

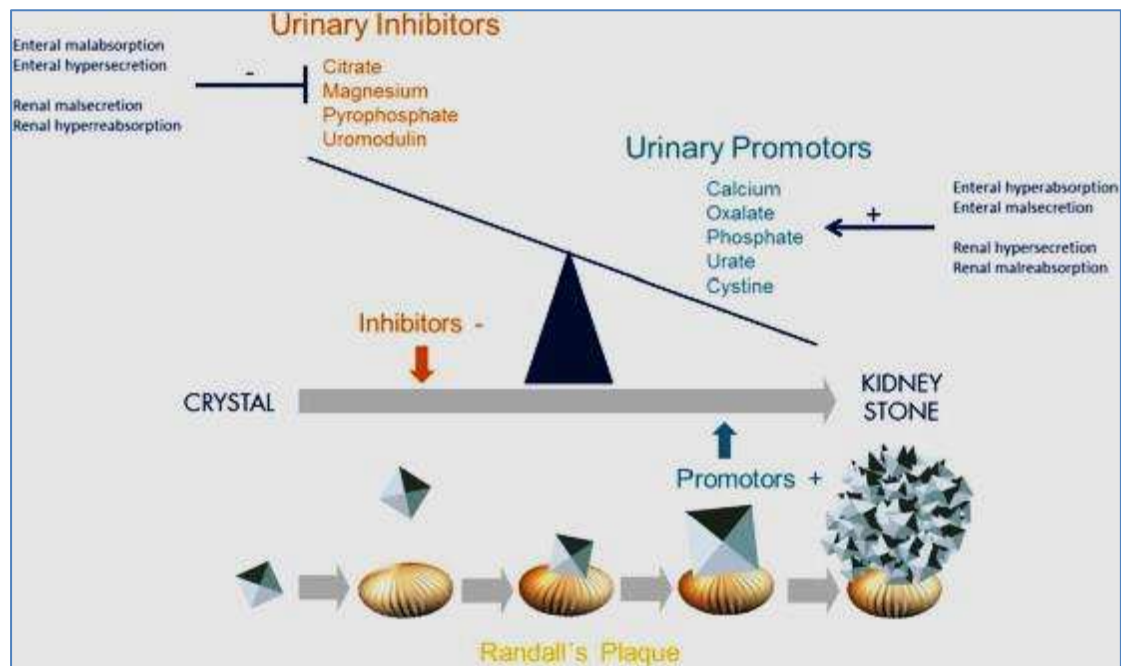
Kidney stones: Mechanism of formation, pathogenesis & possible treatments

Abstract

- With a very high recurrence rate in both males and females, kidney stones also called as **urolithiasis (nephrolithiasis)** are affecting a remarkably significant population around the globe.
- As kidney stones formation largely depends on **urine's oxalate content** rather than the **calcium concentration of the urine**.
- **Kidney stones** are predominantly made up of oxalate and also there are multiple steps involved in the pathogenesis of calcium oxalate including
 - Nucleation,
 - Crystal growth,
 - Crystal aggregation and
 - Crystal retention.
- Although there are several therapies such as
 - Thiazide like diuretic,
 - Allopurinol,
 - Painkillers, and
 - Dietary modifications or
- **In severe conditions**
 - Shock-wave treatment,
 - Ureter-endoscopic and
 - Percutaneous nephrolithotomy
- Are being presently used for kidney stones treatment, however, the side effects that these treatments possess and reoccurrence of the disease have motivated researchers to search for **better and safer options**.
- **Herbal drugs** as well as several medicinal plants display antiurolithiatic activity and thus perform an important role in treatment of kidney stone disease.
- Studies in both humans and animal models of hyperoxaluria-associated calcium oxalate kidney stone disease indicated that **oxalate-degrading microorganisms**, in particular the more widely-studied *Oxalobacter formigenes* are both capable of and important in regulating urinary levels of oxalate.
- Such regulation of oxalate levels in the body have displayed prominent reductions in hyperoxaluria followed by diminished rate of recurrent stone formation.
- The present work discusses the mechanism of stone formation, several forms of kidney stones as well as the various therapeutic measures for treatment of urinary stones including many medicinal plants as herbal option as well as oxalate degrading enzyme.

