Chapter 2

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Kidney stone diseases are a major concern affecting the people all over the world (Rodgers, 2014). After the urinary tract infections and prostate diseases, urinary calculi are the third most common affliction of the urinary tract (Mazdak et al., 2007). About 12% of the global population has been estimated to have renal stone disease (Soundararajan et al., 2006). The considerable economical burden of the disease lies in its treatment and morbidity. Chronic kidney disease is frequently seen among patients with kidney stones and an estimated 10 to 15% of these patients eventually progress to chronic kidney failure. Urolithiasis has affected people of all ethnicity, regions and culture for centuries (Vupputuri et al., 2004). Kidney stone diseases have multifactorial etiologies and a complicated unknown pathophysiology (Worcester et al., 2006). In traditional medicine practice, herbal preparations have been used since ancient times as conservative management (Rodgers et al., 2015).

Urolithiasis is a broad term which refers to the condition of having calculi in the urinary tract including kidneys, ureters, bladder and urethra (Figure 2.1). Nephrolithiasis term arose from Greek words nephros, "kidney" and lithos, "stone" and hence refers to the condition of having kidney stones. Urolithiasis is a multifactorial renal calculi disease, influenced by both intrinsic and extrinsic factors (Einollahi et al., 2013). Persistent urinary tract infection (UTI) is a serious condition caused by infectious urolithiasis due to urease producing bacteria e.g. *Proteus mirabilis* (Torzewska and Rozalski, 2014). Primary hyperoxaluria is a result of inborn errors of metabolism, inherited in the autosomal recessive fashion in which the decrease in enzyme production for glyoxalate results in overproduction of oxalate. Type I primary hyperoxaluria results from a deficiency of peroxisomal enzyme alanine-glyoxylate aminotransferase and the more rare type II, from a deficiency of cytosolic enzyme D-glycerate dehydrogenase. The secondary hyperoxaluria is a result of excessive oxalate intake. End-stage renal disease (ESRD) is a recurrent kidney stone formation and parenchymal renal damage caused by abnormally high concentrations of oxalate in the urine. Oxalosis is the deposition of oxalate in various organs caused by the ESRD (Mitsimponas et al., 2012).
Figure 2.1 Stones at different parts of urinary system

Global Disease Burden: Prevalence and Incidence

Urolithiasis is an important public health issue that predominantly affects people at their productive age. Incidence and prevalence of kidney stones are influenced by genetic, nutritional and environmental factors. Men are affected more commonly than women, though the incidence is rising worldwide in women and also with increasing age (Condemi et al., 2015).

Prevalence of a disease is defined as the total population affected with the disease at a particular time in a given area while incidence is how many new cases have been reported annually. There is approximately 10% lifetime risk of developing kidney stones in the general population. The geographic predisposition to form kidney stones has been highlighted in literature (Lopez and Hoppe, 2010). Regional "stone belts," have been explained in people living in the Southern United States, having an increased risk of stone
formation (Brikowski et al., 2008). Urinary stone disease is a common disease, which affects 10-12% of the population in industrialized countries. Prevalence of urolithiasis has been found to be lowest in Greenland and Japan. Middle east countries has the highest lifetime risk of kidney stone formation (20-25%) followed by United States of America (10-15%), Europe (5-9%) and least in Asia (1-5%) (Najeeb et al., 2013). In males, the highest prevalence of the disease occurs between 20 to 40 years of age, however in females, the highest incidence of the disease occur later in their life. Incidence of nephrolithiasis in the United States has been on the rise during the past several decades (Fwu et al., 2013). Kidney stone disease is rare in the South African black population and more prevalent in the white population (Theka et al., 2012). In Italy prevalence of urolithiasis in 2012 was 4.14%, with male preponderance (4.53% versus 3.78%) and a positive correlation with increasing age. Incidence was higher in age group 65-74 years (Prezioso et al., 2014). Incidence of kidney stones in Iceland has been reported to be 138 per 100,000 which rose significantly from 108 in 5-year interval (Edvardsson et al., 2013).

Epidemiological studies are lacking in India for kidney stone disease. Rizvi et al., (2002) had reported a prevalence rate of 15% in India. In India, upper and lower urinary tract stones occur frequently, but the incidence shows wide regional variations. Two high incidence stone belts have been marked in India. The first belt starts from Amritsar in North and while passing through Delhi and Agra ends up in Uttar Pradesh (Ganesamoni and Singh, 2012). The other belt starts from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been reported in West Bengal and coastal areas of Maharashtra, Karnataka, Kerala, Tamil Nadu and Andhra Pradesh (Aggarwal et al., 2014a). Year-wise distribution revealed that there is steady increase in urolithiasis with inflation in drought years. With respect to climatic parameters, hot days are significantly correlated with urolithiasis. Presence of certain elements in groundwater such as sodium and fluorides are significantly associated with the incidence of urolithiasis and suggest a strong relation between geo-environment and urolithiasis (Kale et al., 2014).
Financial burden

Kidney stone disease imposes a substantial financial burden to the global health care system (Ngo and Assimos, 2007). Since the nephrolithiasis primarily afflicts the working population it places a considerable economic expense on the community. The significant burdens are related to health service costs while personal and pharmaceutical costs representing only a small component. Davidson et al., (2009) had estimated the financial burden to a defined community of Christchurch, New Zealand to be $450,000 per 100,000 general populations.

Recurrence Rates

Kidney stone is a recurrent disease and the rate of recurrence depends mostly on the treated or untreated conditions. If the disease remained untreated, probability of developing another stone after the initial episode generally for all kinds of stone has been averaged 30 to 40% at 5 years (Johnson et al., 1979). Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened. Features associated with recurrences include a young age of onset, positive family history, infection stones and underlying medical conditions (Damasio et al., 2014). The treatment, however, has shown more than 50% reductions in recurrence rates in many randomized control trials (Novak et al., 2009). The decline in the recurrence rates due to dietary or medicinal interventions strongly suggests that urolithiasis may be preventable (Curhan, 2007).
2.1 Chemical composition of kidney stones

Based on the chemical composition kidney stones may have different characteristic morphology. Crystallization process followed by lithogenesis may take place due to different type of solutes in the urine. As per the laws of crystallography if the concentrations of the solutes are higher than the thermodynamic solubility range i.e. if the urine is supersaturated with respect to stone material, crystals do form. The type of stone formed may depend on the urinary supersaturation levels. Thus, by lowering supersaturation the recurrence of stone formation may be prevented. The main component of the stone matrix account for 2-3% of their total dry weight and consists of macromolecules generally present in the urine (Park and Pearle, 2007). Stones are composed of 64% protein, 9.6% non-aminosugars, 5% hexosamine as glucosamine, 10% bound water and the remainder as inorganic ash. Recently lipids have also been documented as an important constituent of stone matrix (Aggarwal et al., 2013b).

![Proportions of kidney stones by types (Monk, 1996)](image)

**Figure 2.2** Proportions of kidney stones by types (Monk, 1996)

### 2.1.1 Calcium stones

Calcium stones are the most common type of kidney stones and constitute approximately 70-80% of all renal stones (Figure 2.2). As discussed previously, the stone
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forming calcium salts supersaturation in the urine are the basis for calcium stone formation. Metabolic abnormalities such as hypercalciuria (>250mg/24hrs), hyperoxaluria (>45mg/24hrs), hypocitraturia (<450mg/24hrs) and hyperuricosuria (>800mg/24hrs) can change the either the composition or saturation of urine to promote stone formation (miller 2007). Calcium stones are generally a mixture of calcium phosphate and calcium oxalate. Depending on the major constituent (>50%), the stones are categorised as either calcium oxalate or calcium phosphate stone. Calcium oxalate is the primary constituent of calcium kidney stones while calcium phosphate stone are less common and only about 15% of kidney stones fall in this category (Parks et al., 2004).

a) Calcium oxalate stones

Oxalate is unavoidable component of human diet as it is a ubiquitous component of plants and plant derived food products. Endogenous oxalate synthesis primarily occurs in liver with glyoxylate as an immediate oxalate precursor. Glyoxylate is derived from oxidation of glycolate by glycolate oxidase or by catabolism of hydroxyproline, component of collagen (Jonassen et al., 2003). Transamination of glyoxylate with alanine by alanine glyoxylate transaferase (AGT), results in the formation of pyruvate and glycine (Figure 2.3).

Calcium oxalate stones can exist in monohydrate and dihydrate forms, with or without phosphate. These are the most common type of urinary stones. Calcium oxalate stones are radiopaque and usually visible on plain film radiography or non contrast CT (Figure 2.4a). Hypercalciuria is one of the most frequent metabolic anomalies associated with stone formation. Some of the other reasons include hyperoxaluria, hypocitraturia, which involve deficiency of citrate and hyperuricosuria, the naturally occurring stone inhibitor (Pietrow and Karellas, 2006).

b) Calcium phosphate stones

Calcium phosphate crystals could be present in urinary stones as either brushite or apatite. Brushite or calcium mono hydrogen phosphate is believed to be the precursor of apatite (Coe et al., 2005). The morphological appearance is depicted in figure 2.4b.
Hydroxy apatite ($\text{Ca}_{10} \text{(PO}_4\text{)}_6\text{(OH)}_2$) is the principal constituent of bones and teeth. Up to fifty percent of bone is made up of a modified form of the inorganic mineral hydroxy apatite. Carbonated calcium-deficient hydroxy apatite is the main mineral of the dental enamel. Hydroxy apatite crystals are also found in the small soft tissue calcifications such as pineal gland and other structures (Coe et al., 2005).

