization, as evidenced by the low urinary magnesium levels observed in stone formers. It has also been reported that magnesium reduces the growth rate of calcium oxalate crystals. [23]

2. **Ethylene glycol and ammonium chloride induced Urolithiasis in rats**

   The lithogenic effect induced by 0.75% ethylene glycol (EG) is primarily attributed to oxidative damage caused by the excessive oxalate produced during its metabolism. Although the ethylene glycol induced rat model has limitations as a general model for renal stone formation, it remains a valuable experimental model for evaluating renal papillary stone development, especially in cases where stone formation is closely linked to oxidative cellular damage. The addition of 2% ammonium chloride (w/v) is used to enhance hyperoxaluria and promote calcium oxalate deposition in the kidneys. [24]

3. **Sodium oxalate (NaOx) induced Urolithiasis in rats**

   Oxalocalcic lithiasis was induced by intraperitoneal administration of sodium oxalate (NaOx). The observed loss in body weight is attributed to anorexia resulting from disturbances in carbohydrate, protein and fat metabolism caused by sodium oxalate injection. The damaging effects are mainly due to the formation of calcium oxalate (CaOx) salts, which are insoluble at physiological pH and lead to CaOx nephrolithiasis. The severity of tissue necrosis, as well as the size, number and distribution of CaOx crystals within the inner medulla, was found to depend on the time interval following NaOx administration. [23]

4. **Caliculi-Producing diet induced Urolithiasis**

   Administration of glycolic acid increases the activity of oxalate-producing liver enzymes, particularly glycolate oxidase. Calcium oxalate (CaOx) stone formation was induced by feeding a normal diet supplemented with 3% glycolic acid. Continuous intake of a commercial diet containing 3% glycolic acid for 40 days led to marked deposition of calcium and oxalate in renal tissues. This excessive accumulation in the kidneys is known to promote papillary calcification and eventually result in kidney stone formation. Hyperoxaluria and CaOx deposition were induced using gentamycin (40mg/kg/day, subcutaneously) in combination with a calculi-producing diet. This diet was prepared by mixing powdered standard rat feed with 5% ammonium oxalate, reforming it into pellets and drying them before administration.

5. **Glyoxylate induced acute lithiasis**

   Acute glyoxylate intoxication-induced glyoxylic lithiasis significantly increases the deposition of stone-forming constituents such as calcium, oxalate and phosphorus in renal tissue. A four-fold elevation in urinary oxalate levels has been reported under these conditions. Glycolate and glyoxylate act as effective precursors of oxalate in isolated rat hepatocytes. However, glyoxylate is the only compound proven to be an immediate precursor of oxalate in humans.

   The glyoxylate model offers two major advantages. First, the amount of toxic substance absorbed can be accurately controlled. Second, since glyoxylate is an immediate precursor of oxalic acid, it allows a more reliable evaluation of true antilithiatic agents. In this model, oxalate stone formation is induced within 24 hours by administering sodium glyoxylate at a dose of 120mg/kg intraperitoneally, given in two divided doses in the morning and evening. [23], [25]

6. **Zinc disc induced Urolithiasis in rats**

   Rats were anesthetized with sodium pentobarbitone (40mg/kg, intraperitoneally) and the urinary bladder was exposed through a suprapubic incision. A small incision was made at the bladder apex and urine was aseptically aspirated for bacteriological analysis and pH determination using narrow-range BDH pH paper. Pre-weighed sterile zinc discs were then inserted into the bladder. The incision was closed with a single suture using absorbable 4-0 chromic catgut (Ethicon) and the animals were allowed to recover for one week. Implantation of zinc foreign bodies into the urinary bladder induce urinary stone formation and hypertrophy of the bladder smooth musculature, which were more pronounced in male rats than in females at 4 and 8 weeks post-surgery, respectively. [23], [26]