Keywords

- Urolithiasis;
- Hypercalciurea;
- Hyperoxaluria;
- Nephrolithiasis;
- Calcium oxalate;
- Oxalate degrading bacteria

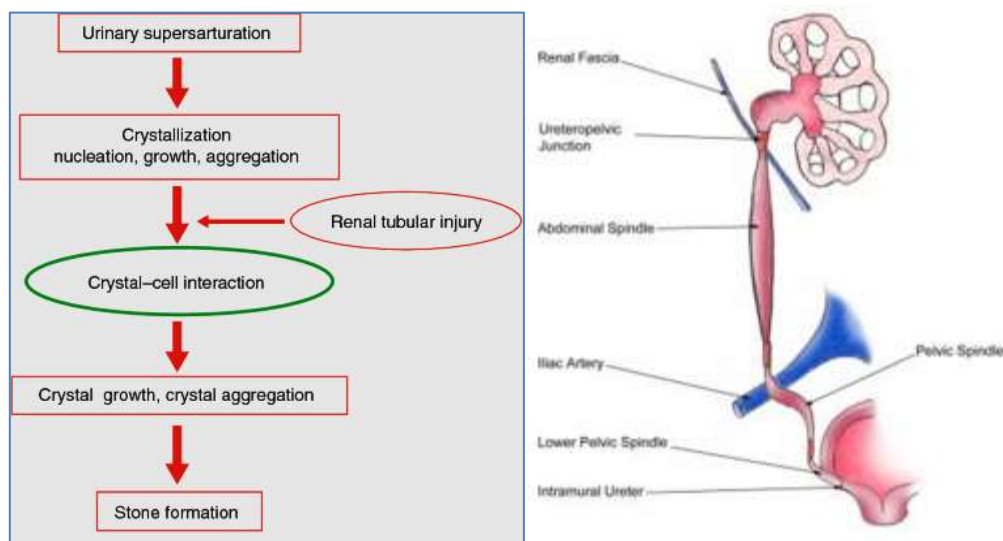


Introduction

- Urolithiasis or kidney-urinary tract stone disease has emerged as a severe health concern throughout the World.
- **With a high recurrence rate** in both, males and females as well as affecting around 12% of the population globally, kidney stones or urolithiasis (nephrolithiasis) is marked **by the formation of urinary calculi in the urinary tract.**
- In certain parts of the world such as the Middle East countries the hazards of urolithiasis are there is much greater.
- Because of the **inhibiting ability of estrogen, high capacity of estrogen and greater muscular mass** kidney stones are more prevalent in males rather than in females and affects all age groups from less than 1 year old to more than 70 years.
- Increase in incidence of nephrolithiasis has caused increased morbidity and economic burden across the world.
- The prime cause of nephrolithiasis is the **super saturation of urine with calcium and oxalate that leads to pathological mineralization in the kidneys.**
- Calcium oxalate accounts for the maximum percentage of kidney stones (approximately 75%) while
 - **Calcium hydroxyl phosphate** (brushite or calcium hydroxyapatite) 50%
 - **Magnesium ammonium phosphate** (struvite or triple phosphate), (10-20) %,
 - **Urate 5% and cysteine** constitute around (1-2) % of the kidney stones.