![Figure 2.3](image) Different routes taken by ingested oxalate or oxalate precursors (Kleinman, 2007)

2.1.2 Non calcium stones

a) Uric acid stones

Uric acid urolithiasis or uric acid kidney stones refer to development of a stone or calculus composed of significant amounts of urate in the renal pelvis, ureter, or bladder. These stones (Figure 2.4c) form when pH of the urine is critically low. Only 90 mg/l
undissociated uric acid dissolves in human urine at 37℃ therefore, at a pH of 5.35, only 180mg/l of total urate species can be dissolved, whereas the total urate concentration of healthy individuals and uric acid stone formers approximates 500mg/l of urine. Low urine pH is due in part to low ammonia excretion (Sakhaee et al., 2002). In the patients of diabetes mellitus, gout and metabolic syndrome, reduced pH of urine and uric acid stones are the common feature due to insulin resistance that may decrease renal ammonia excretion (Maalouf et al., 2004).

Figure 2.4 Morphology of different stones (a) Calcium oxalate; (b) Calcium phosphate; (c) Uric acid; (d) Struvite and (e) Cystine
b) Struvite stones

Struvite stones are made up of ammonium, magnesium and calcium phosphate. These are often known as infection or triple phosphate stones. The occurrence of Struvite stones in females is more than in males and also is the leading cause of staghorn calculi. The cause of Struvite stones is the presence of neurogenic bladders and foreign bodies in the urinary tract. Recurrent urinary tract infections with urea splitting organisms may results in the alkalisation of urine and the addition of ammonium to the milieu. These types of stones are mostly radiopaque on standard radiographic imaging (Figure 2.4d).

c) Cystine calculi

Cystine calculi occurs due to an autosomal recessive disorder which cause the defective transport of dibasic amino acid leading to reduced reabsorption of cystine in the kidney. Only patients with homozygotic condition develop cystine calculi often since childhood. In these patients stone formed may be consist of pure cystine or may be mixed with calcium oxalate (Mattoo and Goldfarb, 2008). The stone formation occurs due to poor solubility of cystine at normal urinary pH. When the level of cystine in the urine rises above 250mg/l, the precipitation initiate and ultimately leads to generation of calculi (Figure 2.4e). A urinary cystine level of more than 250mg/24 hr is diagnostic marker for the disorder (Pietrow and Karellas, 2006).

2.2 Urinary Components

Many of the non-volatile nitrogen containing wastes produced in cellular metabolisms are required to be eliminated from the blood stream. Moreover, the surplus of ingested fluid, electrolytes and minerals must be excreted to maintain body equilibrium. Urine is composed of all of the body’s daily waste products excreted by the kidneys. Because of the extremely diverse chemical nature of urinary factors, it is practically impossible to categorize them as lithogenic or stone inhibiting with respect to urolithiasis etiology. Therefore, some recurrent stone-formers have no obvious
predisposing factors that can be detected by the standard metabolic evaluation techniques. In contrast, many hypercalciuric individuals never develop kidney stones. Uncertain pathophysiology indicates the complexity of physicochemical and biochemical processes as well as the insufficiency of global efforts on metabolic assessment of lithogenesis (Sikora et al., 2009). Kidney stones form as a consequence of tubular or interstitial supersaturation which is due to the elevated concentrations of urinary solutes. Many diverse processes alter urine flow rate or solute excretion rate and hence urinary solute concentrations at any given time.

2.2.1 Urinary flow rate

Urine volume must be large enough and excessive oligouria must be avoided to decrease the risk of precipitation of poorly soluble constituents in the urine (Halperin et al., 2008). Preserving effective circulatory volume is essential and hence control of the rate of water excretion is important for survival (Gowrishankar et al., 1998). The effect of volume on super saturation is amplified by the fact that it would affect both free calcium and free divalent phosphate concentrations.

2.2.2 Urinary pH influencing urinary supersaturation

The main role of pH in calcium phosphate precipitation is its effect on the proportion of divalent/monovalent phosphate. At blood pH around 7.4, approximately 80% of total phosphate exists as HPO$_4^{2-}$. Considering the amount of phosphate excretion (20-30 mmol/dl), the excretion of urine with this pH could imply a significant risk of precipitation. The typical urine pH in healthy individuals is around 6.0. At this pH only 10% of total phosphate exists as HPO$_4^{2-}$ ion. At urinary pH 6.1 one sixth of the urine phosphate is in the form of HPO$_4^{2-}$ whereas at higher pH of 6.8, half of the urine phosphate will be in its HPO$_4^{2-}$ form. The potential risk for precipitation of CaHPO$_4$ enhanced by 3-fold at urinary pH 6.8. In contrast, there is a very less risk when the urine pH rises from 7.1 to 7.5. Studies have evaluated the effect of pH on calcium and
phosphate limit concentrations in a typical parental nutrition regimen (Kamel et al., 2007).

### 2.2.3 Solute influencing urinary supersaturation

Alternative divalent cations to calcium may be considered as inhibitors of urinary supersaturation. Magnesium, a divalent cation, is a complexing agent for oxalate. Magnesium inhibits oxalate absorption and excretion, thus preventing its supersaturation. Magnesium forms oxalate and phosphate salts, which are more soluble compared to those of calcium (Massey, 2005). In addition, citrate and sulfate are anions with which calcium forms soluble complexes as alternatives to phosphate or oxalate. Urine also contains substances to which calcium binds, thereby reducing the free ion activity. Pyrophosphate, nephrocalcin, and osteopontin are other inorganic and organic crystal inhibitory calcium-binding moieties.

### 2.2.4 Stone Formation Inhibitors and Chelating Agents

#### a) Citrate

Citrate is one of the most significant chelating agents of calcium. As it binds calcium, the nucleation and growth of calcium crystals are inhibited. It chelates calcium at 1:1 ratio and when citrate concentration is more than calcium concentration virtually free Ca\(^{2+}\) activity is nil. The same is the reason for hypocitraturia being an important risk factor for developing calcium stones. Citrate has important role in the excretion of urinary bases as the bases can be excreted without raising urine pH with citrate. In this way it helps in preventing calcium phosphate precipitation by maintaining low divalent phosphate concentration (Schlieper et al., 2007).

#### b) Tamm-Horsfall protein

Tamm-Horsfall protein (THP) is expressed by the thick ascending limb of Henle’s epithelial cells. It is also known as uromodulin and is the most abundant protein found in
urine. A normal individual may produce up to 100 mg/day of this protein. THP plays dual role in the formation of CaOx stones. Its inhibitory effect on crystal aggregation and also on growth has been described for CaOx and hydroxyapatite stones. The animal studies reported that 16% of the mice which are deficient for this protein spontaneously developed nephrolithiasis. Interestingly, osteopontin expression is provoked in THP exposed to calcium overload. This suggests the synergistic action of both these proteins. The THP deficiency was also noticed in people with CaOx nephrolithiasis. There are reports about the decreased excretion of THP in urine in patients suffering from stone diseases (Schlieper et al., 2007).

c) Osteopontin

Osteopontin (OPN) is a major component of renal stones. It is also known as uropontin or nephropontin. Roughly 4 mg of OPN is secreted in urine per day. OPN expression is increased in kidneys of rats with ethylene glycol (EG) induced hyperoxaluria and CaOx nephrolithiasis. Interestingly, it can enhance the force of adhesion between a carboxylate tip and a specific crystal surface. The exact contribution of OPN in human nephrolithiasis is less clear. It may inhibit the process of nucleation, growth and aggregation of CaP crystals. If OPN is anchored to collagen or crystal surfaces, it could also serve as a nucleating site for new crystals (Evan et al., 2006). Reduction in OPN expression is reported to be associated with significant decrease in crystal deposition in animal models. Specific suppression of OPN mRNA expression in kidneys of hyperoxaluric rats leads to a decrease in OPN production and simultaneously inhibits renal crystal deposition (Tsuji et al., 2014).

d) Glycosaminoglycans

Glycosaminoglycans are the common name for sulphated biomolecules. The most commonly encountered glycosaminoglycans are chondroitin sulfate, heparan sulfate and hyaluronic acid in relation to stone diseases. The role of chondroitin sulfate is to delay the process of nucleation but dermatan sulfate may inhibit this process completely.
Hyaluronic acid acts as molecular glue for crystals at the surfaces of renal tubular cells. Moreover, association of calcium with these glycosaminoglycans may also reduce urinary inhibitory activity, and hence may enhance the risk of formation of stones (Lemann, 1993). An animal study showed an enhanced expression of heparin sulphate during urolithiasis in both distal and proximal tubules. In a canine tubular cells study it was found that synthesis of glycosaminoglycans may increase protection from toxic insults of CaOx crystals and oxalate ions. There is still ambiguity about the role of these glycosaminoglycans. Some human studies documented enhanced secretion in urine while many studies could not show major relation between glycosaminoglycan excretion in urine and urolithiasis (Schlieper et al., 2007).

e) Other Factors

The prevalence of depression in patients with urolithiasis was substantially greater than societal norms. Multiple stone-related factors were associated with significant psychological distress (Angell et al., 2012). There are some other biomolecules responsible for the formation of stones. Some of the other factors are enlisted in Table 2.1.

2.3 Etiological Factors

Many risk factors including genetic, metabolic, anatomic, dietary and environmental in nature have been identified in urinary tract calculi (Bastug and Dusunsel, 2012).

2.3.1 Family history

The association of family history with the risk of urolithiasis had been document in the past (Park et al., 2010). This risk of occurrence of stone disease is more in males as compared to the females. The family history has been documented in over 25% of the urolithiatic patients in a very old report (Resnick et al., 1968). In addition, in 2008, a study on paediatric patients also suggested the association of family history (Spivacow et al., 2008) with urolithiasis. Lerolle et al., (2002) had also reported a significant dose
dependent association between stone disease and calciuria in patients with family history of hypercalciuria. Moreover, patients with a family history of urolithiasis had also shown enhanced calcium levels in serum and a tendency for excretion of calcium in urine (Park et al., 2010). The role of environmental factors had been one of the important risk factor for the familial affinity of urinary stone disease. These factors mainly include similar diet patterns among family members (Curhan, 2007).

