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## REVIEW OF LITERATURE

The study was undertaken to explore the possibility of developing novel herbal drugs coupled with advancement made in the area of phytochemistry and crystallography for kidney stones, based on the traditional usage of banana corm juice for treatment of kidney stones. An attempt has been made to evaluate the antilithiatic property of selected banana cultivars by conducting and evaluating the *in vitro* crystallization studies. *In vivo* and *in vitro* (using NRK 52E cell lines) studies and the phytochemical characterization were conducted with the cultivar having good antilithiatic potential among the cultivars tested. With this in view, an abstract of relevant literatures are presented here under following headings.

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## 2.1. Kidney stones

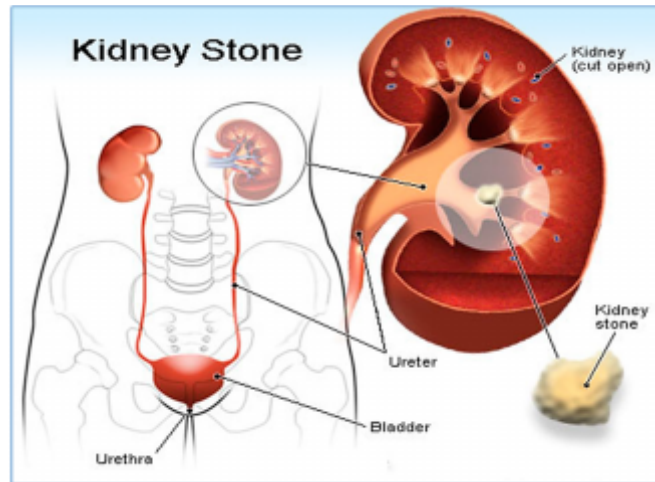
Kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extra cellular fluid volume, and regulation of blood pressure. It excretes a variety of waste products produced by metabolic activities. Formation of urine is the most important function of the kidney (Glodny *et al.*, 2009). Kidneys work as filters in our body, which purifies our blood and remove the impurities from it (Guyton and Hall, 2007). It maintains the proper amount of every constituent in the blood. If any imbalance in the level of water, salt or sugar in the blood, the kidneys excrete the excess amount from it. Stone formation is the process that results from a combination of factors in which the main phenomenon is the super saturation of stone components in the urine that might crystallize forming solid concretion (<http://www.thegeminigeek.com/how-is-urine-formed-in-our-body>).

Renal stone diseases cover kidney and lower urinary tract, caused by a variety of conditions, including metabolic and inherited disorders, and anatomical defects with or without chronic urinary infection. Most cases are idiopathic, in which there is undoubtedly a genetic predisposition, and also environmental and lifestyle factors play an important role. Indeed, it is becoming apparent that renal stone disease is often part of a larger 'metabolic picture' commonly associated with type 2 diabetes, obesity, dyslipidemia, and hypertension (Johri *et al.*, 2010). The condition of having kidney stones is termed as 'nephrolithiasis'. Having stones at any location in the urinary tract is referred to as 'urolithiasis', and the term ureterolithiasis is used to refer to stones located in the ureters (Figure 1).

### 2.1.1. Signs and symptoms of kidney stones

Some kidney stones may not produce symptoms (known as "silent" stones) and people who have kidney stones often report the sudden onset of excruciating, cramping pain in their low back and/or side, groin, or abdomen. Changes in body position do not relieve this pain. The abdominal, groin, and/or back pain typically waxes and wanes in severity, characteristic of colicky pain (the pain is sometimes referred to as renal colic). It may be so severe that it is often accompanied by nausea and vomiting (Kumar *et al.*, 2012).

**Figure 1**  
**Location of stones in urinary system**



Kidney stones also characteristically cause blood in urine (hematuria). If infection is present in the urinary tract along with the stones, there may be fever and chills. Sometimes, symptoms such as difficulty in urination, urinary urgency, penile pain, or testicular pain may occur due to kidney stones (Teichman, 2004; Curhan *et al.*, 2011).

### 2.1.2. Diagnosis

The diagnosis of kidney stones is compulsory when the typical pattern of symptoms are noted and when other possible causes of the abdominal pain are excluded. Imaging tests are usually done to confirm the diagnosis. Many patients are admitted to the emergency room and will have a non-contrast CT scan done. A CT scan however, exposes patients to significant radiation and recently, ultrasound in combination with plain abdominal X-rays have been shown effective in diagnosing kidney stones. In pregnant women or those who should avoid radiation exposure, an ultrasound examination may be done to help establish the diagnosis (Curhan *et al.*, 1997). Amaro *et al.* (2014) evaluated the prevalence of urinary metabolic abnormalities in patients with urolithiasis and their potential risk factors. Urinary metabolic disturbances were diagnosed in 96.8 % of patients in the study. These results warrant metabolic study and follow-up in patients with recurrent lithiasis in order to decrease recurrence rate through specific treatments, modification in alimentary, and behavioural habits.

### 2.1.3. History of nephrolithiasis

Renal lithiasis or urolithiasis or nephrolithiasis or kidney stones can be traced back to the earliest record in human civilization. It is a multifactorial disease, afflicted humans since antiquity (Atmani *et al.*, 2003). Examination of Egyptian mummies have revealed kidney and bladder stone disease (Hesse, 2005). Already in ancient Mesopotamia, knowledge of soluble or insoluble (bladder) stones was available. (Shattock, 1905; Shah and Whitfield, 2002; Eknayan, 2004).

Early literary (3200 - 1200 BC) references to stone disease were made within the medicinal texts called 'Asutu' in Mesopotamia. These medicinal texts present description of symptoms and treatments to dissolve the urinary calculi. For the dissolution of soft kidney and bladder stones, saltpetre and turpentine oil were used which increase the urine production (Dardioti *et al.*, 1997; Bitsori and Galanakis, 2004). The traditional Persian system of medicine dates back to 1000 BC and describes the symptoms of stone disease and suggests avoidance of eggs, meat and fish (Abdel-Halim *et al.*, 2003; Lopez and Hoppe, 2010).

The medical and surgical measures in the management of urological ailments prevailed in ancient India. Medical doctrines are first encountered in the religious texts called the Vedas compiled in successive generations from 3000 to 1000 BC (Mac Donell, 1962). It describes urethral and bladder instillations for certain calculus diseases and for cystitis in women. Various herbal medications are recommended for oral intake as well as to be anointed on the abdomen. In recalcitrant situations it advised referral for surgical interventions. Susruta (an ancient essay in Ayurveda) described various urological ailments with assumption about their pathogenesis followed by their detailed management (Bhishagratna, 1963).

In the Ayurvedic system of medicine, 'Pashanabheda' group of plants were used for the treatment of urinary stones. 'Pashanabheda' is the Sanskrit term used for a collection of plants with diuretic and antiurolithiatic activities (Pashana = stone; Bheda = break). It is believed that these plants have the property of breaking and disintegrating the kidney stones. Its identity however, is yet debatable (Johnston-Saint, 1929).

#### 2.1.4. Epidemiology

The prevalence of urinary calculi is estimated to be 3 million per year. The risk of developing urolithiasis in adults appears to be 12-15% in western hemisphere and 1-5% in eastern hemisphere, although the highest risks have been reported in Asian countries (about 21%). It is associated with high rate of recurrence, which is around 10-23% per year, 50% in 5-10 years and 75% in 20 years. Once afflicted, the subsequent relapse rate is increased and the recurrence interval is shortened. In India, 15% of the population of northern India suffers from kidney stones. Nearly, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage (Lieske, 2014; Roudakova and Monga, 2014).