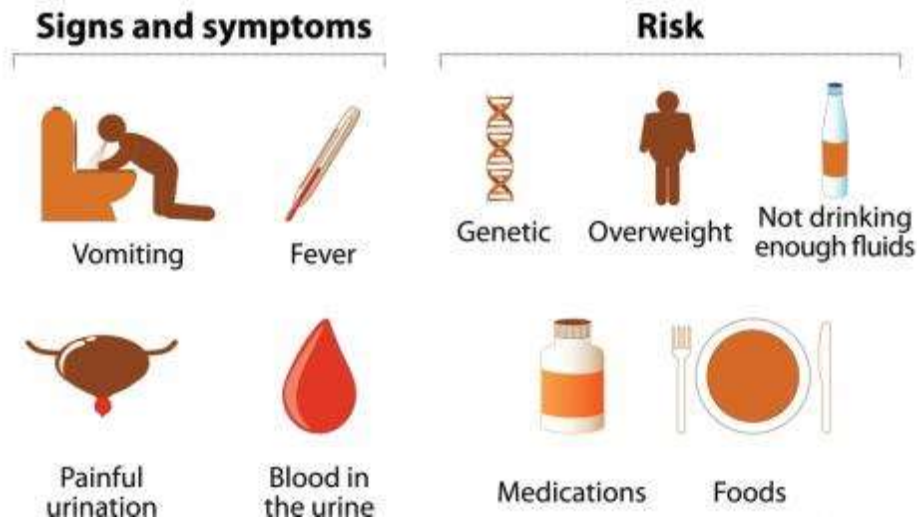
- Kidney or urinary stones **vary widely in their size** from micrometers to several centimeters in diameter and **so remain unnoticed most of the times for long periods** and are often **discovered incidentally** on radiography or ultrasound scanning or by painful manifestations.
- The study of **papillary biopsies** in patients with kidney stones have demonstrated that on the basis of the type of stone and the urine chemistry, the sequence of events leading to stone formation differ greatly.
- Hence involving a **number of physicochemical events** such as
 - **Super-saturation,**
 - **Nucleation,**
 - **Growth,**
 - **Aggregation and**
 - **Retention within the kidneys**
 - *Development of kidney stone or urolithiasis is a complex process.*
- In the urinary tract, stones are located at different sites such as the urinary bladder, ureters and the kidneys.
- **Staghorn (filling numerous major and minor calices)** and **non-staghorn** are the two classes into which the kidney stones have been grouped.
- *Although the proximal, middle or distal are the various locations that have been reported for ureteral stones, the non-staghorn stones are mainly pelvic or calyceal in nature.*
- Small kidney stones of
 - **Less than 5 mm in diameter** possess the largest possibility of being passed out easily
 - Followed by stones **of 5mm to 7 mm** that have about 50% possibility
 - But stones **over 7 mm** do not pass out on their own and often require urological intervention.
- While
- **Only 10% of the stones** in the kidneys have to be removed surgically,
- **90% of them** move out of the urinary tract and as the stone moves down the urinary tract, renal colic or flank pain begins to grow.
- Although elimination of the kidney stones can be accomplished through various methods such as shock wave lithotripsy, percutaneous nephrostolithotomy, ureteroscopy and open or laparoscopic interventions however these therapies exhibit severe side effects are also highly expensive and painful.
- In this regard several medicinal plants demonstrated effective results in treatment of kidney stones disease.

Mechanism of Kidney Stone Formation



- The uneven proportion of **inhibitors and promoters** are responsible to the complex process of stone formation in kidneys or urolithiasis.
- **Supersaturation of the urine** with calcium and oxalate salts followed by crystalline particle formation is the major reason behind kidney stone formation with supersaturation being the driving force.
- The mechanism involved in the process of stone formation (**Figure 1**) include
 - **Nucleation of crystals fractions,**
 - **Growth or gathering of these crystals to a size so that they can interact with some intra-renal structure(s),**
 - **Confinement of these crystals inside the kidney or renal collecting system**
 - **Succeeded by further aggregation and/ or secondary nucleation ultimately forming the clinical stone.**
- Calcium oxalate (CaOx) forms the major proportion of the kidney stones that is around 80%, while calcium phosphate (CaP) forms a small percentage (15%) of these stones.
- The crystals formed either
 - In renal tubular fluid or
 - In the renal interstitial fluid that is supersaturated
 - With respect to these constituents, which in turn might be a consequence of reduced urine volume,
 - An alteration in urine pH,
 - Mechanisms causing increased secretion of stone forming constituents such as hypercalciuria, hyperuricosuria, hypocitraturia, hyperoxaluria or a combination of these factors.
- *The urine and presumably, the tubular fluid of stone formers are often more highly supersaturated than that of normal healthy adults, which favors nucleation and growth of crystals.*

- *Long-term accretion of additional elements, crystalline as well as organic matrix produces the clinical stone.*
- As already explained extreme excretion of calcium or oxalate either alone or in combination as well as low volume of urine leads to an enhanced calcium oxalate supersaturation.