**Table 2.1 Factors associated with Urolithiasis**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrocalcin (osteocalcin family)</td>
<td>Tightly binds apatite and calcium and hence may inhibit nucleation of COM crystals</td>
</tr>
<tr>
<td>Calgranulin</td>
<td>Effective inhibitor of CaOx crystal growth</td>
</tr>
<tr>
<td>Urinary prothrombin</td>
<td>CaOx crystallization inhibitor in urine <em>in vitro</em></td>
</tr>
<tr>
<td>Bikunin</td>
<td>Prevents the adhesion of calcium oxalate crystal to renal tubular cells in human urine</td>
</tr>
<tr>
<td>Pyrophosphates</td>
<td>Natural inhibitors preventing hydroxyapatite precipitation <em>in vitro</em></td>
</tr>
<tr>
<td>Phytate</td>
<td>Inhibitor of CaOx crystal formation <em>in vitro</em></td>
</tr>
</tbody>
</table>

**2.3.2 Systemic disorders**

Many systemic disorders have been linked to kidney stone formation. Primary hyperparathyroidism, Crohn’s disease and renal tubular acidosis are the most common amongst others. Interestingly in 5% of people with some form of stone disease, primary hyperparathyroidism is the frequently reported morbidity. Enhanced body mass index (more than 30 Kg/m$^2$) and gain in weight are the crucial risk factors for urolithiasis
Both uric acid and CaOx stones formation risk is double in individuals with history of gout (Kramer et al., 2003). Furthermore, the risk of urolithiasis is increased by 30-50% in females with a history of type II Diabetes mellitus. No such association has been reported in men (Taylor et al., 2005). One longitudinal study reported a 31% increased risk for myocardial infarction in patients with a history of kidney stones (Rule et al., 2010). Possible reasons for such association include shared risk factors, an increased incidence of heart disease among patients with a history of kidney stones due to abnormalities of calcium metabolism (Cheungpasitporn et al., 2014).

### 2.3.3 Environmental factors

There is a strong correlation between certain environmental conditions and risk of developing kidney stones. One study suggested the role of heat and climate as significant risk factors for lithogenesis (Fakheri and Goldfarb, 2011). Global warming has been found to be an important risk factor of urolithiasis. Those individuals who work in a hot environment, have limited access to water or other fluids have been at increased risk of developing kidney stones (Atan et al., 2005).

### 2.3.4 Dietary factors

Diet has been an important aspect in health and well being. Many of the eating habits have been correlated with nephrolithiasis. The role of metals in urinary stone formation has been reported previously (Kuta et al., 2013). Several other nutrients have been reported to predispose individuals to stone formation. Phytate is a natural plant product and was also reported to substantially reduce the risk of stone formation in younger women (Curhan et al., 2004). The occupational exposure to cadmium had been associated with increased risk of stone formation. The primary source of cadmium is the food in the general population. The smoking is also an important additional source due to tobacco (Thomas et al., 2013). Higher dietary zinc intake is associated with increased risk of kidney stone disease (Tang et al., 2012). Increasing magnesium intake was reported to be associated with decreasing hyperoxaluria in patients with stone formation. Magnesium can make complexes with oxalate and significantly decline oxalate absorption in the
gastrointestinal tract and also calcium oxalate supersaturation in urine (Eisner et al., 2012a). Animal protein intake however, was not independently associated with stone diseases (Sorensen et al., 2012).

a) Calcium

The role of calcium is contradictory in predisposing individuals to kidney stone formation. Hypercalciuria has been identified as a predominant risk factor for urolithiasis. However, calcium supplements in the treatment of osteoporosis alone or in combination with another type of treatment does not significantly increase the risk of nephrolithiasis or renal colic (Candelas et al., 2012). Although high calcium intake in diet is strongly suspected of enhanced risk of urolithiasis, Curhan et al.,(2004) had reported that very low calcium intake can actually predispose to kidney stone formation as well. Greater dietary calcium intake significantly decreased the risk of incident kidney stones (Sorensen et al., 2012).

b) Vitamins

High vitamin C intake and vitamin B₆ deficiency may significantly enhance the chance of CaOx stone formation. Vitamin B₆ deficiency increases oxalate production, whereas vitamin C can be metabolized to oxalate and both can cause hyperoxaluria (Curhan et al., 2004). Vitamin D is involved in the body's calcium homeostasis. A previous study reported a relationship between serum vitamin D level and 24h urine calcium excretion in stone-formers (Eisner et al., 2012b).

c) Oxalate

Most kidney stones consist of calcium oxalate, and higher urinary oxalate increases the risk for calcium oxalate nephrolithiasis (Taylor and Curhan, 2007). Moreover, enhanced consumption of starchy foods and food with high content of oxalate was detected to be associated in the 10-16 years age group (51%) by Alaya et al (2013). Also, about one-third of patients with CaOx kidney stones may have increased absorption
of oxalate. In few individuals a deficiency of oxalate degradation by *Oxalobacter formigenes* may enhance the risk (Holmes and Assimos, 2004).

d) Fluid Intake and Beverages

Supersaturation of the urinary environment with stone forming constituents is a prerequisite for calculus formation and increased fluid consumption results in excretion of higher volume of urine which is less supersaturated with stone forming constituents (Filgueiras Pinto Rde *et al.*, 2013). Studies have been proposed that the urine output of less than 1L per day increases the risk of stone formation than those with higher urine output. On the other hand some beverages like grape fruit juice and soft drinks have been shown to increase the risk of stone formation (Curhan, 2007). Grape fruit juice intake has been associated with a 40% higher risk of stone formation. Also, with the soda intake there is an increased risk of kidney stone formation (Park and Pearle, 2007). Wang *et al.*, (2015) have reported the beneficial effects of alcohol and coffee intake in urolithiasis. The dose-response meta-analysis indicated that the rate of urolithiasis decreased by 10% for 10 g/day increase in alcohol intake. Epidemiologic studies have reported various results relating coffee to urolithiasis. The overall current literature suggests that coffee intake is inversely associated with risk of urolithiasis (Wang *et al.*, 2014).

2.3.5 Urinary Factors

Some urinary factors have been suggested to be the potent risk factors for renal stone formation. Hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia have been associated with enhanced risk of urolithiasis (Meschi *et al.*, 2011). Urinary volume has also been associated in the process of stone formation. An *in vitro* study suggested that urinary dilution, obtained through good hydration by means of soft mineral water reduces the crystallization tendency of calcium oxalate induced by an oxalate load (Guerra *et al.*, 2006). Alteration in pH is known to influence the stone formation by affecting the solubility of certain alkaloids. Grases *et al.*, (2012) reported that 34.1 and 41.5% of calcium oxalate dihydrate calculi were present in patients with urinary pH<5.5 and >6.0, respectively. At urinary pH<5.5, uric acid becomes insoluble and forms crystals of anhydrous or dihydrate uric acid, depending on concentrations.
2.3.6 Climatic Factors

Temperature and relative humidity have a strong correlation with calculi presentation rate and relative humidity has a trend towards overall calculi presentation rate (Sirohi et al., 2014). A significant association has been observed between exposure to ambient heat and urolithiasis among outdoor working populations (Luo et al., 2014). The role of a significant human migration has been reported to be greater than global warming on the observed worldwide increasing prevalence rate of nephrolithiasis (Fakheri and Goldfarb, 2011).

2.3.7 Vitamin D

High serum levels of vitamin D may play an important role in the pathogenesis of urolithiasis in infants with hypocalcaemia (Fallahzadeh et al., 2012). Vitamin D is involved in maintaining calcium homeostasis. The role of vitamin D in nephrolithiasis has not been documented in a systematic manner. The observational studies showed contradiction in the correlation between higher nutritional vitamin D store and enhanced risk of urolithiasis (Tang et al., 2012). Increased urinary calcium and oxalate excretion are among the risk factors for kidney stone formation. Calcitriol increases oxalate absorption as well as its urinary excretion by promoting calcium absorption.

2.3.8 C-Reactive Protein

C-reactive protein (CRP) is a pointer of low-grade inflammation. Serum CRP levels have been found to be associated closely with self-reported kidney stones in younger individuals. In patients with renal colic due to urolithiasis, CRP provides an objective and useful parameter over leukocytosis or serum creatinine level for deciding placement of urinary stent (Angulo et al., 2010). Urolithiasis, a state of low-grade inflammation, a significant increase in CRP levels is reported in diabetic urolithiasis patients (Hasna et al., 2015). A significant relationship between serum CRP and self-reported kidney stones in younger individuals has been highlighted (Shoag and Eisner, 2014). However, further studies are needed to understand the mechanisms underlying these epidemiological findings.
2.3.9 Microbial infections

A correlation between Randall’s Plaques and the calcifying nanoparticles (CNP, Nanobacteria) which are similar to snowballs had been documented in the past. The occurrence of these had been detected in various pathological calcifications including kidney stones, prostatic and gallbladder stones. CNP are calcified self-propagating entities which are similar in mineral composition to spherical bodies observed in Randall’s plaques (Ciftcioglu et al., 2008). The common microbes reported to be associated with infection stones are *Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Providentia*. Urease produced by these miro-organisms splits urea into ammonia and carbon dioxide in the urine (Bichler et al., 2002). Further, ammonia and carbon dioxide are hydrated to ammonium ions and bicarbonate respectively. This metabolic process elevates the urine pH and at alkaline conditions phosphate ions are precipitated in the form of magnesium ammonium phosphate, commonly known as Struvite.

2.4 Mechanism of stone formation

Stone formation is a multifactorial process and occurs due to imbalance between promoters and inhibitors. A physicochemical theory of urolithogenesis considers urine as supersaturated solution in which homogeneous or heterogeneous nucleation can lead to initial crystal formation, which can then aggregate and grow (Coe et al., 2010). The stone formation includes but not limited to the following steps. The outline of mechanisms proposed for the crystal formation is depicted in figure 2.5.