Few cases of urinary calculi are found in southern India when compared to other parts of the country, which may be due to regular dietary intake of tamarind (Mohamed *et al.*, 2006). Global warming also contributes significantly to the increase in kidney stone formation in future (Trinchieri *et al.*, 2006). There a positive correlation between mean annual temperature in a region and stone formation. Theoretically, global warming could influence stone formation (Brawer *et al.*, 2008; Manjula and Hoppe, 2012). It has been documented that the incidence of urinary stones is higher in countries with hot climates, probably due too low urinary output and scant fluid intake. Also, the stone recurrence is higher in summer than in winter and spring. These are some of the factors that contribute to the geographical pattern that has characterized the Afro-Asian and North American stone belts. (Conдеми *et al.*, 2014; Adriano *et al.*, 2000; Curhan, 2007; Michelle and Bernd, 2010).

Nephrolithiasis is more common in men (12%) than in women (6%) and is more prevalent between the ages of 20 to 40 in both sexes (Worcester *et al.*, 2008). In most populations the occurrence of urolithiasis in men is two to three times higher than in women as testosterone enhances whereas estrogen inhibits stone formation. Women have a bimodal age on onset with episodes peaking at 35 and 55 years (Biren *et al.*, 2011; Reilly, 2005).

### 2.1.5. Etiology of stone formation

Several factors which increase the risk of developing kidney stones, include insufficient fluid intake, dehydration, reduced urine flow/volume, high calcium, oxalate rich diet or low citrate in urine and several medical conditions (Kumar *et al.*, 1991). Renal lithiasis is a multifactorial disease and is strongly related to dietary lifestyle habits and practices. Obesity and weight gain increases the risk of kidney stone formation (Taylor *et al.*, 2005; Lieske, 2014). Increased rate of hypertension and diabetes which are linked to nephrolithiasis, also contribute to an increase in stone formation.

Dietary factors in general which increase the risk of stone formation include low fluid intake, a high dietary intake of animal protein, sodium, refined sugars, fructose, high fructose corn syrup, oxalate, grapefruit juice, apple juice, and cola drinks (Borghi *et al.*, 2006). A protein-rich diet that enhances the risk for calcium oxalate and upper urinary tract stones. A nutritionally-poor diet that is low in animal protein, calcium and phosphate but high in cereal also leads to the development of bladder stones in children in under-developed countries (Rizvi *et al.*, 2002; Grases *et al.*, 2006).

Stone formers consumed less calcium, presumably to prevent more stones, and displayed a bone mineral responsiveness to calcium loss. This lowered calcium consumption alters bone responses in a direction that can predispose to mineral loss and eventual fracture. Sodium intake has a major risk factor for stone formation in view of the fact that high urinary sodium excretion has been repeatedly associated with hypercalciuria in adult and pediatric population (Menon and Resnick, 2002). An inverse relationship occurs between renal potassium and calcium excretion, which brings attention to the role of potassium rich foods such as vegetables and fruits in the prevention of stone formation. People with body mass index (BMI) greater than or equal to 27 had lower estimated Glomerular Filtration Rates (GFR) and had kidney stones (Asplin *et al.*, 2003; Grases *et al.*, 2002).

Oxalic acid present in foods such as chocolate, rhubarb or sweet potato and beverages is poorly absorbed from the intestine. The exact etiology of increased urinary oxalate excretion remains to be elucidated. Increased dietary protein intake, altered renal excretion and increased hepatic oxalate production have all been postulated as possible causes. Renal, urologic, endocrine and metabolic disorders may also lead to the

development of crystallized materials in the urinary system ([www.emedicine.com/PED/topic/2371.htm](http://www.emedicine.com/PED/topic/2371.htm); Menon and Koul, 1992; Holmes and Kennedy, 2000).

Metal and non-metal present in hard water at higher concentration might influence the outcome of the disease. Experiments in rats have shown that fluoride when fed at high levels accelerated the incidence of CaOx crystalluria and enhanced the incidence of bladder stone diseases considerably. The studies suggest that other condition being conducive, excess intake of fluoride (through water) might aggravate the situation. This is supported by reports that in Punjab the incidence is high in areas where fluoride content in drinking water is high (Chitme *et al.*, 2010).

#### **2.1.6. Pathogenesis of kidney stones**

Pathophysiologic mechanisms of stones are complex, mainly because stone disease is a polygenic, multifactorial disorder that involves an interrelationship between the kidney, bone and intestine. There are distinct stone phenotypes and the cascade of events leading to kidney stone formation vary depending on this phenotype.

Kidney stones result from a complex physical and chemical process, which involves two major opposing forces. One is urinary supersaturation (SS) that provides the driving force for stone formation. The other is urinary inhibitors and soluble molecules that protects from formation of calculi.

SS means that the concentration of a stone-forming salt exceeds its solubility in a solution and once reached, nuclei of its solid phase can form. SS can rise up to eightfold, depending on the crystal involved. It is the driving force for a phase change from dissolved salt to solid phase. Existing stones may aggregate and grow in the metastable zone but new stone cannot form without a nidus (Cotran *et al.*, 2005; Miller and Lingeman, 2007).

#### **2.1.7. Nucleation and crystal formation**

Nucleation is the establishment of the smallest unit of crystal formation. There are two forms of nucleation namely homogenous and heterogeneous nucleation. In pure solution, nuclei will form when SS rises above the formation product, called homogenous nucleation and usually requires high SS levels. In human urine the chemical environment

is diverse and crystal nuclei tend to form on structures such as cellular debris, urinary crystals, urinary casts and existing urinary membranes. This form of nucleation is called heterogeneous nucleation and occurs in much lower level of SS (Khan, 2004).

#### **2.1.8. Crystal aggregation and epitaxy**

Stone crystals bind to one another through a process known as aggregation or agglomeration. Strong chemical and electrical forces promote the aggregation process. Once crystals adhere to one another, they are held in place and cannot be easily separated. Crystal aggregation is thought to have an important role in stone formation since a single crystal would never be large enough to be retained in the collecting system. The ability of one crystalline lattice to grow on another is called epitaxy, and together with crystal aggregation is thought to play an important role in the formation of urinary calculi (Grases and Costa-Bauza, 1990; Chetan and Joshi, 2013).

#### **2.1.9. Crystal retention**

Within the time frame of transit of urine through the nephron, crystals must grow and aggregate in order to form urinary calculi. Since the transit time from collecting duct to bladder is estimated to be around 5 to 7 min, crystal retention is necessary for stone formation of clinically significant size. There have been two mechanisms proposed to account for crystal retention; the free particle hypothesis and fixed particle hypothesis (Kok and Khan, 1994). The former suggests that the process of nucleation occurs entirely in the tubular lumen. As the crystal moves through the renal tubules, rapid aggregation generates a crystal large enough to occlude the tubular lumen (Finlayson and Reid, 1978). The fixed particle hypothesis relies on adherence of crystals to a fixed point, such as renal epithelial cells or Randall's plaque (Evan *et al.*, 2006). In idiopathic calcium oxalate stone formers, when examined, the renal papilla have sites of plaques, as whitish calcifications located at the papillary tip called Randall's plaques. This plays a dominant role in the pathogenesis of calcium oxalate renal calculi (Kuo *et al.*, 2003).