- The reciprocal actions between **genetic susceptibility and environmental factors** in different proportions promote both hypercalciuria and hyperoxaluria.
- A few genetic disorder which a rare autosomal recessive disorder,
 - Dietary routine including
 - Greater intake of oxalate and
 - Low calcium intake,
 - Potential abnormalities of anion transporters found in both gut and kidney,
 - Enhanced absorption of oxalate in the intestines other than mal absorptive diseases (enteric hyperoxaluria),
 - *Changes in the normal flora of the gut thereby decreasing degradation of oxalate in the colon* may cause one form of hyperoxaluria called primary hyperoxaluria types 1 and 2.
- **Some rare genetic disorders such as**
 - Adenine phosphoribosyl transferase (APRT) deficiency,
 - Cystinuria, and
 - Dent disease,
 - Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) and
 - Primary hyperoxaluria (PH) may lead to severe or chronic kidney stone diseases in children.
- The above mentioned processes in humans are influenced by several factors known as **promoters and inhibitors** that either support or prevent the process of stone formation thus affecting a person's ability to promote or prevent stone formation.
- Stone formation is facilitated by promoters while inhibitors prevent it (**Table 1**). Among the promoters of calcium oxalate stones are high oxalate, sodium, calcium, urate, low urine pH and low urine volume while the inhibitors include organic substances such as nephrocalcin and urinary prothrombin fragment-1, osteopontin and various inorganic substances such as citrate, magnesium.

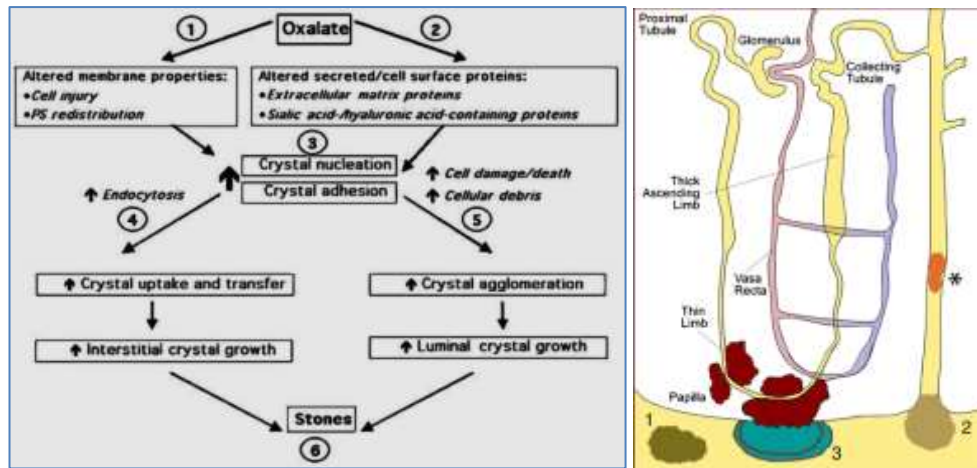
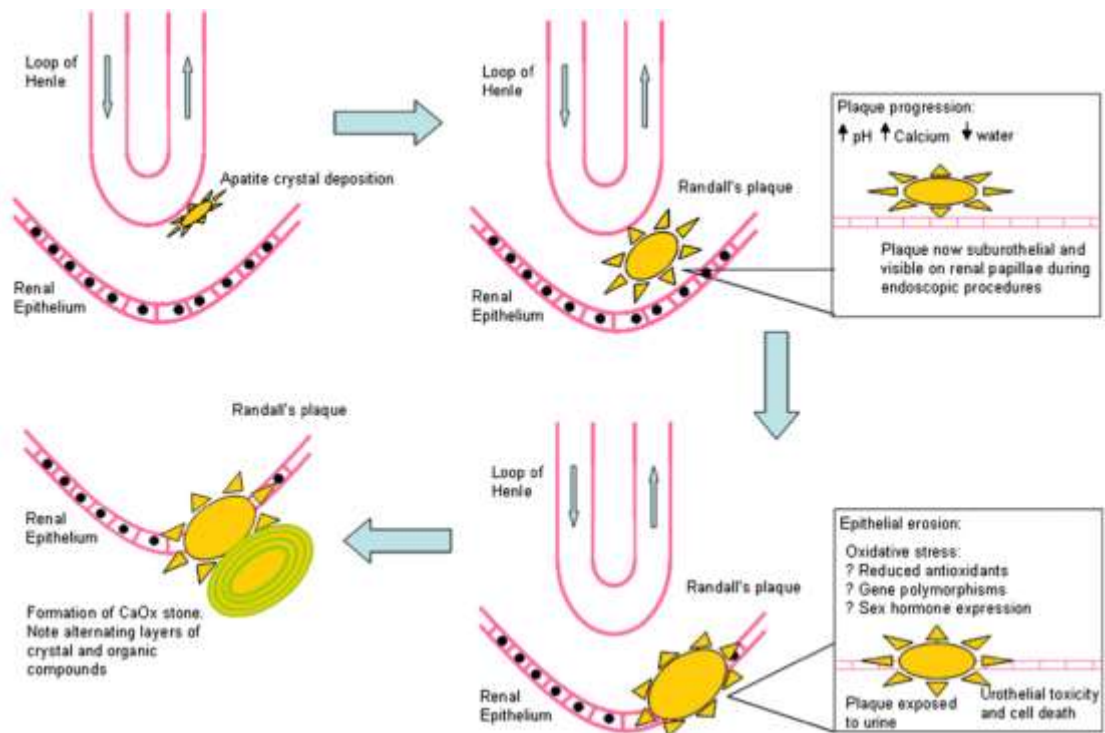