2.4.1 Nucleation

Nucleation describes the process that occurs when the activity of calcium salts reaches the level at which the solid phase begins to appear. Although materials acting as nidus for kidney stone growth remain unclear, it has been suggested that COM crystal deposition may occur on a preformed calcium phosphate nidus, known as Randall’s plaques (Lingeman et al., 2009). However, Randall’s plaques are not found in all individuals suffering from kidney stones (Ryall, 2008). Studies on knockout mice showed that the presence of calcium phosphate is not necessary for calcium oxalate crystal
deposition (Khan and Glenton, 2008). The renal tubular injury may also promote the retention of crystals leading to the formation of stone nidus (Evan et al., 2006). Moreover, renal tubular injury also increase the nucleation process at low supersaturation (Lu et al., 2012).

**Figure 2.5** Mechanism of stone formation (Aggarwal et al., 2013b)

### 2.4.2 Randall’s plaque Formation

Alexander Randall noted the presence of interstitial papillary deposits, which he referred to as plaque, and hypothesised that these are the sites of calcium nephrolithiasis (Kuo et al., 2003). When these deposits become exposed to the urinary space following erosion of the overlying urothelium, they become nidus for the formation of calcium stones (Miller, 2011).

### 2.4.3 Crystal Growth

Microscopic nuclei are too small to cause any obstruction in the urinary tract so it is the crystal growth which is critical to stone disease. Once a crystal nucleus has achieved 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 (Aggarwal et al., 2013b). Crystals are regular lattices, composed of...
repeating subunits and they grow by the incorporation of calcium and oxalate or phosphate, into new subunits on their surfaces. In meta-stable solutions at 37°C, growth rates of calcium oxalate and the stone-forming calcium phosphate crystals are rapid and depend on the extent of super saturation (Baumann and Affolter, 2014).

Many of calcium oxalate stones are attached to the renal papillae, over a whitish deposit which is Randall’s plaque. Evan et al., (2006) had shown 100% plaques deposits in patients of idiopathic calcium stone. Though in only 48% cases, calculi were stick to the Randall’s plaque. The histopathological evidence suggests that plaque cultivates stones.

2.4.4 Crystal Aggregation

The crystals stick together in solution to form large particles. The procedure is called aggregation. This is a crucial step in urolithiasis and it has a significant effect on the size of particles. The crystal aggregates are often present in urine and renal stones. Crystal aggregation is enhanced due to various macromolecules present in urine like Tamm-Horsfall glycoprotein that serves as molecular glue because of the presence of multiple binding sites (Aggarwal et al., 2013b).

2.4.5 Crystal-Cell Interaction

Supersaturation of the urine is the primary cause of crystallization. Crystal cell interactions means the the process of attachment or endocytosis of crystals to renal tubular cells (Aggarwal et al., 2013b). CaOx monohydrate crystals quickly get adhered to microvilli present on the surface of cells and are later on internalized. This association of crystals to the cell membranes is inhibited by various polyanionic molecules in urine such as glycosaminoglycans, glycoproteins, and citrates.

2.4.6 Endocytosis of CaOx Crystals

Lieske et al. (1994) had reported the process of internalisation of crystals into tubular epithelial cells and cell proliferation in a transplanted kidney. They also proved and confirmed the process of engulfment by using cell culture assays.
2.4.7 Hyperoxaluria

Exposure to oxalate, a major component of kidney stones, elicits a cascade of responses in renal epithelial cells that often leads to cell injury or death (Jonassen et al., 2003). Some of these responses are elicited by one or more lipid signaling pathways. Especially, the exposure of oxalate may activate phospholipase A2 which hydrolyzes acyl group from the sn-2 position of phospholipids and produces free fatty acids such as arachidonic acid and lysophospholipids. This molecules generally act as intracellular signaling molecules (Miller et al., 2000). Formation of ceramide, another lipid that serves multiple intracellular signaling functions, also enhances due to oxalate exposure (Cao et al., 2004). Oxalate exposure may also enforce an oxidative stress on renal epithelial cells by either promoting the lipid peroxides accumulation or/and reducing the cellular antioxidants availability including reduced glutathione (Khand et al., 2002). Oxidative stress in the kidneys is considered a major cause of renal injury and inflammation, giving rise to a variety of pathological disorders. The abundance of ROS (Oxidative stress) may lead to permanent damage to macromolecules and may also be responsible for interference in redox-dependant signaling processes (Figure 2.6) (Khan, 2014).

Figure 2.6 Oxidative stress mediated damage to DNA and protein (Joshi et al., 2013)
In vitro cell culture techniques revealed that the oxalate and CaOx crystal exposures to renal epithelial cells may be responsible for the amendment in expression of various subunits involved in activation of NADPH oxidase leading to ROS production and may damage renal cells (Hanna et al., 2002). Significant correlation has been established between CaOx crystal-induced up regulation of p22phox and p47phox and NADPH oxidase activation and associated cell injury. It has been proposed that activation of Renin–angiotensin system leads to the activation of NADPH oxidase upon exposing the cells to high oxalate and/or CaOx/CaP crystals (Umekawa et al., 2004) and further generation of ROS (Figure 2.7).

2.4.8 Oxidative stress

Oxidative stress is best defined in broad terms as an alteration in pro-oxidant-antioxidant balance in favour of the former that leads to potential damage. Free radicals are atoms, molecules or ions with unpaired electrons that are highly unstable and, therefore, are active towards chemical reactions with other molecules. They are derived from three elements: oxygen, nitrogen and sulphur, thus creating reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) respectively. Reactive oxygen species include free radicals like the superoxide anion (O$_2^-$), hydroperoxyl radical (HO$_2^-$), hydroxyl radical (’OH), nitric oxide (NO) and other non-radical species like hydrogen peroxide (H$_2$O$_2$), singlet oxygen ($^1$O$_2$), hypochlorous acid (HOCl) and peroxynitrite (ONOO$^-$) (Lu et al., 2010).

ROS may lead to conformational changes due to protein modification, like caused by kinases and phosphatases. They may also activate nuclear factor κB (NFκB) which further has important roles in the regulation of immune response to infection (Anrather et al., 2006). Activation of NFκB leads to over expression of adhesion molecules such as intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1 and e-selectin on the endothelium. ROS have also been reported to stimulate inflammasome (different cytosolic molecular complexes) that have enzymatic activity mediated by the activation of caspase-1(Dworakowski et al., 2008). Inflammasomes are documented to be involved
in maturation and cleavage of cytokines mainly IL-1\(\beta\) involved in many of the inflammatory reactions (Joshi et al., 2013).

![Diagram showing the generation of reactive oxygen species in hyperoxaluria](image)

**Figure 2.7** Schematics of hyperoxaluria induced generation of reactive oxygen species, involvement of mitochondria and their effect on cellular physiology and pathology (Khan, 2005).

Most of the reactive oxygen intermediates are produced as a by product of respiratory chain complex reactions in mitochondria. During healthy metabolism, cytoplasmic antioxidants as well as those present in mitochondria check cell damage caused by these reactive oxygen intermediates. However, all those biochemical circumstances that enhance reactive oxygen intermediates or diminish antioxidant availability may cause cellular damage (Khan, 2005). There is evidence suggesting that oxalate and/or CaOx crystal exposure leads to mitochondrial up regulation of ROS production in renal cells (Khand et al., 2002). However, the mechanism of action of
oxalate exposure in enhancing mitochondrial ROS production is not very well established.

One of the proposed mechanisms suggests that the ROS production is mediated by the enhanced intracellular production of the arachidonic acid. Arachidonic acid enhances the production of hydrogen peroxide by uncoupling electron transport via complexes I and III in the mitochondrial respiratory chain. Moreover, arachidonic acid enhances the production of hydrogen peroxide in isolated rat diaphragm mitochondria by interacting with complex I (Nethery et al., 2000). It has also been suggested that these ROS can impaired the critical active sites of all the enzymes involved in the respiratory chain and hence suggest the role of mitochondria as a source as well target for ROS (Cao et al., 2004).

(a) Chemistry of ROS

The chemical structure of ROS influences the ability of different species to react with specific cellular substrates within the microenvironment in which they are produced. Due to the unpaired electron, free radicals are very unstable and will react with any atom or molecule in their vicinity. In biological systems, the most important free radicals are derivatives of oxygen (Satriano et al., 1993). Single-electron reduction of oxygen either by enzymatic catalysis or by "electron leaks" from various electron transfer reactions produces superoxide (O$_2$ + e$^-$ → O$_2^-$). In contrast to its remarkable stability in many organic solvents, O$_2^-$ in aqueous solution is short-lived. This "instability" in aqueous solutions is the basis of rapid dismutation of O$_2^-$ to H$_2$O$_2$ through the Haber–Weiss reaction (2 O$_2^-$ + 2H$^+$ → H$_2$O$_2$ + O$_2$). If H$_2$O$_2$ reacts with an iron catalyst like Fe$^{2+}$, the Fenton reaction can take place (Fe$^{2+}$ + H$_2$O$_2$ → Fe$^{3+}$ + 'OH + OH$^-$) forming the most reactive and damaging form of oxygen free radicals, the hydroxyl radical HO'. (Flora, 2009). Although H$_2$O$_2$ is relatively stable and can diffuse through plasma membranes, hydrogen peroxide is generally converted into H$_2$O rapidly by catalase or glutathione peroxidase (GPx, Figure 2.8).
(b) **Sources of ROS**

Reactive oxygen species are produced from both exogenous and endogenous sources. Exogenous sources include inflammatory cytokines, microbes, environmental carcinogens, dietary factors, various xenobiotics, metal ions, ultraviolet light and ionizing radiation (Lim *et al.*, 2010). Endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes, NADPH oxidases and xanthine oxidases (Perez-Matute *et al.*, 2009).