### **2.2. Types of stones**

Renal stone disease is not a single disorder, since stone composition varies, which reflects constitutional, environmental and genetic factors. Kidney stones are composed of organic and inorganic crystals and are combined with proteins. Urinary stones can be

classified according to stone composition as calcium stone, uric acid stone, struvite stone, cystine stone and some other types including xanthine stone. Approximately 80% of the kidney stones are primarily composed of calcium oxalate (Stamatiou *et al.*, 2006) and calcium phosphate, 9% uric acid, 10% struvite (magnesium ammonium phosphate produced during infection with bacteria that possess the enzyme urease), and the remaining 1% is composed of cystine or ammonium acid urate (Vyas, 2010) which are diagnosed as drug related stones (Fedric *et al.*, 2005).

### **2.2.1. Calcium oxalate stones**

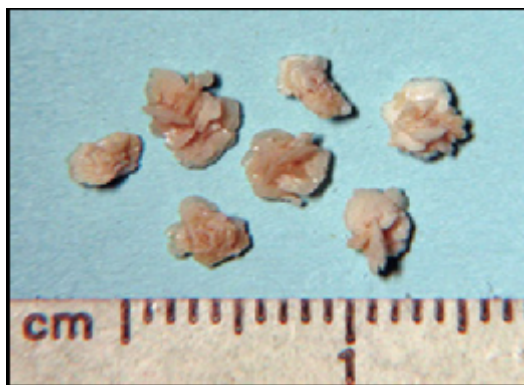
Roughly four out of five kidney stones are calcium stones, usually in the form of calcium oxalate. Oxalate is found in some fruits and vegetables, but the liver produces most of the body's oxalate supply. Dietary factors, high doses of vitamin D, intestinal bypass surgery and several different metabolic disorders can increase the concentration of calcium or oxalate in urine. Most stones contain calcium combined with oxalate, phosphate, or occasionally uric acid. All calcium stones are radio-opaque, and calcium oxalate and calcium phosphate stones are black, grey, or white and small (1 cm in diameter), dense and sharply circumscribed on radiographs (Figure 2a). Calcium oxalate is the primary component of 70-80% of calcium stones. Oxalate plays an important role in stone formation and has about 15-fold greater effect than urinary calcium. Increased oxalate concentration is responsible for precipitation and deposition of calcium oxalate crystal. Calcium oxalate exists in three different hydrated forms namely Calcium Oxalate Monohydrate (COM,  $\text{CaC}_2\text{O}_4\cdot\text{H}_2\text{O}$ ), Calcium Oxalate Dihydrate (COD,  $\text{CaC}_2\text{O}_4\cdot 2\text{H}_2\text{O}$ ), and Calcium Oxalate Trihydrate (COT,  $\text{CaC}_2\text{O}_4\cdot 3\text{H}_2\text{O}$ ). The monoclinic COM is the thermodynamically most stable phase, followed by the triclinic COT and the tetragonal COD (Pietrow and Karellas, 2007; Philip and Hall, 2009; Yadav *et al.*, 2011).

COM phase of calcium oxalate is the principal crystalline constituent in human kidney stones, with plate like morphology of urinary calculi. When compared to COD and COT, COM has strong affinity for renal tubule cell membranes and its difficulty in ejection along with urine makes it a possible source of urinary and kidney stones. The urinary proteins play an important role in transforming COM phase to COD, which is not developed into urinary and kidney stones. COD crystals are routinely flushed out

by urinary flow of both healthy people and stone-formers. COD is less cell-adherent and less injurious to cell membranes than COM (Yoreo *et al.*, 2006; Gangu *et al.*, 2014).

**Figure 2a**

**Calcium oxalate stones**



Studies by Finlayson and Reid (1978) have shown that once nucleated, a crystal would pass into urine before it is grown sufficiently large to be retained in the nephron and subsequently form a stone. However, if not stabilized by adsorbed urinary compounds and excreted, COD crystals will undergo phase conversion to COM. Prevention of COD dissolution, even for short reaction times, could, therefore, help to suppress crystal deposition and kidney stone formation. It appears that cell membranes, stones, membrane vesicles, protein fragments and magnesium decrease the activation energy for COD formation and thereby catalyze the COD phase via heterogeneous nucleation. It has been described that the *Sargassum fusiforme*, a sulfated polysaccharide isolated from marine algae, could decrease the size of COM and COD crystals, inhibits the aggregation of COM and induces the formation of COD (Neira-Carrillo *et al.*, 2010).

### **2.2.2. Calcium phosphate stone**

Calcium phosphate (CAP) kidney stones include apatite or carboxyapatite or hydroxyl apatite (HAP), Brushite, and Octacalcium Phosphate (OCP). HAP is thermodynamically most stable form of calcium crystals with the occurrence rate of apatite 4-10%, brushite 2-6% and octacalcium phosphate less than 1%. The first product that precipitates is an amorphous calcium phosphate, which subsequently is converted to the crystal phases OCP and HAP or occasionally brushite (Amy *et al.*, 2010). Among the

majority of kidney stones, CaOx is the main constituent and Cap is present in amounts ranging from 1% to 10%. When Cap becomes the main constituent (>50%) of stones (Evan *et al.*, 2005), the stones are called cap stones (Coe *et al.*, 2005).

### 2.2.3. Uric acid stones

These stones are formed of uric acid, a byproduct of protein metabolism. If the diet is rich in high protein, it may lead to the development of uric acid stones. Gout also leads to uric acid stones (Kramer *et al.*, 2003). Certain genetic factors and disorders of the blood-producing tissues also influence the uric acid stone formation. This type of stones are smooth, round, yellow-orange and nearly radiographically transparent unless mixed with calcium crystals or struvite (Figure 2b). Diets high in purines, especially those containing meats and fish, result in hyperuricosuria, and, in combination with low urine volume and low urinary pH, can exacerbate uric acid stone formation (Tiwari *et al.*, 2012).

**Figure 2b**  
**Uric acid stones**



### 2.2.4. Struvite or triple phosphate stones

Found more often in women, struvite stones are almost always the result of urinary tract infections. Struvite stones may be large enough to fill most of a kidney's urine-collecting space, forming a characteristic stag's-horn shape (Figure 2c). It is a crystalline substance composed of magnesium ammonium phosphate. Signs of struvite stones include urinary pH greater than 7 and staghorn shape calculi (Chetan and Joshi, 2013).

**Figure 2c**  
**Struvite Stones**



### 2.2.5. Cystine Stones

These stones represent only a small percentage of kidney stones. They form in people with a hereditary disorder that causes the kidneys to excrete (Font-Liitjos *et al.*, 2005). Excessive accumulation of cystine due to improper dietary factors can result in cystinuria, which ultimately ends up in stone formation. Formation of cystine stones is the only clinical expression of cystinuria, an autosomal recessive disorder. People who are homozygous for cystinuria excrete more than 600 mg per day of insoluble cystine. The stones are greenish-yellow, flecked with shiny crystallites, and are moderately radio-opaque in appearance (Figure 2d). Their occurrence is mostly rare. Cystine is one of the building blocks that make up muscles, nerves, and other parts of the body. Excessive accumulation of cystine due to improper dietary factors can result in cystinuria which ultimately ends up in stone formation (en. Wikipedia. Org /wiki / cystinuria).

**Figure 2d**  
**Cystine Stones**



### **2.2.6. Other uncommon stones**

Other uncommon type of stones include xanthine stones, dihydroxyadenine stones, silicate stones and matrix. Xanthine stones is an autosomal recessive type and due to deficiency of xanthine oxidase leads to xanthinuria. Dihydroxyadenine stone occur due to efficiency of enzyme adenine phospho ribosyl transferase, whereas silicate stones are rare in humans (excess intake of antacid with magnesium trisilicate). Matrix occur due to infection by proteus. It is radiolucent (all calculi have some amount (3%) of matrix but matrix calculus has 65% Matrix content in calculi) (Moron, 2014).