***In-vitro* studies on Urolithiasis**

1. **Determination of effect on calcium oxalate (CaC_2O_4) crystallization**

   Calcium oxalate (CaC_2O_4) crystallization was assessed by monitoring time-dependent changes in turbidity resulting from crystal nucleation and aggregation under metastable concentrations of calcium and oxalate ions. Stock solutions of $CaCl_2$ (8.5 mM) and $Na_2C_2O_4$ (1.5mM), containing 200 mM NaCl and 10 mM sodium acetate, were adjusted to pH 5.7 [23],[27]. Crystallization was monitored at 620nm using a platelet aggregometer. $CaCl_2$ solution (0.5ml) was continuously stirred at 37°C in the absence or presence of various concentrations of the test material or potassium citrate. After a stable baseline was obtained, $Na_2C_2O_4$ (0.5ml) was added to achieve final concentrations of 4.25 mM Ca^{2+} and 0.75 mM oxalate. Turbidity changes were recorded for 15 minutes at a chart speed of 30 mm/h. All experiments were performed in triplicate and slopes of nucleation (S_N)

and aggregation (S_A) phases were calculated by linear regression. Percentage inhibition was determined using $[(1 - S_m/S_c) \times 100]$.

For morphological evaluation, $CaCl_2$ solutions containing test material or potassium citrate were incubated with $Na_2C_2O_4$ under identical conditions in 24-well plates at 37°C for 45 minutes with shaking. Crystals were examined under an inverted microscope, and crystal number and morphology were assessed in five randomly selected fields (200×). [23]

2. **Nucleation Assay**

Calcium chloride (3 mM) and sodium oxalate (0.5 mM) solutions were prepared in Tris buffer (0.05 M) containing NaCl (0.15 M) at pH 6.5 and filtered through a 0.22 µm membrane. Calcium chloride solution (33 ml) was mixed with 3.3 ml of the test sample at different concentrations. Crystallization was initiated by adding 33 ml of sodium oxalate solution. The reaction mixture was magnetically stirred at 800 rpm using a PTFE-coated stir bar and maintained at 37 °C. Turbidity was measured at 620 nm at 1-min intervals. Percentage inhibition was calculated as [1 − (Tsi/Tsc)] × 100, where Tsc represents the turbidity slope of the control.

3. **Growth Assay**

Inhibitory activity against calcium oxalate (CaOx) crystal growth was evaluated using a seeded solution-depletion assay. A test solution of 10 mM Tris-HCl containing 90 mM NaCl was adjusted to pH 7.2 with 4 N HCl. Calcium oxalate monohydrate crystal seeds were prepared as a stone slurry (1.5 mg/mL) in 50 mM sodium acetate buffer (pH 5.7). The crystal seeds were added to a reaction mixture containing 1 mM $CaCl_2$ and 1 mM sodium oxalate ($Na_2C_2O_4$). Interaction of calcium and oxalate ions with the crystal seeds resulted in CaOx deposition on the crystal surface, leading to a decrease in free oxalate concentration, which was measured spectrophotometrically at λ 214 nm. In the presence of the test sample, inhibition of CaOx crystal growth reduced the depletion of free oxalate ions. The rate of oxalate reduction was calculated from baseline values and readings obtained after 30 seconds of incubation, with and without the test sample. Percentage relative inhibitory activity was calculated using the formula:

% Relative inhibitory activity = $[(C - S)/C] \times 100$,

Where C represents the rate of oxalate reduction in the absence of the test sample and S represents the rate in its presence. [23]

4. **Calcium phosphate Assay**

The calcium phosphate (CaP) assay was performed in vitro to evaluate mineralization, crystal growth, and demineralization using a 5ml homogeneous system. The system consisted of 0.5ml KH_2PO_4 (50mM), 0.5ml $CaCl_2$ (50mM) and 2.5ml Tris buffer (210 mM NaCl + 0.1 mM Tris-HCl), with test solution volumes ranging from 0.2–1.5 ml adjusted by distilled water. After centrifugation at 4500 rpm, precipitates were dissolved in 0.1 N HCl for mineralization analysis. For crystal growth, standard CaP systems were re-grown with increasing volumes of the test solution. Calcium and phosphate contents were estimated from the dissolved precipitates. Control systems contained no test solution. For demineralization, CaP precipitates were initially formed without the test and then treated with Tris buffer and varying test volumes. Following centrifugation, calcium and phosphate were estimated in the supernatant.