(c) **Mitochondria and ROS**

Reactive oxygen species are mainly produced within the mitochondria of a cell. Along the electron transport chain, electrons derived from the oxidation of NADH or FADH$_2$ can “leak” and directly react with oxygen to produce ROS. Free radical production occurs primarily at complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome c reductase), with the latter being the major site of ROS production (Ma *et al.*, 2010). About 1-3% of the oxygen molecules in the mitochondria are converted into ROS (Valko *et al.*, 2004). Monoamine oxidase (MAO) belongs to the
class of flavoprotein and is localized in the outer layer of mitochondrial membrane. It is another important source of free radicals (i.e. $H_2O_2$). The mechanism of free radical involving MAO includes the oxidative deamination of amines including primary aromatic amines, diamines with long chains etc. Being neutral, $H_2O_2$ can effectively cross the mitochondrial membranes and elevate the intracellular concentration in cytoplasm.

(d) Cytochrome P450 and ROS

The cytochrome P450 enzymes are terminal oxidases located within the membrane bound microsomal mono-oxygenase system, which is localized in the endoplasmic reticulum of most animal tissues. This enzyme utilizes oxygen for oxidation of foreign compounds in the detoxification process, and performs hydroxylation reactions to remove or inactivate toxic compounds in the body. During both reactions, electrons may leak and affect oxygen molecules, resulting in the formation of ROS (Froy, 2009).

(e) NADPH Oxidases and ROS

The family of NADPH oxidases is a group of plasma membrane associated enzymes that have been implicated as a major source of ROS generation (Nauseef, 2008; Perez-Matute et al., 2009). These oxidases have the ability to produce ROS in the reactions involving the transport of electrons through the cell membrane. When a phagocytic cell is exposed to foreign compounds such as microbes or cytokines (e.g. TNF-α, IL-1 or IL-6), the defense enzyme undergoes a series of reactions called “respiratory burst” that enable the cell to provide oxidizing agents to destroy such compounds (Ma et al., 2010).

(f) Cellular Damage by ROS

An imbalance between ROS and antioxidants resulting from the increased production of ROS and/or reduction in the amount of antioxidants, generates a state of oxidative stress in the cell (Ma et al., 2010). Oxalate-induced oxidative cell injury is one of the major mechanisms implicated in calcium oxalate nucleation, aggregation and growth of kidney stones. Oxalate-induced NADPH oxidase-derived free radicals play a
significant role in renal injury. The main targets of ROS, RNS and RSS are lipids, proteins, nucleic acids, sugars and lipids (Carocho and Ferreira, 2012) (Figure 2.9).

**Figure 2.9** Cellular Targets of ROS. Modified from Carocho and Ferreira, (2012)

(g) **Lipid peroxidation**

Lipid peroxidation (LPO) is often the first chemical reaction caused by free radicals, as these free radicals are usually generated near membranes (Figure 2.10). Lipid peroxidation consists of three steps: initiation, propagation and termination (Fritz and Petersen, 2011). (i) Free radicals react with the double bonds found in polyunsaturated fatty acids (PUFAs). (ii) The free-radical mediated abstraction of a hydrogen atom from one of the double bonds yields a carbon-centered lipid radical species that can readily interact with O$_2$. (iii) This results in the production of a lipid peroxyl radical which can abstract a hydrogen atom from another fatty acid yielding (iv) a new radical and a lipid hydro-peroxide, hence it establish a chain reaction. Potent antioxidants like Vitamin E can terminate the chain reactions and prevent the peroxidation of lipids (De Bandt et al.,
The hydroxyl radical is one of the main radicals in lipid peroxidation and acts according to the following generic reaction:

\[
LH + \cdot OH \rightarrow H_2O + L\cdot
\]

Where LH represents a generic lipid and L· represents a lipid radical.

Many of the by-products are generated during lipid peroxidation such as Malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), glyoxal and methylglyoxal (Navarro-Compan et al., 2013), which are often used to estimate the extent of LPO in biological system.

**Figure 2.10** Intracellular mechanism of Lipid peroxidation
(h) Detoxification of Reactive Oxygen Species

There are number of antioxidants present in the cell to inhibit the harmful effects of ROS. Natural antioxidant system is divided into enzymatic and non-enzymatic antioxidants. These are tabulated in figure 2.11.

Superoxide dismutase (EC 1.15.1.1)

Superoxide dismutase (SOD) is a secretory, tetrameric glycoprotein with a high affinity to certain glycosaminoglycans, such as heparin and heparan sulphate. It requires copper and zinc as co-factors for showing its enzymatic activity. It plays an important role in maintaining vascular tone, lung function and metabolism of nitric oxide. Also, SOD has shown important association with the pathology of many diseases such as diabetes, atherosclerosis, arthritis (McCord, 1974) and kidney stone diseases. It converts superoxide anions into hydrogen peroxide which is further used as a substrate of catalase (Rahman, 2007) in the interstitial spaces of tissues and in extracellular fluids (plasma, lymph, and synovial fluid). This mechanism helps to prevent the formation of ROS and their derivatives.

Figure 2.11 Classification of natural antioxidants. Modified from Carocho and Ferreira, (2012)
Catalase (EC 1.11.1.6)

Catalase (CAT) catalyzes the breakdown of \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \) and \( \text{O}_2 \). It is a homotetramer and is present commonly within the whole cell (Kirkman and Gaetani, 2007). It helps to reduce the levels of harmful \( \text{H}_2\text{O}_2 \) produced within the cells. Both CAT and GPx catalyse the degradation of \( \text{H}_2\text{O}_2 \), but catalase has a much lower affinity for \( \text{H}_2\text{O}_2 \) at low concentrations compared with GPx. The value of \( K_m \) for GPx is 1µM while for CAT is 1 mM, therefore, CAT does not show significant activity under physiological conditions. Due to its comparatively low affinity for hydrogen peroxide CAT becomes an key enzyme when the concentration of \( \text{H}_2\text{O}_2 \) is elevated e.g., by exposing cells to drugs and chemicals that increase intracellular \( \text{H}_2\text{O}_2 \) generation (Powers and Jackson, 2008).

Glutathione peroxidase (EC 1.11.1.9)

The enzyme glutathione peroxidase (GPx) catalyzes the reduction of \( \text{H}_2\text{O}_2 \) or organic hydroperoxide (ROOH) into water (\( \text{H}_2\text{O} \)) and alcohol (ROH), respectively. Reduced glutathione (GSH) or in some cases thioredoxin or glutaredoxin are used as electron donor (Holmgren et al., 2005). When GSH is the electron donor, it donates a pair of hydrogen ions and itself is oxidized into glutathione disulfide (GSSG) as follows:

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O}
\]

\[
2\text{GSH} + \text{ROOH} \rightarrow \text{GSSG} + \text{ROH} + \text{H}_2\text{O}
\]

The enzyme has a lower \( K_m \) value for \( \text{H}_2\text{O}_2 \) than CAT and is considered more important when low amounts of \( \text{H}_2\text{O}_2 \) are generated (Powers and Jackson, 2008).

Reduced glutathione (GSH)

Reduced Glutathione (GSH) is a tripeptide also known as \( \gamma \)-glutamyl-cysteinyglycine. It is present in all mammalian tissues as the most abundant non-protein thiol. It protects against oxidative stress and is a major determinant of redox signaling. It has vital role in detoxification of xenobiotics, and regulates various important cellular functions (Lu, 2012). Cytosol is the major source of GSH (80–85%) followed by mitochondria (10–15%) and a small percentage is also found in endoplasmic reticulum
Biosynthesis of GSH occurs in the cytosol in a tightly regulated manner. The deregulation of GSH biosynthesis may lead to a number of diseases such as Diabetes mellitus, alcoholic liver disease, pulmonary and liver fibrosis, cholestatic liver injury and drug-resistant tumour cells (Lu, 2012). Levels of GSH have an effect on the expression or activity of caspases and other signaling molecules important in cell death (Ballatori et al., 2009; Garcia-Ruiz and Fernandez-Checa, 2007).

**Vitamins**

Vitamin E is chemically known as α-tocopherol has been known to be a radical-chain breaker. Due to its hydrophobic nature, Vitamin E operates in a lipid environment. The effects of α-tocopherol as an antioxidant are thus restricted to its direct effects in membranes and lipoprotein domains (Azzi, 2007). Vitamin E is a major lipid soluble antioxidant, protecting lipids against peroxidative damage. Vitamin C, also known as Ascorbic acid, on the other hand is a water soluble vitamin and is considered to be one of the most important antioxidant in extracellular fluids (El-Gendy et al., 2010). It can vary efficiently detoxify superoxide, hydrogen peroxide, hydroxyl radical and peroxyl radicals. Vitamin C may protect cell membranes against peroxidation by promoting the activity of tocopherol (Fuchs-Tarlovsky, 2013).

**2.5 Clinical Implications & Diagnosis of Urolithiasis**

The agonizing pain in the flank and lower abdomen from kidney stone is most often compared with the pain of normal labour and delivery. However, kidney stone colicky pain usually appears suddenly, without any warning sign and cripples the patient owing to intense sharp, stabbing and shooting pain. Kidney stones can also cause nausea, vomiting, fever in addition to blood in the urine, pain with urination and other symptoms of infection as well (Foxman, 2002).

**2.5.1 Parenchymal Kidney injury**

In essence, kidney stones are not merely a mechanical disease or an extreme painful condition, but they can cause parenchymal damage and reduce kidney function by
different mechanisms which include either direct kidney tissue injury or indirectly by urinary obstruction and infection. Moreover, iatrogenic renal injury by procedures needed to remove stones sometimes cause serious problem (Wood et al., 2011). Evidence is accumulating that nephrolithiasis is associated with decreased glomerular filtration rates and creatinine clearances (Curhan, 2007).

2.5.2 Diagnosis

The focused history of the patient is the primary diagnostic tool of the urinary tract calculi. Systemic abnormalities in urolithiasis often include but not limited to intestinal disease, calcium homeostasis disorders mainly as primary hyperparathyroidism, conditions associated with granulomatous diseases, type II Diabetes, obesity, recurrent urinary tract infection, bariatric surgery, medullary sponge kidney and various drug treatments.