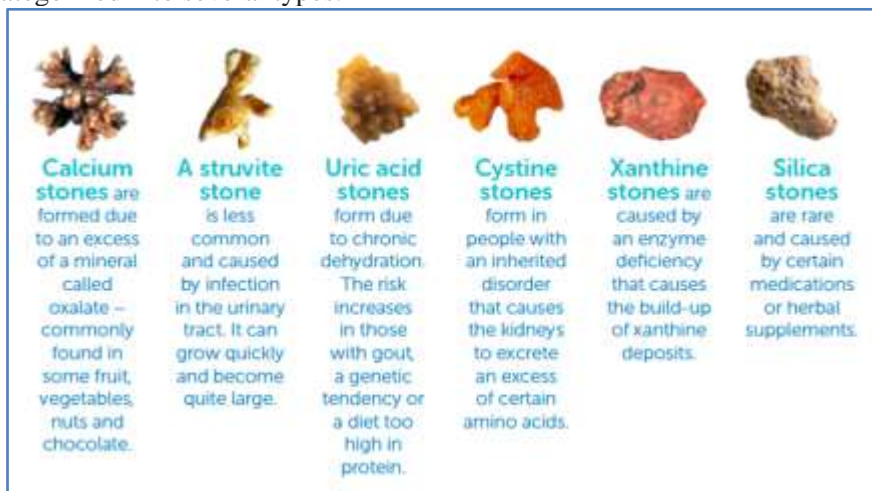
Figure 1: Mechanism of kidney stone formation.

Components	Pathophysiological mechanism
Promoters:	
Uric acid or urate	Has an Antagonistic effect on substances in the urine. Promotes heterogeneous nucleation by uric acid or monosodium urate. Binding of calcium oxalate to cells is increased.
Urine pH	Highly acidic pH enhances calcium oxalate crystallization. Formation of uric acid crystals is enhanced. Promotes secondary nucleation of calcium oxalate by formation of calcium phosphate precipitates.
Urine volume	Promotes crystallization
Hypercalciuria	Increased intestinal calcium absorption which may be 1, 25-dihydroxyvitamin D [1, 25(OH) 2D] dependent or independent. Decreased renal calcium reabsorption. Increases movement of calcium from bones. Enhances urine supersaturation and hence crystallization of calcium.
Hyperoxaluria	Because of an innate error in metabolism oxalate overproduction occurs. Increased dietary intake and bioavailability Increased intestinal oxalate absorption. Urinary supersaturation and formation of calcium oxalate crystals.
Inhibitors:	
Alkaline pH	Inhibits cystine and uric acid stone formation
Citrate	Forms complexes with calcium and therefore lower calcium oxalate supersaturation, inhibits aggregation of preformed crystals and attachment of crystals to urinary epithelium.
Pyrophosphate	Formation of both calcium oxalate and calcium phosphate crystals is inhibited.
Phytate	Inhibits calcium crystallization both in the intra-papillary tissue and urine.
Magnesium	Inhibits of growth (and presumably nucleation) of crystals as well as aggregation. While for the attachment of calcium oxalate crystals its supra-physiologic concentrations are required.
Glycoproteins	Inhibits spontaneous nucleation from metastable solutions as well as the growth of preformed crystals.