Percentage inhibition was calculated as % inhibition $[(C - T)/C] \times 100$, where C is control and T is test.

5. **Calcium oxalate crystal Assay**

The inhibitory effect of the test sample on calcium oxalate crystal growth was evaluated using a 4 ml reaction system. The system contained 1 ml each of 4 mM calcium chloride and 4 mM sodium oxalate, added to 1.5 ml of NaCl (90 mM) solution buffered with Tris-HCl (10 mM, pH 7.2). Crystal growth was initiated by adding 30 µl of calcium oxalate monohydrate (COM) crystal slurry (1.5 mg/ml in acetate buffer). Oxalate consumption began immediately and was monitored for 600 seconds by measuring the decrease in absorbance at 214 nm. In the presence of the test sample, inhibition of crystal growth was indicated by reduced depletion of free oxalate ions. The rate of oxalate reduction was calculated from baseline values and values obtained after 30 seconds of incubation, with or without the test sample. The percentage inhibitory activity was calculated using the formula: $((C - S) / C) \times 100$, where C represents the rate of oxalate reduction in the control and S represents the rate in the presence of the test sample. [28]

6. **Lactate dehydrogenase leakage**

NADH (6.6mM) and sodium pyruvate (30mM) were prepared in Tris buffer (0.2M, pH 7.3). The reaction was initiated by adding 50µL of the test sample and the decrease in NADH absorbance was monitored at 340nm for 5 minutes at 1-minute intervals. The percentage of LDH release was calculated by dividing the LDH activity in the supernatant by the total LDH activity measured after complete cell lysis induced by sonication. [23]

DISCUSSION

Urolithiasis remains a significant global health concern due to its increasing prevalence and high recurrence rate. Urolithiasis is the formation of calculi/stones in urinary tract. It is also known as nephrolithiasis, if the stones are formed within the kidney. The main cause of stone formation is supersaturation of urine when the individual's biological system fails to maintain calcium homeostasis. Factors including urine pH, urinary tract infections, medications, dietary factors (poor oral fluid intake, excess intake of animal protein, oxalate rich foods) contribute to stone formation. Stone formation is a complex process driven by the interaction of both physiochemical and biological factors. It begins with urinary supersaturation followed by crystal nucleation, growth, aggregation and crystal retention within the renal tubules, where oxidative stress and inflammation play a crucial role in this process, as reactive oxygen species damage renal epithelial cells and facilitates crystal attachment. Proper

diagnosis is essential to determine the type, size and location of stone. In this review, the treatment of Urolithiasis consisting surgical interventions, pharmacotherapy and phytotherapy were discussed. According to the individual's stage of condition, type of treatment is recommended either with drugs or surgical interventions. The primary strategy recommended for all patients with Urolithiasis is to increase adequate physical activity and fluid intake to minimize the risk of recurrence. Among the available treatment options, including surgical interventions, pharmacotherapy and phytotherapy, patients increasingly prefer herbal drugs over synthetic drugs. However, severe cases are managed with synthetic drugs and surgical interventions. Both *in-vitro* and *in-vivo* models are essential for understanding the pathogenesis of urolithiasis and for evaluating potential antiurolithiatic agents. These models replicate key stages of stone formation, including crystal nucleation, growth, aggregation and retention where *in vivo* models closely resemble the way urolithiasis develops in the human body.

CONCLUSION

Urolithiasis is a common and recurring condition caused by multiple factors, making its management challenging. Although modern diagnostic tools and treatment options have improved patient care, stone recurrence is still frequent. Studying urolithiasis using *in-vitro* and *in-vivo* models is crucial for developing strategies to control the disease and prevent its recurrence over the long term. Urolithiasis treatment includes medications to dissolve or prevent stones, plant-based remedies to support kidney health and surgical or minimally invasive procedures for larger or obstructive stones. Combining these approaches with lifestyle and dietary measures helps manage stones effectively and lowers the chance of them coming back.

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