(a) Urinalysis

Urinalysis must be performed in all of the expected patients of calculi. Apart from typical microhematuria, many of the important findings to be recorded are the pH of urine and also the presence of crystals, which may help to identify the stone composition (Abdel-Gawad et al., 2014; Kazi and Benz, 2014).

(b) Renal Ultrasound

Ultrasonography can be used as a screening tool for hydronephrosis or stones within the kidney or renal pelvis. A renal ultrasound can also determine the amount of renal parenchyma present in an obstructed kidney, in addition to the presence of stones. The ultrasound can be used in combination with plain abdominal radiograph to determine hydronephrosis or ureter dilation (Colella et al., 2005). The advantages of ultrasonography include it being readily available, quickly performed and sensitive to renal calculi. However, it is practically blind to ureteral stones with a sensitivity of only 19% (Carter and Green, 2011).
(c) Plain-Film Radiography

Plain film radiography of kidney, ureter and bladder (KUB) is sufficient most of the times to know the size and location of radio plaque urinary stones. For radiography calcium containing stones, like CaOx and CaP stones are easiest to detect (Portis and Sundaram, 2001). Uric acid stones and the stones which are mainly composed of cystine or magnesium ammonium phosphate are less radio plaque calculi and hence are difficult to detect on plain-film radiographs. Although 90% of urinary calculi have historically been considered to be radio plaque, the sensitivity and specificity of KUB radiography alone remain poor. KUB radiographs are useful in the initial evaluation of patients with known stone disease and in following the course of patients with known radio plaque stones (Levine et al., 1997).

(d) Intravenous pyelography

Intravenous pyelography is the technique which involves intravenous fusion of radio-opaque contrast media into the body. It has been considered the standard imaging modality for urinary tract stones. For a surgeon, the pyelogram may provide very helpful information regarding size, location and the environment around calculi. It has a greater sensitivity (64-87%) and specificity (92 to 94 %) as compared to ultrasonography and KUB radiography for the detection of renal calculi (Niall et al., 1999). Often liquid iodine and barium sulphate are used as contrasting media and have potential for adverse effects (Katzberg, 1997).

(e) Non contrast Helical CT

The use of non contrast helical CT is increased in the initial assessment of renal colic (Pearle et al., 1999). This imaging modality is not only fast and accurate but also identifies all stone types in all locations. Its sensitivity (95 to 100%) and specificity (94 to 96%) suggested that it may definitively exclude stones in patients with abdominal pain (Mitterberger et al., 2007). Computed tomography kidneys, ureter and bladder (CTKUB) is the accepted gold standard investigation for suspected renal colic.
2.6 Management of kidney stones

The management of urolithiasis is heterogeneous with various aspects and depends on the severity of the disease and patients conditions. There are three levels of treatment, first about dietary and drinking habits of patients with calcium stone disease, the next level involves specific dietary commendations and the third level always includes intervention (Surgical procedures and pharmacological treatment). There is much interest among physicians and patients to identify effective measures to promote stone passage, stone dissolution and stone prevention. The last two decades have seen a revolution as far as the management of urolithiasis is concerned. Though the interventional techniques are very advanced for stone removal, the recurrence of urolithiasis is a major concern. From the 15% rate of recurrence may reach to as high as 70% within 9 years of initial stones (Barnela et al., 2012). The management may be divided into surgical and non-surgical procedures. The surgical procedures include following techniques.

2.6.1 Extracorporeal Shock Wave Lithotripsy (ESWL)

Extracorporeal Shock Wave Lithotripsy (ESWL) helps to rupture renal calculi by employing high energy acoustic pulses also called shock waves. These waves are generated outside the body using an external device. The high energy sound waves generated in this way pointed at the site of the calculi to break it into small pieces (Figure 2.12). The whole process may require to be repeated a number of times to shatter the calculi completely. A slight injury with internal bleeding may occur due to the presence of shattered fragments of stones that leads to the appearance of blood in the urine (Aboumarzouk et al., 2011). The one of the limitation of employing ESWL for the treatment of stones is that sometimes fragments of the ruptured stone left behind can provide site for new stones development (Carr et al., 1996). But this disadvantage is limited to the cases where multiple treatment sessions were involved (McAteer and Evan, 2008).

2.6.2 Ureteroscopic Procedure

This is another regularly used procedure to remove the renal calculi. The urologists capture the stone through an invasive technique. A tube is being inserted up the
urethra through the bladder to the actual location of the calculus. At that point in time the stone can be ruptured using laser or merely captured with a basket device (Schuster et al., 2002). This procedure is extremely uncomfortable to the patient. Tissue damage and injury to urinary tract are the major limitations of this procedure. A complicated surgical procedure may be required if the calculi do not break properly (Wolfe et al., 2013).

Figure 2.12 Extracorporeal Shock Wave Lithotripsy (http://www.dreamstime.com/)

2.6.3 Percutaneous Nephrolithotomy

This procedure is a more invasive and involves making an incision in the backside region and channelling to the stone within the kidney (Figure 2.13). PCNL has become the standard treatment of care for complicated upper track urolithiasis (Honeck et al., 2009). This procedure cannot be performed repeatedly due the permanent damage to the kidney tissue to some extent. PCNL is contra indicated for patients with obesity, pelvic kidney, or spinal deformities, such as scoliosis, kyphosis, and lordosis (Veeratterapillay et
Despite being minimally invasive it is associated with complications (Li et al., 2009c) including visceral injury, renal vascular injury, urinary tract perforation/leakage, urosepsis, and hydrothorax (Li et al., 2012).

Figure 2.13 Percutaneous Nephrolithotomy
(http://www.ccmurology.com/surgery/pcnl.php)

2.6.4 Allopathic Management of Urolithiasis

Medical therapy has been demonstrated to significantly decrease stone recurrence rates and may be cost effective as well. Acute therapy of nephrolithiasis mainly intended to manage pain, forcing the diuresis and hydration. In acute conditions non steroidal and narcotic analgesics seem to be prominent therapeutic choice in the management of pain (Table 2.2). In calcium stones-hypercalciuria, thiazide diuretics administration can enhance the tubular reabsorption of calcium and ablate the intestinal calcium absorption (Bihl and Meyers, 2001). The effect of administration of thiazides in reducing urinary calcium is well documented but a number of side effects like fatigue, dizziness, impotence, musculoskeletal symptoms or gastrointestinal complications may occur due to long term usage. Administration of thiazide also results in intracellular acidosis and hypocitraturia due to depletion of potassium (Heilberg and Schor, 2006; Sayer et al., 2010). In recurrent idiopathic conditions of calcium stones-hypercalciuria, indapamide stood to be the best choice for its management (Martins et al., 1996). Potassium citrate reduces urinary saturation of calcium salts and is an effective agent for uric acid stones although, adverse effect of gastrointestinal origin including epigastric pain, abdominal distension or diarrhoea are common (Moe, 2006).
Pyridoxine, which promotes the conversion of glyoxylate to glycine, is the only medicine available to treat primary hyperoxaluria. Oxabsorb is a marine hydro-colloid that bonds with oxalate and calcium supplements. Administration of oxasorb has been found to be in patients with enteric hyperoxaluria (Tiselius, 2003).

The principles of treatment for uric acid stones include effective alkalinisation of urine to 6.5 (pH), dietary restriction of purine rich foods and adequate water intake. Treatment options include potassium citrate and acetazolamide for effective alkalinisation; and administration of allopurinol and febuxostat to minimize the uric acid synthesis (Kenny and Goldfarb, 2010). Allopurinol helps in the blockage of uric acid production. It also reduces heterogenous nucleation of CaOx by both uric acid and monosodium urate. In addition, the adsorption of normally occurring macromolecular inhibitors of CaOx crystallization by uric acid or monosodium urate could possibly be averted when using this drug (Heilberg and Schor, 2006). The antioxidant effect of allopurinol in nephrons also has been suggested. It helps in neutralizing the negative effects of free radicals (Tiselius, 2003).

As the struvite stones are the infectious stones manifested by the bacterial infections, the antibiotic therapy should be recommended for a period of at least three months (Sodimbaku and Pujari, 2014). Specifically, the urease inhibition is preferred in certain cases of patients with acetohydroxamic acid or flurofamide (Griffith et al., 1991; Williams et al., 1984). The other treatment options include the therapy of tiopronin (α-mercaptopropionyl glycine), ascorbic acid and angiotensin converting enzyme (ACE) inhibitors (Fattah et al., 2014). Moreover, the treatment option of captopril, an ACE inhibitor still remains to be unclear (Koll and Pearle, 2013).

2.7 Phytotherapy

Medicinal flora have been known for millennia and are being used as a rich source of therapeutic agents worldwide. More than 3/4th of global population, mostly from the developing countries, depends on herbal medicines for their basic healthcare needs with around 800 plants being used in indigenous systems of medicines. The last two decades have seen a continuous increase in the use of herbal medicine due to their less toxicity and side effects.
Table 2.2 Medical management of urolithiasis dependent on pathophysiological factors (Moe, 2006)

<table>
<thead>
<tr>
<th>Pathophysiological Factor</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low urine Volume/ Hypercalciuria</td>
<td>Hydrochlorothiazide or Indapamide + potassium alkali</td>
</tr>
<tr>
<td>Hypocitrateruria</td>
<td>Potassium Citrate</td>
</tr>
<tr>
<td>Hyperoxaluria</td>
<td>Pyridoxine for primary hyperoxaluria</td>
</tr>
<tr>
<td>Hyperuricosuria</td>
<td>Allopurinol</td>
</tr>
<tr>
<td>Low urinary pH/ Cystinuria</td>
<td>Pottasium citrate</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>β-mercaptoproprionyl glycine</td>
</tr>
<tr>
<td></td>
<td>Antibiotic</td>
</tr>
</tbody>
</table>

Urolithiasis has been a matter of concern to clinicians since the ancient times. Different formulations have been tried and used during the ages to treat urinary stones. In the traditional system of medicine, most of these medicines were plant based and were very useful. However, the rationale behind their usage was never well established through systematic pharmacological and clinical studies except for some composite herbal drugs. Some of the popular marketed composite antiurolithiatic herbal formulations such as Cystone (Himalaya Herbal Healthcare, India), Calcury (Charak Pharma Pvt. Ltd.), Neeri (Aimil Pharmaceuticals, India), Uriflow (BioNeutrix Healthcare of Brooklyn, New York), Uriflush (Global Biosciences, Indonesia) and Culdisol (Ganga Pharmaceuticals, India), Divya Vrikkdoshhar Kwath (Swami Ramdev's Divya Pharmacy) have been used globally.