### **2.3. Medical management of urinary stone**

Management of stone disease needs individualization. Clinical presentation, proper history and laboratory tests help to identify whether one needs urgent surgical or medical treatment. Medical management includes laboratory evaluation, dietary management, disease specific therapies, and medical expulsion therapy of stone and herbal therapies (Gettman and Segura, 2005; Hussain *et al.*, 2009).

#### **2.3.1. Treatment options**

A stone can form only when urine is supersaturated with respect to its constituents such as CaOx, the most abundant element in analyzed kidney stones. To reduce the propensity of the salts to crystallize in urine, patients with kidney stones, regardless of their composition, should take some measures including dietary restrictions and increasing fluid intake (Atmani *et al.*, 2003).

#### **2.3.2. Stone inhibitors and promoters**

Inhibitors are defined as molecules that decrease the super saturation required to initiate nucleation, decrease crystal growth rate and aggregation, and inhibit secondary nucleation. In contrast, promoters induce the formation product of the supersaturated solution. An imbalance between urinary-promoting and inhibiting factors has been suggested as more important in urinary stone formation than a disturbance of any single substance. These substance include inorganic compounds, proteins, and glycosaminoglycans. Abnormal function and or concentration of these compounds in urine may modify physicochemical conditions to promote stone formation (Gupta *et al.*, 2011).

**Inhibitors**

Inhibitors of calcium stone formation prevent crystal growth and aggregation by coating the surface of growing calcium crystals or by complexing with calcium and oxalate (Frietas et al., 2002).

**Citrate**

Citric acid is a tricarboxylic acid that circulates in blood complexed to calcium, magnesium and sodium at physiological pH at 7.4. Most of the circulating citrate is derived from endogenous oxidative metabolism. The most established effect of citrate in urine is to complex with calcium, thereby reducing the concentration of CaOx. Citrate is an inhibitor of crystallization and has also been shown to be an important inhibitor of CaOx agglomeration (Kok *et al.*, 1986).

Treatment using potassium citrate has serious potential drawbacks including the development of hyperkalemia in patients with type IV renal tubular acidosis, or those who have renal impairment. Another potential complication is gastric bleeding, intestinal obstruction, diarrhea, nausea, burning that may occur during the use of liquid potassium citrate. Long term potassium citrate therapy may cause aluminum toxicity (Singh *et al.*, 2010).

**Pyrophosphate**

Urinary pyrophosphate and diphosphate have shown to inhibit the precipitation of CaOx and CaP and inhibit the growth of apatite crystals (Dardamanis, 2013).

**Magnesium**

It is the fourth most abundant mineral in the body and is largely found in the bones. Magnesium can form complexes with oxalate and decreases SS. However, magnesium oxide, tested at 200 mg/100g of rat chow showed no significant changes on CaOx nephrolithiatic rat. Overall, the use of magnesium in therapy of lithiasis patients seemed to be limited (Su *et al.*, 1991; Tiselius, 2003).

**Osteopontin (Uropontin)**

Osteopontin (OPN) is a negatively-charged aspartic acid-rich protein that inhibits growth of CaOx crystals in a supersaturated solution. A relation between OPN and renal stone disease are inconclusive based on clinical studies (Aburto *et al.*, 2013).

### **Tamm-Horsfall protein**

Tamm-Horsfall protein (THP), also known as uromucoid, is an 80-kDa glycoprotein, is the most abundant protein in the urine of normal mammals. THP is on the first line of host defences against both renal stone formation and bacterial infection. Much controversy exists about whether THP is a promoter or an inhibitor of crystal aggregation (Carvalho *et al.*, 2002). Most authors believe that it is an effective inhibitor of COM crystal aggregation in solution with high pH, low ionic strength and low concentration of divalent ions and THP (Farmanesh *et al.*, 2014).

### **Glycosaminoglycans (GAGs)**

GAGs identified as one of the macromolecules present in the stone matrix, as heparin sulphate and hyaluronic acid. GAGs concentration in the urine is too low to decrease calcium SS. The side effects restricted the use of GAGs especially for long term treatment. All these inhibitor molecules level at physiological state were found to be normal in the urine of non-stone formers. Abnormal function and or concentration of these compounds in the urine may modify physicochemical conditions to promote stone formation (Torzewska, and Różalski, 2014).

### **Promoters**

On the cell surfaces of the kidney, cell debris, protein aggregates and other crystals may provide analogous site for nucleation and promote it. Crystals of COM, uric acid and calcium phosphate may promote heterogeneous nucleation. Ionic calcium is also a factor that may promote the formation and growth of intra renal calcium. Hypercalciuria can decrease inhibitor function and lead to crystallization. Furthermore, cellular responses to newly formed crystals and factors that modulate these crystal-cell interactions could stimulate initiation of an intra-renal stone formation (Gupta *et al.*, 2011).

### **2.3.3. Other drugs**

Based on their hypercalciuric action, thiazides are used to treat hypercalciuric nephrolithiasis patients. Thiazide therapy is limited by side effects like fatigue, malaise, impotence and constipation (Heilberg and Schor, 2006)

Sodium cellulose phosphate is used to restore normal calcium excretion by reducing intestinal calcium absorption. This treatment however, is associated with two potential complications namely hypermagnesiuria and hyperoxaluria involving several mechanisms (Penniston, 2014).

Deficiency of inhibitors and /or an abundance of promoters in the urine are almost certain to predispose to stone disease. The role of cell injury may be an even more important determinant in the promotion and progression of kidney stones. Perhaps the presence of the stone itself initiates an inflammatory response, leading to further epithelial disruption and amplification of stone formation. This urges all urologist to take this phenomena into consideration when treating lithiasis patients (Basavaraj *et al.*, 2007).

#### **2.3.4. Surgical therapies**

For kidney stones that do not pass on their own by pharmacological management, the most widely preferred technique is the lithotripsy. In this procedure, shock waves are used to break up a large stone into smaller pieces that can then pass through the urinary system. In case of failure with all other treatments, surgical invasive techniques have also been used like percutaneous nephrolithotomy or through ureteroscopy (Ligeman *et al.*, 2006; Tiselius *et al.*, 2008; Lawrence and Koya, 2009).

##### **2.3.4.1. Extracorporeal shock wave lithotripsy (ESWL)**

ESWL uses non-electrical shock waves that are created outside the body to travel through the skin and body tissues until the shockwaves hit the dense stones. The stones become sand-like and are passed out. For this procedure, patient's acre is placed in a tub of warm, purified water or onto a water cushion machine that acts as a medium for transmitting these non-electrical shockwaves. There are several types of ESWL devices. In one device, the patient reclines in a water bath while the shock waves are transmitted. Other devices have a soft cushion on which the patient lies. ESWL is not ideal when stones are larger than 2cm, about 0.8 inches. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. These procedures however, are highly expensive and with these procedures recurrence is quite common (Coe *et al.*, 1977; Evan and Willis, 2007; Prasad *et al.*, 2007 Srinivas *et al.*, 2012).