Major Types of Kidney Stones

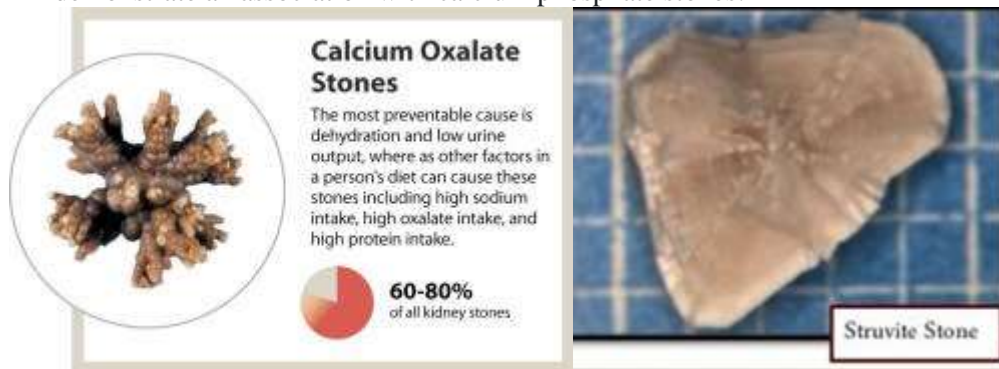
- There are several locations for the stones along the urinary tract such as the kidneys, ureters and urinary bladder.
- Kidney stones are categorized as either **staghorn** (filling numerous major and minor calices) or **non-staghorn**.
- Non-staghorn stones are described as calyceal or pelvic in location, while ureteral stones are defined as proximal, middle or distal.
- Small stones of the kidney, i.e., less than 5 mm in diameter have a greater possibility of passing out, while this possibility reduces as the size of the stones increases with those of 5 mm to 7 mm having only 50% chance, and the ones over 7 mm cannot pass out and always require urological intervention .
- With differences in their pathogenesis and composition kidney stones have been categorized into several types.





Calcium stones:

- Calcium in combination with oxalate, phosphate and also with uric acid constitutes the major proportion of kidney stones.
- Oxalate being a naturally occurring substance is found in various food sources such as some fruits, vegetables, nuts, and chocolate which exhibit high oxalate levels.
- It is also produced metabolically by the liver.
- Normal oxalate level in a healthy adult is around 20-40 mg/d.
- A number of dietary components such as elevated vitamin D doses, oxalate rich diet, various metabolic disorders and intestinal bypass surgery lead to increased concentrations of oxalate and calcium in the urine.
- Calcium oxalate and calcium phosphate stones are white, grey or black colored therefore demonstrating radio-opaque appearance, being roughly of 1 cm in diameter and in the radiographs these stones appear as dense and sharply circumscribed structures.
- Medical conditions such as renal tubular acidosis and hyperparathyroidism demonstrate an association with calcium phosphate stones.



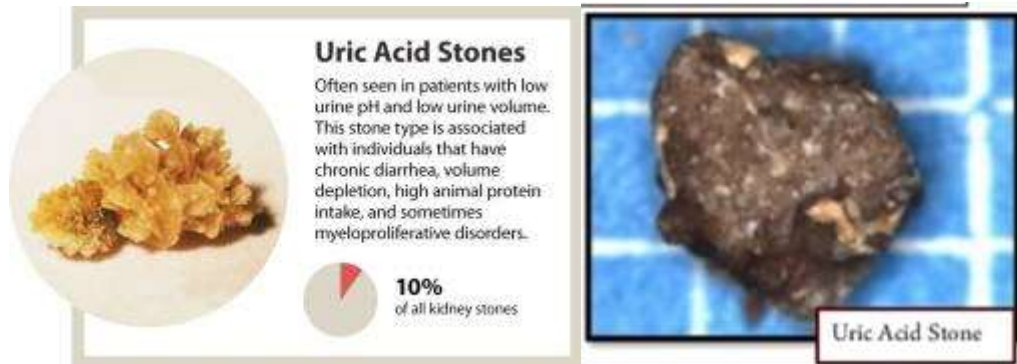
Struvite stones:

- A second type comprising about 10-15% of kidney stones are **triple phosphate or struvite stones** which are formed in presence of bacterial infection and is a crystalline substance made up of **magnesium ammonium phosphate**.
- The development of struvite stones is favored by the **bacterial enzyme urease that splits the urea into ammonia and carbon dioxide** thereby making the urine alkaline.
- Mostly found in humans with certain metabolic diseases including gout, idiopathic hypercalciuria and hyperparathyroidism, the struvite stones are large, glazed and laminated.