2.7.1 Herbal plants used in urolithiasis

Synthetic chemical drugs used in the management of urolithiasis cause different adverse effects such as haemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney which are sufficient enough to cell injury and may often be associated with recurrence of renal stone formation (Terlecki and Triest, 2007). Herbal
drugs are reported to be safer and have created interest among the people by their clinically proven effects like immunomodulation, adaptogenic and antimutagenic. Urolithiasis management include a number of medicinal plants that show antiurolithiatic activity and play vital role in the prevention of disease (Table 2.3). The seeds of Trachyspermum ammi and Dolichos biflorus have been proven to contain antilithiatic proteins (Kaur et al., 2009; Peshin and Singla, 1994) and are being used as a part of naturopathy.

Table 2.3 List of Plants Showing Antiurolithiatic Activity by dissolving the stones

<table>
<thead>
<tr>
<th>Plant (Component)</th>
<th>Potential Beneficial Actions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herniaria hirsuta</td>
<td>Eliminates crystals attached to renal cell surface, excrete more COD than COM</td>
<td>(Atmani et al., 2004)</td>
</tr>
<tr>
<td>Cranberry juice</td>
<td>Increases excretion of urinary citrate, decreases excretion of urinary oxalate and calcium ion</td>
<td>(Gettman et al., 2005; Kessler et al., 2002)</td>
</tr>
<tr>
<td>Grapefruit juice</td>
<td>Increases urinary citrate excretion</td>
<td>(Trinchieri et al., 2002)</td>
</tr>
<tr>
<td>Dolichos biflorus</td>
<td>Decreases calcium phosphate precipitation</td>
<td>(Atodariya et al., 2013)</td>
</tr>
<tr>
<td>Bergenia ligulata</td>
<td>Decreases calcium phosphate precipitation</td>
<td>(Aggarwal et al., 2014b; Bashir and Gilani, 2009)</td>
</tr>
<tr>
<td>Costus spiralis</td>
<td>Decreases size of stone with unknown mechanism,</td>
<td>(Jarald et al., 2011)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Effect</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td><em>Amni visnaga</em></td>
<td>Prevent cell damage caused by oxalate and renal crystal deposition</td>
<td>(Vanachayangkul et al., 2010; Vanachayangkul et al., 2011)</td>
</tr>
<tr>
<td><em>Phyllanthus niruri</em></td>
<td>Modulate urinary inhibitors of CaOx crystallization and other factors associated with renal stone formation, stabilize elevated urinary calcium levels</td>
<td>(Barros et al., 2006; Freitas et al., 2002)</td>
</tr>
<tr>
<td><em>Tribulus terrestris</em></td>
<td>Cytoprotective potency in cell culture and decrease renal epithelial damage, inflammation and restored normal glomerular morphology</td>
<td>(Aggarwal et al., 2012b; Kamboj et al., 2011)</td>
</tr>
<tr>
<td><em>Pomegranate juice</em></td>
<td>Potent antioxidant cause reduction in expression of oxidative stress markers elevated due to hyperoxaluria</td>
<td>(Ilbey et al., 2009)</td>
</tr>
<tr>
<td><em>Trachyspermum ammi</em></td>
<td>Prevent calcium oxalate deposition, maintain kidney functioning by preventing renal injury and decrease crystal retention in renal tissues.</td>
<td>(Kaur et al., 2009)</td>
</tr>
<tr>
<td><em>Coconut water</em></td>
<td>Protect against impaired kidney function and development of oxidative stress in the kidneys</td>
<td>(Gandhi et al., 2013)</td>
</tr>
<tr>
<td><em>Moringa olifera</em></td>
<td>Stone dissolving properties</td>
<td>(Karadi et al., 2008; Sachan et al., 2011)</td>
</tr>
<tr>
<td><em>Terminalia arjuna</em></td>
<td>Inhibit the formation of both calcium phosphate and calcium oxalate crystals</td>
<td>(Chaudhary et al., 2010)</td>
</tr>
<tr>
<td><em>Rubia cordifolia</em></td>
<td>Prevent the impairment of renal functions</td>
<td>(Divakar et al., 2010)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Effect</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em></td>
<td>Prevent and eliminate CaOx deposition into kidneys.</td>
<td>(Atmani et al., 2009; Khajavi Rad et al., 2011)</td>
</tr>
<tr>
<td><em>Sesbania grandiflora</em></td>
<td>Antioxidant and diuretic</td>
<td>(Doddola et al., 2008)</td>
</tr>
<tr>
<td><em>Nigella sativa</em></td>
<td>Reduce the number and size of CaOx stones</td>
<td>(Hadjzadeh et al., 2011)</td>
</tr>
<tr>
<td><em>Berberis vulgaris</em></td>
<td>Protective renal function and antioxidant</td>
<td>(Bashir et al., 2010)</td>
</tr>
<tr>
<td><em>Achyranthes aspera</em></td>
<td>Maintain architecture of kidney tissue and reduce the size of crystals</td>
<td>(Aggarwal et al., 2012a)</td>
</tr>
<tr>
<td><em>Crataeva nurvala</em></td>
<td>Lupeol isolated from plant carry antioxidant and antilithiatic properties</td>
<td>(Shirwaikar et al., 2004; Sudhahar et al., 2008)</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td>Decrease urinary excretion of calcium and oxalate also increase citrate</td>
<td>(Shah et al., 2012)</td>
</tr>
<tr>
<td><em>Costus igneus</em></td>
<td>Lower calcium and oxalate deposition in kidney</td>
<td>(Manjula et al., 2012)</td>
</tr>
<tr>
<td><em>Aerva lanata</em></td>
<td>Reduce the oxalate synthesizing enzymes, diminished the markers of crystal deposition in the kidney</td>
<td>(Soundararajan et al., 2006)</td>
</tr>
<tr>
<td><em>Mimusops elengi</em></td>
<td>Decrease stone forming constituents and antioxidant</td>
<td>(Ashok et al., 2010)</td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em></td>
<td>Diuretic and restore urinary parameters and pH to normal value</td>
<td>(Pareta et al., 2011)</td>
</tr>
</tbody>
</table>
Terminalia chebula
Reduce levels of oxalate and maintain kidney architecture
(Pawar et al., 2012; Saha and Verma, 2015; Tayal et al., 2012)

Punica granatum
Reduce the urinary oxalate, calcium and phosphate, normalize renal tissue oxalate concentration and serum creatinine, urea and uric acid levels
(Rathod et al., 2012)

Medical management strategies include removal of stones and preventing their recurrence. Based on the data obtained from several in vitro, in vivo experiments and clinical trials, it could be concluded that herbal agents could serve as an alternative or adjunct therapy to available therapies. The reviewed studies had revealed that the mechanisms of action of these plants or plant products might be attributed to being diuretic, increasing urinary citrate, antioxidant, antimicrobial and inhibitory properties (Aggarwal et al., 2014a). In the recent years, new proteins demonstrating biological activity towards urolithiasis have been discovered (Aggarwal et al., 2012b).

2.7.2 Antioxidants: Anti-hyperoxaluric Agents

Among the natural antioxidants polyphenolic compounds like flavonoids, flavonols and terpenoids from plant source have emerged as preferred alternative. These compounds have ability to donate electrons to free radicals generated during oxidative stress condition and hence neutralize these chemical species (Gill and Tuteja, 2010). The study of the antioxidant potential of polyphenols from ethnomedicinal plants may also be necessary because this property is among desired therapeutic properties of plants due to their pharmaceutical effects. A list of various antioxidant anti-hyperoxaluric agents and their reported mechanism of action has been enlisted in table 2.4.
Table 2.4 Anti-oxidant compounds and their mechanism of action (Aggarwal et al., 2013a)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>Major lipid per oxidation chain-breaking antioxidant</td>
<td>(Huang et al., 2006)</td>
</tr>
<tr>
<td>Phycocyanin</td>
<td>Free radical scavenger and antioxidant activity</td>
<td>(Farooq et al., 2004)</td>
</tr>
<tr>
<td>Lupeol</td>
<td>Antioxidant activity</td>
<td>(Sudhahar et al., 2008)</td>
</tr>
<tr>
<td>PGG</td>
<td>Protect against ROS induced renal cell injury and reduce renal hyaluron expression</td>
<td>(Lee et al., 2011)</td>
</tr>
<tr>
<td>Gallotannin</td>
<td>Inhibit COM crystal growth and adhesion to renal epithelial cells</td>
<td>(Lee et al., 2012)</td>
</tr>
<tr>
<td>Berberine</td>
<td>Antioxidant activity</td>
<td>(Bashir and Gilani, 2011)</td>
</tr>
<tr>
<td>Apocynin</td>
<td>NADPH oxidase inhibitor</td>
<td>(Joshi et al., 2012)</td>
</tr>
<tr>
<td>Rottlerin</td>
<td>PKC-δ inhibitor</td>
<td>(Maioli et al., 2012)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>PKC-δ inhibitor</td>
<td>(Antunes et al., 2001)</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>Antioxidant and antibacterial activity</td>
<td>(Hadjzadeh et al., 2008)</td>
</tr>
<tr>
<td>Fucoidans</td>
<td>Normalize the redox status</td>
<td>(Veena et al., 2008)</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Inhibit renal crystal retention</td>
<td>(Tsujihata et al., 2011)</td>
</tr>
<tr>
<td>Taurine</td>
<td>Antioxidant activity</td>
<td>(Li et al., 2009b)</td>
</tr>
<tr>
<td>Losartan</td>
<td>Competitive Angiotensin II type 1 receptor antagonist</td>
<td>(Li et al., 2009a)</td>
</tr>
<tr>
<td>N-Acetyl cysteine (NAC)</td>
<td>Antioxidant activity</td>
<td>(Bijarnia et al., 2008; Sharma et al., 2015)</td>
</tr>
</tbody>
</table>
2.7.3 Bergenia ligulata

*Bergenia ligulata* is a small plant (Figure 2.14) with approximately 32-35 cm long leaves and 2.5 cm in diameter obovate to suborbicular, hairy, midgreen, obtuse, tapering at base, glabrous on both sides, margin subentire and ciliate leaves. Flowers are white to rose or purple, petal clawed panicle like cymes of shallowly, funnel shaped, 5 petalled flowers, usually 1.5-2.5 cm across on short, branched othenned or purple flower stems are produced mainly in early spring. It belongs to Saxifragaceae family and is popularly known as a ‘stone flower/stone breaker’ (Ruby *et al*., 2012). It is also known as *Saxifraga ligulata*. As per the Wikipedia descriptions the classification of *B. ligulata* has been described in table 2.5.