#### **2.3.4.2. Ureteroscopic surgery**

Open surgery involves opening the affected area and removing the stone(s). Another, less known procedure (called coagulum pyelolithotomy) also removes kidney stones. This procedure involves the injection of a liquid containing calcium chloride, cryoprecipitate, thrombin and indigo carmine into the kidney. This injection forms a jelly like clot that traps the stones inside. Through an incision made in the kidney, the doctor extracts the stone with forceps. Some stones are partially amenable to dissolution therapy; these include uric acid and cystine stones. Dissolution therapy is based on the solubility characteristics of the calculus in urine (pKa) and the manipulation is that most of the uric acid or cystine is soluble. Citrate based medications such as potassium citrate are used as dissolution therapy and can be used to successfully manipulate the pH of the urine and pKa of the solutes with resultant dissolution of calculi. If implemented, the pH of the urine has to be monitored, as it gets altered dramatically (increased to an alkaline state), and can cause the precipitation of calcium based stones. Ureteric stones greater than or equal to 5mm should be referred for a urological opinion. They have a less likelihood of passing spontaneously and hence a long term sequel should be managed surgically (Fine *et al.*, 2010).

#### **2.3.4.3. Percutaneous nephrolithotomy**

Percutaneous nephrolithotomy (PCNL) is a surgical procedure to remove stones from the kidney by a small puncture wound (up to about 1 cm) through the skin. It is most suitable to remove stones of more than 2 cm in size and which are present near the pelvic region (Viers *et al.*, 2014). Surgical management has become increasingly tolerable, medical prevention of recurrent struvite calculi is feasible and greatly desirable. In such a condition, it is the need of scientists to discover such drugs, which can inhibit struvite growth. In addition high success rates, excellent safety profile with low side effects, and ease of use of such a drugs are necessary for management of calculi ([en.wikipedia.org / wiki / percuraneow – nephron lithotomy](http://en.wikipedia.org/wiki/percuraneow_nephron_lithotomy)).

### **2.3.5. Non-surgical therapies/ Prevention options**

#### **2.3.5.1. Fluid intake therapy**

One of the best proven means to decrease kidney stones is by increasing the fluid intake, which decrease urinary supersaturation. But the recurrence rate was too high in this method (Bijarnia *et al.*, 2010).

#### **2.3.5.2. Diuretic therapy**

Therapies that increase renal fluid output such as diuretics might theoretically facilitate stone passage and elimination because of associated increased hydrostatic pressure within the water. The potential positive impact from diuretic treatment is not significant. Also diuretics are not routinely recommended in acute ureteric stone (Singh *et al.*, 2014).

#### **2.3.5.3. Probiotic therapy**

The role of probiotic in the treatment of recurrent calcium oxalate nephrolithiasis, is after the discovery of oxalate-degrading bacteria within the human gastrointestinal tract. Kaufman *et al.* (2008) evaluated the stool samples from recurrent CaOx stone formers and controls, and found a occurrence of *Oxalobacter formigenes* which is 17 per cent in stone formers and 38 per cent in controls. Indeed, the results from uncontrolled studies using administration of *O. formigenes* in enteric-coated capsules led to significant decrease in urinary oxalate, but were not confirmed in a prospective randomized, double-blind, placebo-controlled trial in stone formers (Goldfarb and Asplin, 2001; Hoppe *et al.*, 2011).

All the preventive methods and chemical medications have not given expected results, not to speak of the undesirable side-effects and recurrence still remains a problem (Pareta and Watson, 2011). Thus, an alternative to these conventional methods is very much needed. At this juncture, phytotherapy that can overcome the problem of side-effect and recurrence of the ailment requires to be considered.

### **2.4. Phytotherapy**

The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs have been prized for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the

modern age outshined their importance for a short time. But the blind dependence on synthetics is over and people are now returning to the natural products with a hope of safety and security (Serhat and Kupeli, 2006).

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes. The annual production of medicinal and aromatic plant's raw material is worth about Rs.200 crores. This is likely to touch US \$5 trillion by the year 2050 (Jawla *et al.*, 2009).

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to the rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care of rural population which depends on indigenous system of medicine. Of the 2,50,000 higher plant species on earth, more than 80,000 have medicinal value (Devi *et al.*, 2010).

India is one of the world's 12 biodiversity centres having of over 45,000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal preparations traditionally from the days of yore. In India, herbal drugs of herbal are in traditional system of medicines such as Unani and Ayurveda (Zaidi *et al.*, 2006). The Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, Amchi 600 and modern medicine around 30 species. The drugs are derived either from the whole plant or from different parts like leaves, stem, bark, root, flowers and seeds etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacology. Some important chemical intermediates needed for manufacturing modern drugs are also obtained from plants (E.g. diosgenin,

solasodine,  $\beta$ -ionone). Not only that plant-derived drug offers a stable market worldwide, but also plants continues to be an important source for new drugs due its diverse nature (Senthilkumar *et al.*, 2010).

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide range of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford to buy the products of the western pharmaceutical industry and have to rely upon traditional medicines mainly derived from plant material. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species (Williams *et al.*, 1996). In spite of the overwhelming influences and dependence on modern medicines and tremendous advances made in synthetic drugs, a large segment of the world population still depends on drugs derived from plants. In many developing countries, the use of plant drugs is increasing because modern life-saving drugs are beyond the reach of common man and such countries spend 40-50% of their total wealth for drugs and health care. As part of the strategy to reduce such financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future (Diana, 2013).

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurveda. The Rigveda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvanaveda (4500-2500 BC) 290 species and Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively, and many such drugs are still being used in the classical formulations, in the ayurvedic system of medicine. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests impairing the availability of raw materials, ayurveda, like other systems of herbal medicines has reached a very critical phase (Deshpande *et al.*, 1982; Subrat, 2002; Nagori *et al.*, 2011).

### 2.4.1. Socio-economic impact

A decade ago, alternative medicine was a distinctly counter culture phenomenon, but now it has become an established presence in mainstream culture. The use of plant products as medicine is widespread and growing due to generate awareness. A national survey reported that the use of unconventional therapies for various health problems (Ventola, 2010). One among the unconventional therapy that we will focus on, is phytotherapy or the use of plants in the treatment of diseases especially kidney stone formation, Herbal treatment has been a favorite choice of naturopathically inspired practitioners. According to the World Health Organization, approximately 75% of the global population, mostly in developing world, depends on botanical medicines for their basic healthcare needs. Substances first isolated from plants accounts for approximately 25% of the pharmacopoeia, with another 25% derived from modification of chemical first found in natural products (WHO, 2005; Jiofack *et al.*, 2010).

At present, ethnobotany has become more and more valuable for the development of health care and conservation programs across of the world. In India, studies on ethnobotany were initiated by the Economic Botany Section of Botanical Survey of India (Howrah) in 1954. From 1960, Jain started intensive field studies among tribal areas of central India. An All India Coordinated Research Project on Ethnobiology (AICRPE) came into operation from 1982 at NBRI, Lucknow, and four centres (Shillong, Howrah, Coimbatore and Port Blair) of Botanical Survey of India (Jain, 1963<sup>a, b & c</sup>; Balick, 1996).

### 2.4.2. Antiurolithiatic studies using medicinal plants

As far as urolithiasis is concerned, acupuncture, herbal medicine, natural products and homeopathy have been used to treat and/or to alleviate symptoms of lithiatic patients. Concerning herbal medicines, there is a large number of species described in many pharmacopoeia of several countries in the world as remedies for urolithiasis. Few investigators however, have devoted their efforts to study these plants by using objective and scientific mechanism by which these plants function and identify the active principles involved. Few of them were described below.

A reduction in calcium oxalate crystallization and decrease in excretion of calcium were noticed with the treatment of various plant extracts namely rice-bran for

idiopathic hypercalciuria (Ohkawa *et al.*, 1984), *Rosa canina* infusion (Grases *et al.*, 1992), banana stem juice (Prasad *et al.*, 1993), Kampou extracts (Koide *et al.*, 1995), *Phyllanthus niruri* (Freitas *et al.*, 2002), *Aerva lanata* (Nirmaladevi *et al.*, 2013), Spirulina (Al-Attar, 2010), *Adonis aestivalis* Linn. (Parameshwar *et al.*, 2011) and polyherbal formulation (Baheti and Kadam, 2013).