Uric acid stones:

- Yellow-orange, round and smooth stones made up of uric acid constitute around 5-10% of the kidney stones which in the radiographs appear nearly transparent unless they have been mixed with calcium crystals or struvite.
- These stones are usually formed of square, diamond or rod shaped pleomorphic **crystals** which are polarizable.
- People with abnormalities such as gout syndrome, obesity or those taking a diet rich in proteins, purines especially ones eating meat and fish possess uric acid kidney stones.



Protease-related stones:

- This type of stone is usually observed in HIV positive patients using **protease inhibitor indinavir sulphate drug**.
- The use of this drug may lead to the formation of such stones in 4-12% of the patients under treatment.

Cysteine stones:

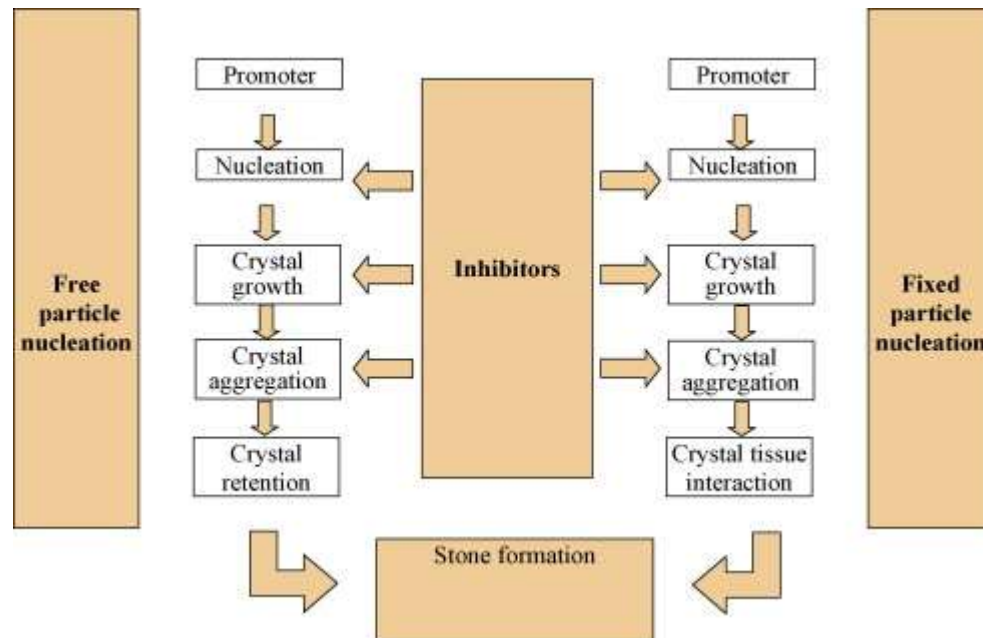
- These stones are caused due to hereditary disorders which cause the kidneys to secrete excessive amino acids (cystinuria) and are rare.
- With a moderate radio-opaque and rounded appearance these stones are marked with shiny crystallites and are greenish-yellow in color.



Silica stones:

- Medicines as well as herbal products including zonisamide, sulfa medications, indinavir, guaifene sin, laxatives (when abused), acetazolamide, ciprofloxacin, triamterene, ephedrine, loop diuretics, topiramate, and products containing silica promote the formation of such stones which are also known as drug-induced stones.