![Figure 2.14 Bergenia ligulata](image)

**Table 2.5 Classification of Bergenia ligulata** (www.wikipedia.org)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>Super division</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Manoliopsida</td>
</tr>
</tbody>
</table>
Subclass | Rosidae  
---|---  
Order | Saxifragales  
Family | Saxifragaceae  
Genera | Bergenia  
Species | ligulata  

(a) Vernacular names

As *B. ligulata* has been used in different regions of the world for various ailments since ancient time, different vernacular names do exist in different local languages. Some of the commonly found names are tabulated in table 2.6.

**Table 2.6 Vernacular names of *Bergenia ligulata* (Gurav and Gurav, 2014)**

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assamese</td>
<td>Patharkuchi</td>
</tr>
<tr>
<td>Bengali</td>
<td>Himasagara, Patharchuri, Patrankur</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Pakhanbheda, Pashanbheda</td>
</tr>
<tr>
<td>Hindi</td>
<td>Dakachru, Pakhanabhed, Pakhanabheda, Patharcua, Silparo, Silpbheda</td>
</tr>
<tr>
<td>Kannada</td>
<td>Alepgaya, Hittaga, Hittulaka, Pahanbhedi, Pasanberu</td>
</tr>
<tr>
<td>Kashmiri</td>
<td>Pashanbhed</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Kallurvanchi, Kallurvanni, Kallorvanchi</td>
</tr>
<tr>
<td>Marathi</td>
<td>Pashanbheda</td>
</tr>
<tr>
<td>Mizoram</td>
<td>Khamdamdawi, Pandamdawi</td>
</tr>
<tr>
<td>Oriya</td>
<td>Pasanbhedi, Pashanabheda</td>
</tr>
<tr>
<td>Punjabi</td>
<td>Batpia, Dharposh, Kachalu, Pashanbhed</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Ashmabheda, Nagbhita, Pashaanbheda, Silabheda</td>
</tr>
<tr>
<td>Tamil</td>
<td>Sirupilai</td>
</tr>
<tr>
<td>Telugu</td>
<td>Kondapindi, Telanurupindi</td>
</tr>
<tr>
<td>Urdu</td>
<td>Kachalu, Pakhanabheda</td>
</tr>
</tbody>
</table>
(b) Geographical Distribution

Bergenia is found in temperate Himalayas from Kashmir to Bhutan between 7,000-10,000 feet and Khasia hills at 4,000 feet, Kashmir to Kumaon from 2,600-4,600 meter, North East Garhwal Himalayas from lateral 29°26’ to 31°28’ North and long 77°49’ to 80°6’ East and with a total area of about 3,090 sq. km. It includes five districts Chamoli, Pauri, Dehradun, Tehri and Uttarkashi. In North-West Himalayas lies between 30°4’ to 30°52.5’ lateral North and 70° 56’ -79° 3’ East long (Kirtikar and Basu, 1983).

(c) Macroscopic features

The rhizomes are compact solid, barrel shaped, somewhat cylindrical, measuring 1-3 cm long and 1-2 cm in diameter. The outer surface is brown coloured with small roots, ridges, furrows wrinkles and covered with root scars. It possesses aromatic odour and astringent taste (Ruby et al., 2012).

(d) Microscopic features

Transverse section of rhizome shows cork divided into two zones; outer and inner. Outer zone is with few layers of slightly compressed and brown coloured cells whereas inner zone is multilayered consisting of thin walled, tangentially elongated and colourless cells. Cork is followed by single layered cambium and two to three layers of secondary cortex. Cortex consists of a narrow zone of parenchymatous cells containing a number of simple starch grains whereas most of cortical cells contain large rosette crystals of calcium oxalate (CaC$_2$O$_4$) and starch grains (Jani et al., 2013).

(e) Phytochemistry

The chemical constituents of B. ligulata are diverse and have been reported to have wide applications. The major chemical constituents identified and reported of the plant are Bergenin, α-Sitosterol, α -Sitosterol-D-glucoside (Jain and Gupta, 1962; Srivastava and Rawat, 2008), Leucocyanidin, Gallic acid, Methyl gallate and Catechin (Dixit and Srivastava, 1989). The rhizomes are found to contain higher concentration of
bergenin, catechin and gallic acid as compared to other plant parts (Dhalwal et al., 2008). Besides, the rhizome also contains mucilage, wax, glucoside, albumin, starch (Zhang et al., 2011) and 4(4’-β-D-glucopyranosyloxy1’-benzoyloxy)–6-methyltetrahydropyran-2 also named as Paashaanolactone (Chandrareddy et al., 1998). Inhibitory activity of afzelechin against α-Glucosidase was reported from rhizome of *Bergenia ligulata* (Saijyo et al., 2008).

(f) Biological properties

*Bergenia* sp. has been used for centuries in South Asia (mainly India and Pakistan) for a wide range of complaints (Guo-Yan et al., 2007). However, the most important medicinal activities are its diuretic and lithotriptic effects. *Bergenia ligulata* is used as a cure for dysuria and strangury and for stones in the kidney and ureter. Some preliminary studies had evaluated the antiurolithic potential of *B. ligulata* rhizome. The aqueous extract of *B. ligulata* rhizome inhibited not only the homogenous precipitation of calcium oxalate crystals (Garimella et al., 2001) but also the *in vitro* growth of calcium oxalate and calcium hydrogen phosphate dihydrate crystals have been suppressed (Joshi et al., 2005a; Joshi et al., 2005b). Moreover, *in vivo* studies suggest that the alcoholic extract of *B. ligulata* is effective in dissolving the calculi developed in the bladder of rats by foreign body insertion and reduced idiopathic hyperoxaluria in stone formers (Bashir and Gilani, 2009). In a modified animal model (male wistar rats) of urolithiasis developed by addition of 0.75% ethylene glycol in drinking water, methanolic extract (5–10 mg/kg) of *B.ligulata* rhizomes prevented CaC$_2$O$_4$ crystal deposition in the renal tubules. Methanolic extract of *B. ligulata* exhibited free radical scavenging activity with IC value of 50µg/ml by DPPH assay (Roselli et al., 2012). The alcoholic extract (250 mg/kg body weight) of roots of *B. ligulata* exhibited hypoglycemic activity. The (+)- afzelechin isolated from rhizomes of *B. ligulata* was found to be an inhibitory compound of alpha-glucosidase activity (Saijyo et al., 2008). These studies revealed antidiabetic potential of *B. ligulata* and could be helpful to develop medicinal preparations or nutraceutical and
functional foods for diabetes and related symptoms. Animals treated with alcoholic extract (500 mg/kg body weight) of roots of *B. ligulata* showed significant decrease in the levels of Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP) and total bilirubin as compared to control and confirmed the hepatoprotective action of the same. Alcoholic extract (500mg/kg body weight) of roots of *B. ligulata* was found to be effective in increasing urinary electrolyte concentration of K\(^+\) and Cl\(^-\) which indicates its significant diuretic activity (Singh *et al.*, 2009).

**g) Medicinal value**

1) *Bergenia ligulata* is used as medicinal plant in Ayurvedic medicine. Its extract has whitening ability, anti-oxidant, anti-aging and anti-inflammatory abilities. Also, the alcoholic extract has significant analgesic, antibacterial and diuretic properties (Sajad *et al.*, 2010; Sinha *et al.*, 2001a).

2) Sinha *et al.*, (2001b) reported the anti tussive activity of rhizome extract in mice.

3) The aqueous and methanolic extract of rhizome of *Bergenia* possesses potent radical scavenging activity (Rajkumar *et al.*, 2010). The catechin from the rhizome (Ivanov *et al.*, 2011) and bergenin had been shown to posses anti-oxidative potential (Rastogi and Rawat, 2008).

4) β-Glucan extract, made of β-Glucan, polysaccharide, has acceleration effect on collagen synthesis and immunology power enhancement effect and also it makes cosmetics viscous (Yanaginuma *et al.*, 2003).

**Rational of the Thesis**

The literature review highlighted the urgent need of better treatment modalities of urolithiasis. Present methods of management include non-surgical methods such as ESWL, surgical interventions and allopathic drugs but their undesirable side-effects, surgical complications, recurrence of disease still pose a challenge to scientific
community. Phytotherapy have been used since ancient times and have also been gaining priority in the management of various other ailments. The only disadvantage of the phytotherapy in the forms of some formulations is the unknown metabolite actually responsible for the therapeutic effect. The unnecessary exposure to other metabolites and batch to batch variations in the formulations may give different levels of outcomes. The present study hence is undertaken to investigate the role of *B. ligulata* rhizome in the management of urolithiasis and also to isolate novel metabolites actually responsible for this therapeutic effect, which then may lead to their commercial usage with similar potency worldwide.