Many herbal formulations are commercially available which are used for kidney stone management. The marketed composite herbal formulations, Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India), Uriflush (Inti Sumatera Global, Indonesia) and Uriflow (Discovery Herbs, USA) have been used clinically to dissolve urinary stones in the kidney and urinary bladder.

Cheryl (2006) made an investigation on ethnomedicines used in Trinidad and Tobago and Canada, for urinary problems and diabetes mellitus based on ethnobotanical interviews conducted from 1996–2000. He reported the usage of the following fourteen plants for kidney and other urinary problems viz., *Kalanchoe pinnata*, *Mimosa pudica*, *Chamaesyce hirta*, *Flemingia strobilifera*, *Peperomia rotundifolia*, and *Lepianthes peltata*.

The plants used for kidney problems as reported by Ankur *et al.* (2010) are *Alium sativum*, *Apium graveolens*, *A Armoracia lopathifolia*, *Barbarea vulgaris*, *Capsella bursapastori*, *Citrus japonica*, *Ficus carica*, *Olea europeae*, *Pimpinella anisum*, *Rosmarinus officinalis*, *Chamaesyce hirta*, *Flemingia strobilifera*, *Peperomia rotundifolia*, *Petiveria alliacea*, *Nopalea cochinellifera*, *Apium graveolens*, *Cynodon dactylon*, *Eleusine indica*, *Gomphrena globosa*, *Pityrogramma calomelanos* and *Vetiveria zizanioides*.

According to the folk-medicine system of Kumaun Himalaya of Uttarakhand, India, *Bergenia ligulata* (Wall.) is useful in dissolving kidney and bladder stones (Gangwar *et al.*, 2010). Nandagopalan *et al.* (2011), had made an attempt to identify medically important folklore plants frequently used by rural communities of sacred groves of Pudukkottai district. He had reported the use of *Punica granatum*, *Musa paradisiaca* and *Solanum torvum* for the elimination of kidney stones.

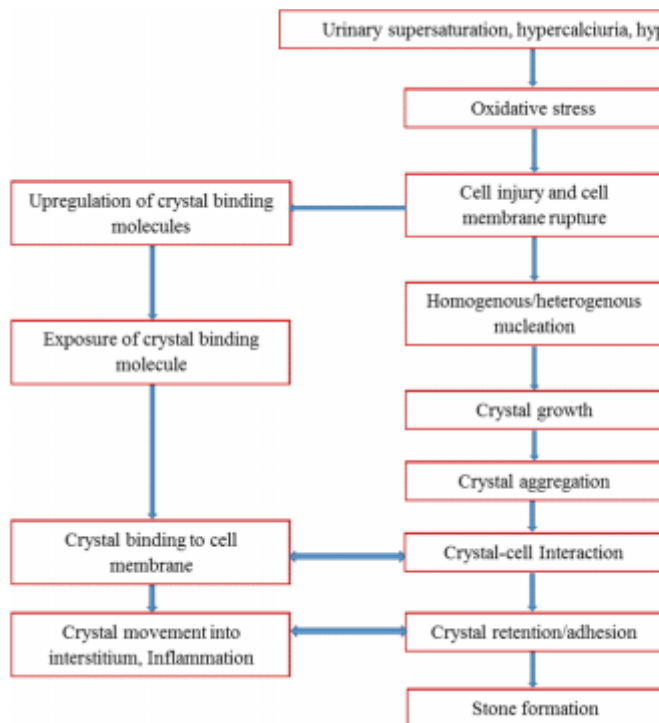
The information on medicinal uses obtained from local healers and herbalists, practicing traditional system of medicine, revealed the use of *Abutilon indicum*, *Aerva lanata*, *Boerhaavia diffusa*, *Bryophyllum pinnatum*, *Crataeva nurvala*, *Cynodon dactylon*,

*Daucus carota*, *Equisetum debile*, *Gomphrena celosioides*, *Musa balbisiana*, *Ricinus communis*, *Solanum surattense*, *Trianthema portulacastrum*, *Tribulus terrestris* and *Zea mays* as antiurolithiatic agents in local remedies (Diana, 2013). The use of *Scoparia dulcis* for the treatment of urinary calculi was also reported in another study conducted among Nadars of Atoor village of Kanyakumari district, Tamilnadu, India (Jeeva and Femila, 2012).

The antilithiatic property of *Moringa oleifera* was reported by a number of researchers. Karadi *et al.* (2006) and Sachan (2012) had evaluated the effectiveness of alcoholic extract of *Moringa oleifera* on calcium oxalate urolithiasis *in vivo* in male Wistar albino rat model experiments. The increased deposition of stone forming constituents in the kidneys of calculogenic rats were significantly lowered by curative and preventive treatment using aqueous and alcoholic extracts. The results indicate that the root-wood of *Moringa oleifera* is endowed with antiurolithiatic activity.

## **2.5. Stone formation**

The steps involved in stone formation includes nucleation, growth and aggregation. The physico-chemical mechanism of stone formation via precipitation, growth, aggregation, and concretion of various modulators were represented in Figure 3 (Finlayson *et al.*, 1984). The methodology for testing the *in vitro* stone formation was given in chapter 3.

**Figure 3****Schematic representation of various cellular events during stone formation.****2.5.1. *In vitro* model for lithiasis**

*In vitro* calcium oxalate crystallization has been, and will continue to be, of fundamental importance to urolithiasis research. Crystallization is a physiochemical process involving a change of state from solution to solid. The supersaturation, which is a measure of the chemical energy available for this, is a crucial factor and governs all aspects of crystallization such as nucleation, growth and aggregation processes (Kavanagh, 2006). These three important stages of stone formation were simulated *in vitro* in the present study.

**2.5.1.1. Crystal nucleation**

The initial step in the transformation from a liquid to solid phase in a supersaturated solution is called nucleation. Nuclei form the first crystals that do not dissolve and have a characteristic lattice pattern. Once a nucleus is created and principally if it is anchored, crystallization can occur at lower pressures than required for the formation of the initial nucleus (Boskey, 1981).

### 2.5.1.2. Crystal growth

Once the nucleus has reached a critical size and relative supersaturation remains above one, the overall free energy is decreased by adding new crystal components to the nucleus. This process is called crystal growth. Crystal growth is one of the prerequisites for particle formation and thus for stone formation. Although crystal growth is definitely a step in CaOx renal stone formation, the process of growth is so slow that crystals cannot become large enough to obstruct the renal tubules (Finlayson *et al.*, 1984).

### 2.5.1.3. Crystal aggregation

The process whereby crystals in solution stick together to form larger particles is called aggregation. Crystal aggregation is probably involved in crystal retention within the kidneys, since aggregation of crystals can have a considerable effect on particle size. It is important to study the different stages of the development of renal stones by using artificial models that closely reproduce all the conditions prevailing in the upper urinary tract (Grases *et al.*, 1998). With this aim, the above explained experimental systems were used for studying *in vitro* renal stone formation to reproduce the conditions found *in vivo*.