Therapeutic Options and Methods of Prohibition of Kidney Stones



- Spontaneous passage of the urinary stones is the **only objective of conventional management**, the most persistent option for treatment of renal colic.
- Kidney stones can be prevented and treated by **drinking plenty of water, eating vegetarian diet and a diet heavy on herbs**.
- The dreadful nature of kidney stones urges for improved treatment options although dietary restrictions with reduced calcium and oxalate intake and greater fluid intake has been one of the most usual method for the inhibition of kidney stones.
- Several investigations reveal that kidney stones can be prevented by intake of diet rich in vegetables and fruits.
- **Green tea, pomegranate, oregano, parsley, common madder, raspberry, yellow-fruit nightshade, khela, black cumin** etc. are the some of the dietary plants that have been observed to be significantly effective in preventing the formation of kidney stones and treating hyperoxaluria.
- **Narcotic analgesic, thiazide like diuretic, allopurinol and potassium citrate** are the main drugs which are prescribed as treatment strategy for preventing kidney stones.
- For patients in pain treatments such as shock-wave therapy, endoscopic management such as both ureter endoscopic and percutaneous nephrolithotomy offer efficient ways to treat stones and provide rapid relief, while open surgery or laparoscopic treatment is usually performed in combination with the treatment of other diseases e.g., renal pelvic stenosis.
- Treatment of kidney stones with diuretics and analgesics although is a common approach but their excessive consumption leads to intense side effects while shock waves cause traumatic side effects as well as may lead to infections and formation of residue stone fragments and hence have inspired researchers to move towards natural and safer remedies like **oxalate degrading enzymes and herbal medications**.

- Management of the initial processes of stone formation such as the crystallization events is the ideal method for treatment and prevention of urolithiasis.
- Medicinal plants such as *Amaranthus spinosus*, *Bambusa nutans*, *Abutilon indicum*, *Phyllanthus emblica*, *Cinnamomum bejolghota* and *Amaranthus viridis* play important part in the prevention of kidney stones and exhibit good antiurolithiatic property and therefore due to their clinically manifested antimutagenic, immunomodulatory as well as adaptogenic effects, herbal drugs have generated great curiosity among the people.

Oxalate-Degrading Bacteria as new Therapeutic Tools for Preventing Kidney Stones

- The discovery of oxalate-degrading bacteria in the human gastrointestinal tract has opened the way to explore several investigations regarding their potential role in reducing the urinary excretion of oxalic acid.
- By selectively enhancing the natural oxalate degrading microflora of the gut, i.e., *Bifidobacterium* sp., *Bacillus* sp., *Oxalobacter formigenes* and *Porphyromonas gingivalis*, humans as well as animals can counter hyperoxaluria and oxalate rich diets as has been affirmed by several researches.
- An oxalate degrading bacterial enzyme namely oxalate decarboxylase from *Bacillus subtilis* proved to be a possible therapeutic option for treating calcium oxalate stone disease in human.
- Oxalate decarboxylase breaks down oxalate thereby producing formate and carbon dioxide.
- Oral therapy of a cross-linked formulation of oxalate decarboxylase has proved to be an effective therapeutic option for hyperoxaluria as it strongly reduced the oxalate content in urine in hyperoxaluria model of mice.
- Previously, the heterologous expression of this enzyme in *Lactobacillus plantarum* (*L. plantarum*) was developed and utilized as a potential probiotic for depletion of intestinal dietary oxalate.
- For herbivores, transgenic plants expressing fungal oxalate decarboxylase may significantly reduce nutritional stress due to diminished oxalate content in the feed.

Conclusion

- **Attributed to a number of factors such as**
 - Nutritional disorders,
 - Genetic disorders and
 - Physiological disorders
- Renal urolithiasis or kidney stones are a common pathological condition.
- **The process of stone formation encompasses a number of steps such as**
 - Crystal nucleation,
 - Aggregation,
 - Binding to the kidney,
 - More aggregation and
 - Secondary nucleation.
- **Although kidney stone formation can be prevented by**
 - Controlling the dietary intake of oxalate,
 - Adequate nutrient intake, also
 - Suitable medication and
 - Various therapies are available for treating renal stones

- But re-occurrence of kidney stones as well as the side effects posed by these treatments has become a great issue that has attracted the people to use
- **better and safer medications such as**
 - Herbal drugs and
 - Medicinal plants
- Which play vital roles in kidney stone diseases treatment.
- Treatment with oxalate-degrading bacteria or purified oxalate-degrading enzymes (isolated from bacterial and fungal sources) could be promising new therapeutic options for patients with hyperoxaluria.
- Novel and practical techniques can be developed in the near future that can easily analyze the physicochemical process involved in stone formation that will surely be a boon for this field.
- Based on the elemental pathophysiological mechanisms of nephrolithiasis, targeted therapy using microbial oxalate degrading enzymes **for prevention and treatment of nuclei formation or salt crystallization** may prove to be highly successful with or without using herbal formulations.