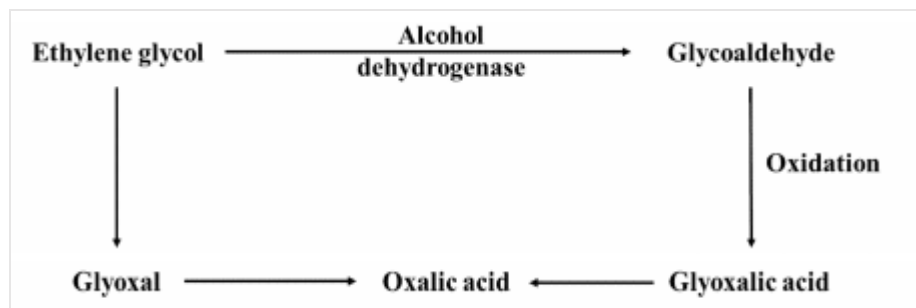
### 2.5.2. *In vivo* studies using animal model –male Wistar rats

Rats are the most frequently used animals in models of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in human. The rate of occurrence of stone formation is three times higher in men than women. Male albino Wistar rats are used for lithiatic studies because of enhancing capacity of testosterone and inhibiting capacity of estrogens in the stone formation. *In vivo* models for urolithiasis are commonly divided into three categories namely chemical induced urolithiasis (ethylene glycol 0.75% in drinking water by oral administration), diet induced (oxalate calculi producing diet and high calcium diet) and minor surgical methods (insertion of zinc disc into the urinary tract and calcium oxalate seed model) (Vijaya *et al.*, 2014).

In the present study the chemical induced urolithiasis model was utilized and 0.75% ethylene glycol was fed to the experimental albino male Wistar rats. The mechanism underlying is presented in Figure 4, in which ethylene glycol is converted to glycolaldehyde

by the enzyme alcohol dehydrogenase. Glyoxalic acid is formed by oxidation of glycolaldehyde. Oxalic acid is formed upon further oxidation of glyoxalic acid. Hence, stone formation in ethylene glycol fed rats is induced by hyperoxaluria which increases renal retention and excretion of oxalate (McQuade *et al.*, 2014).

**Figure 4**  
**Mechanism of stone induction by ethylene glycol (0.75%)**



### Standard Drug Cystone<sup>®</sup>

Cystone<sup>®</sup> is traditionally used for relief of a variety of urological problems mainly for nephrolithiasis. Its purported effect is to ‘prevent supersaturation of lithogenic substances, control oxamide (a substance that precipitates stone formation) from the intestine and correct the crystalloid-collide imbalance. Cystone<sup>®</sup> inhibits calculogenesis by reducing stone-forming constituents and causes their expulsion (Mohanty *et al.*, 2010; Erickson *et al.*, 2011). Cystone<sup>®</sup> causes disintegration of the calculi and crystals by acting on the mucin, which binds the particles together. Cystone<sup>®</sup>’s antispasmodic and anti-inflammatory activities relieve ureteric colic and alleviate symptoms of painful, burning and micturition (<http://himalayahealthcare.com/products/cystone.htm>). It is manufactured and sold by Himalaya Health Care.

### 2.5.3. *In vitro* assays using cell lines

#### 2.5.3.1. NRK 52E cells

The role of animal investigation to biomedical research is of undoubted value though the real usefulness of animal models is still being debated. The ‘3R rule’ proposed by Russell and Burch (1959) explains Refinement, Reduction and Replacement is now widely accepted and has a major impact on animal experimentation procedure.

Refinement clarifies the severity of procedures applied to animals and has been extended to the entire lives of the experimental animals. Reduction elucidates on the number of animals necessary to obtain statistically significant data and may be improved by good experimental design and statistical analysis of data. Replacement refers to the development of validated alternative methods.

Shamoo and Resnik (2003) discuss the fourth and fifth R. The fourth would be Relevance which explains that the research protocol should have some scientific, medical or social relevance and all risks/harms to animals should be balanced against benefits to humans. The fifth R is Redundancy avoidance, which clarifies to avoid redundancy in animal research whenever possible and ensure that the experiment has not already been done and if it has already been done, provide justification for repeating the work. The 6<sup>th</sup> R that some have suggested, to be added to the list, is Regulations.

With this back drop, the present study was carried out to understand the interaction between stone crystals and cell membranes with an alternative model for lithiasis using cultured Normal Rat Kidney cells (NRK 52E).

Renal epithelial cells occupy approximately 80% of the total kidney volume and particularly vulnerable to the changing environment in the glomerular filtrate and tubular fluid. The interstitium containing a variety of resident and infiltrating cells, is associated with the tubular epithelium as well as the vascular network of the kidney, and is greatly influenced by tubulointerstitium leading to inflammation, renal fibrosis and progressive renal failure (Lemley and Kriz, 1991).

Tissue-culture studies using NRK 52E cells could explain the interaction between the crystals and renal cells to better understand tubulointerstitial damage caused by crystal deposition in the kidneys. Both oxalate and calcium oxalate crystals are injurious to renal epithelial cells in culture (Khan *et al.*, 1999; Khan, 2012a). Epithelial injury promotes attachment of calcium oxalate crystals and this attachment is mediated by oxalate induced exposure of phosphatidylserine (PS) on the cell surfaces. Several studies have, however, indicated that injury and exposure to PS may not be essential for the attachment of calcium oxalate crystals to epithelial cells (Verkoelen *et al.*, 1997 and 1999; Lieske *et al.*, 1994). Results of studies of renal epithelial cell exposure to calcium

oxalate also confirmed the involvement of free radicals in oxalate toxicity (Scheid *et al.*, 1996; Jonassen, *et al.*, 1999; Liang *et al.*, 2014).

### **2.5.3.2. Oxidative stress and antioxidants**

Exposure to high levels of oxalate and calcium oxalate crystals can induce oxidative stress such as an increase in free radical generation, increased lipid peroxidation and a decrease in cellular antioxidant status. Oxalate crystal injures the cells when there is a sustained exposure to high levels of oxalate and calcium oxalate (Umekawa, *et al.*, 2005). Mitochondria have been demonstrated to show excessive uptake of calcium when the cytoplasm level of free calcium markedly increases, causing abnormalities in the respiratory chain and increasing the mitochondrial production of ROS (Reactive Oxygen Species). Calcium-induced mitochondria injury can be prevented by antioxidants suggesting that oxidative stress may be an important event in its development (Kawai *et al.*, 2006; Huang *et al.*, 2000).

Renal tubular cell injury is induced by the oxidative stress, which is produced during the attachment of crystals to renal tubular cells. Exposure to high concentrations of oxalate can give rise to the generation of ROS, which are known to mediate many toxin induced renal tubular injuries (Thamilselvan *et al.*, 2014). In the present study the effects of banana corm for its antioxidant properties on the renal calcium crystallization was examined.

## **2.6. Cultivars analysed in the study**

Banana is a large herbaceous flowering plant of the genus *Musa*, which is native to India. Bananas and plantains are being grown in every humid tropical region and constitute the 4<sup>th</sup> largest food crop of the world after rice, maize and wheat. With increasing urbanization bananas and plantains are becoming more and more important as cash crops, in some cases providing the sole source of income to rural populations (Frison and Sharrock, 1999). Thus, playing an important role in poverty alleviation. Bananas and plantains are one of the cheapest food to produce. It is the staple food in many countries in Africa. The cost of production of one kg of plantain is less than for most other staples, including sweet potato, rice, maize and yam. Consequently banana and plantains can be very cheap food to buy and are, hence, an important food for low income families (Hailu *et al.*, 2013).

Banana fruit is an excellent source of potassium and a single banana can provide 23% of daily potassium requirement. Bananas are also good source of vitamins namely vitamin A, B<sub>6</sub>, C and D. The fruit has a mild laxative property, and is the remedy for constipation in children and help in curing diarrhea and dysentery. It also heals intestine ulcers, stomach upset and worm problems in kids. Banana flower is used to treat ulcers and bronchitis, cooked flowers are considered a good for diabetics. Roots and seeds cure digestive disorders. Peel and pulp were shown to have antifungal and antibacterial components and found to contain neurotransmitters norepinephrine, serotonin and dopamine. Plantain juice is used as an antidote for snake bite. Studies in rats demonstrate effectiveness for stone lysis. The roots can arrest hemoptysis and possess strong astringent and anthelmintic properties (Kumar *et al.*, 2012). Various parts of banana has been utilized and value added products prepared from them (Narayana and Ramajayam, 2003).

Taxonomically cultivated bananas are referred to by their genome constitution. The crop encompasses a range of diploids, triploids and tetraploids. Cheesman (1948) first suggested that cultivated bananas originated from intra and interspecific hybridization between the two wild diploid species *Musa acuminata* Colla. and *Musa balbisiana* Colla., each contributing the A and B genomes respectively. The identification of *Musa* cultivars has traditionally been based upon various combinations of morphological, phenological and floral criteria.

Simmonds and Shepherd (1955) devised a scoring technique based on 15 diagnostic morphological traits to differentiate clones of *Musa acuminata* from *Musa balbisiana* and their hybrids into genome groups. According to this system of classification, cultivated dessert bananas are classified as AA, AB, AAA, AAB, ABB, ABBB, AABB, AAAB and AAAA. They occur naturally or are produced by artificial hybridization.

*Musa* species are grouped according to 'ploidy' level based on the number of chromosome they contain, and the relative proportion of *Musa acuminata* (A) and *Musa balbisiana* (B) in their genome makeup. The most familiar, seedless cultivated varieties (cultivars) of banana are triploid hybrids e.g., Robusta /Grand Naine, Red banana, Poovan, Rasthali, Nendran, Karpooravalli, Monthan and so on. Common diploid forms include Sannachenkadali (AA), Neypoovan (AB) and Bhimkol (BB), a wild clone.

The natural tetraploid forms AAAA, AAAB, AABB and ABBB are much rarer (Sathiamoorthy and Balamohan, 1993; Stover and Simmonds, 1987).

The cultivars selected in the present study includes Poovan (AAB), Red banana (AAA), Neypoovan (AB), Karpooravalli (ABB), Sannachenkadali (AA) and Bhimkol (BB), which represents the six different genome and ploidy levels.

### **2.6.1. Poovan (AAB)**

Poovan internationally known as Mysore. It is a leading commercial cultivar grown throughout India with location specific synonyms like Palayankodan in Kerala, Poovan in Tamil Nadu, Karpura Chakkarakeli in Andhra Pradesh and Alpan in North Eastern Region. Tamil Nadu is the leading producer of Poovan cultivar owing to its high demand and ideal agro-climatic conditions. Poovan is also commercially cultivated for leaf purposes throughout Tamil Nadu and in certain parts of Kerala. Fruit is medium sized, firm and has typical sour-sweet taste. Poovan corm and flower are cooked and eaten in certain parts of Kanyakumari districts of Tamilnadu ([http://agritech.tnau.ac.in/expert\\_system/banana/season&variety.html](http://agritech.tnau.ac.in/expert_system/banana/season&variety.html)).

### **2.6.2. Red banana (AAA)**

Red banana also known as Red Dacca bananas in Australia. It is the most relished and highly prized variety of Kerala and Tamil Nadu. The plant is tall and robust statured. Its commercial cultivation is prominent in Kanyakumari, Coimbatore, Dindugal and Tirunelveli districts of Tamil Nadu. It is also popular in Karnataka, Andhra Pradesh and to some extent in Western and Central India. In Bihar and other regions, it is popular as Lal Velchi while in Karnataka as Chandra Bale. The colour of the pseudostem, petiole, midrib and fruit rind is purplish red. Fruits are sweet with orange yellow coloured pulp and with a characteristic pleasant aroma. The corm of the Red banana is less fibrous and easy to slice off (Kumar *et al.*, 2012).

### **2.6.3. Neypoovan (AB)**

The synonyms of Neypoovan (Tamilnadu), include 'Elakki bale' (Karnataka), 'Safet Velchi' / 'Chini Champa' (North India), 'Ranel' (Sri Lanka), 'Kisubi' (Uganda), 'Apple', 'Farine France'. It is also known in English as Sugar banana, Finger banana,

Fig banana, Date banana, Sucrier etc. Neypoovan is the choicest diploid cultivar, which is under commercial cultivation on a large scale especially in Karnataka and Tamil Nadu. In Kerala it is grown in backyards and now shifting to large-scale cultivation. Neypoovan is a slender plant bearing bunches of 15-20 kg with a crop duration of 12 months. Dark green fruits turn golden yellow on ripening with a very good keeping quality. Fruit has piquant flavor and taste with a firm flesh. Neypoovan is tolerant to leaf spot diseases but susceptible to *Fusarium* wilt and Banana Bract Mosaic Virus (Patil, 2011).

#### **2.6.4. Karpooravalli (ABB)**

It is a popular hardy variety grown for table purpose and can be grown even in less fertile soils. Its commercial cultivation is spread over in central and southern districts of Tamil Nadu. In Bihar, it is cultivated under the name 'Kanthali'. Internationally it is known as Pisang Awak, which is extensively grown in Africa, Thailand and Sri Lanka. Karpooravalli is a tall, robust plant well suited to marginal lands and soils, produced under low input conditions. It is also the sweetest among Indian bananas. Its ashy coated and ripens to yellow. Karpooravalli is susceptible to wilt disease and pulp which is stodgy tolerant to leaf spot disease (Anand *et al.*, 2006).

#### **2.6.5. Sannchenkadali (AA)**

It is a hard diploid variety. The plant resembles that of Red banana with red coloured pseudostem, petiole and midrib. The bunch is medium sized and fruits are small, slender, pronounced tip and peel red colour. The pulp is light orange yellow. It performs well even in shaded condition and suitable for planting in coconut grooves. It is tolerant to leaf spot (Uma and Sathiamoorthy, 2007).

#### **2.6.6. Bhimkol (BB)**

The scientific name is *Musa balbisiana* clone Bhimkol /Bheem Kol (BB). It is a wild type grown in northeastern states of India. It is a seedy fruit with 60 to 80 seeds per fruit and they are dried, pounded and the endosperm of the seed is sieved. This is used as baby-food in northeastern states of India. In Assamese tribal culture, the fruit skin is dried and burnt to form 'khar' which is a natural alternative to baking soda. Khar is a favourite ingredient in many Assamese dishes. The flower called 'kol-dil' and

the tender interior parts of the main stem, called 'kol-posola' are eaten. The outer layers of the stem are used as plates and bowls, and the leaves are used during religious rituals and traditional feasts. The corm is made into paste and applied for skin infection, as it possesses antimicrobial and antifungal activities. It is chopped and cooked with pulses (Purkayastha and Nath, 2006). It is the most robust among the wild species and the stems is used as floats during floods. In fact it is said that no part of this plant is wasted.

Regardless of such studies being reported, there are no studies reported on the antilithiatic and antioxidant properties of the corm banana cultivars. The present study aimed at an extensive search into the antilithiatic property of banana clones. The cultivar with better antilithiatic activity among the selected banana clones was tested for its antioxidant property besides a detailed phytochemical characterization.

The layout of the study, the materials used and the methodology adopted are explained, with appropriate references quoted, in the following chapters.