Chapter 1
Review of Literature
1.1. KIDNEY STONE

Kidney stones also called renal calculi, are solid concretions (crystal aggregations) of minerals present in urine. These calculi can form anywhere in the urinary tract, from kidneys to the bladder but in the industrialized and affluent countries, they are generally restricted to the kidneys. The term urolithiasis refers to the presence of calculi in the urinary tract, as shown in figure 1.1. It is a widespread disease afflicting mankind and continues to pose a universal health problem even today.

![Kidney Stone Diagram]

**Figure 1.1.** Common location of Kidney Stones

1.1.1. Overview

Kidneys are a pair of organs that are primarily responsible for filtering metabolites and minerals from the circulatory system. These secretions are then passed to the bladder and out of the body as urine. Some of the substances found in urine are able to crystallize, and in a concentrated form these chemicals can precipitate into a solid deposit attached to the kidney walls. These crystals can grow
through a process of accretion to form a kidney stone and the fundamental cause for all stones is supersaturation of urine [1]. In medical terminology these deposits are known as renal calculi (Latin *renal*, "kidney" and *calcoli*, "pebbles") [2].

Renal calculi can vary in size from as small as grains of sand to as large as a golf ball [3]. Kidney stones typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size before passage (on the order of at least 2-3 millimeters) they can cause obstruction of the ureter. The resulting obstruction with dilation or stretching of the upper ureter and renal pelvis as well as spasm of muscle, trying to move the stone, can cause severe episodic pain, most commonly felt in the flank, lower abdomen and groin (a condition called renal colic). Renal colic can be associated with nausea and vomiting due to the embryological association of the kidneys with the intestinal tract. Hematuria (bloody urine) is commonly present due to damage to the lining of the urinary tract.

Kidney stones affect approximately 12 percent of men and 5 percent of women by age 70. Recurrence can occur at a rate of up to 5 percent per year in people who are not treated. Within the United States, about 10–15% of adults are diagnosed with a kidney stone [4] and the total cost for treating this condition was US$2 billion in 2003 [5]. An unanticipated result of global warming is the likely northward expansion of the present-day southeastern U.S. kidney stone "belt." The fraction of the U.S. population living in high-risk zones for nephrolithiasis will grow from 40% in 2000 to 56% by 2050, and to 70% by 2095 [6].

1.1.2. History

The existence of kidney stones has been recorded since the beginning of civilization, and lithotomy for the removal of stones is one of the earliest known surgical procedures [7]. In 1901, a stone was discovered in the pelvis of an ancient Egyptian mummy, and was dated to 4,800 BC. Medical text from ancient Mesopotamia, India, China, Persia, Greece and Rome all mentioned calceulous disease. Part of the Hippocratic oath contains an admonition about the dangers of operating on the bladder for stones. The Roman medical treatise *De Medicina* by Cornelius Celsus contained a description of lithotomy, and this work served as the
basis for this procedure up until the 18th century [8]. New techniques in lithotomy began to emerge in 1520, but the operation remained risky. It was only after Henry J. Bigelow popularized the technique of lithopaxy in 1878 and after that the mortality rate dropped from about 24% down to 2.4%. However, other treatment techniques were developed that continued to produce a high level of mortality, especially among inexperienced urologists [8, 9]. In 1980, Domier MedTech introduced extracorporeal shock wave lithotripsy for breaking up stones via acoustical pulses, and this technique has come into widespread use [10]. Among the famous leaders who were kidney stone sufferers are Emperor Napoleon Bonaparte, Emperor Napoleon III, Peter the Great, Louis XIV, George IV, Oliver Cromwell, and former U.S. President Lyndon B. Johnson. Other notable individuals who endured stones include Benjamin Franklin, the philosopher Sir Francis Bacon, the scientist Sir Isaac Newton, the civil servant and diarist Samuel Pepys, the physicians William Harvey and Herman Boerhaave, and the anatomist Antonio Scarpa [9]. Interestingly, astronauts seem to have a higher risk of developing kidney stones during or after long duration space flights.[11].

1.1.3. Types of kidney stones

There are about six major types of crystalline substances involved in kidney stone formation (Figure 1.2). These are (A) Calcium oxalate (monohydrate-Whewellite, dihydrate-Weddellite), (B) Calcium phosphate (Brushite, Apatite, Whitlockite, Octacalcium-phosphate), (C) Magnesium ammonium phosphate (Struvite, Newberryite), (D) uric acid (uric acid dihydrate, urate monohydrate, ammonium acid urate, xanthine), (E) Cystine and others (F) miscellaneous types, occurs with drug metabolites such as xanthine, Crixivan etc. [12].

A. Calcium oxalate (monohydrate & dihydrate)

About 70% to 80% of all kidney stones are composed of calcium, usually combined with oxalate. Calcium oxalate (CaOx) develops in acidic urine with pH less than 6.0. Hypercalciuria or increased calcium in the urine may lead to calcium stone formation. It is stated that the amount of calcium in a person's urine is an important contributing factor in the formation of both types of kidney stones.
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<th>Calcium oxalate monohydrate</th>
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<td>Uric Acid Dihydrate</td>
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**Figure 1.2.** Types of Kidney stones
But high levels of oxalate in the urine, or hyperoxaluria, is even more important to stone formation than high levels of calcium or hypercalciuria, since oxalate forms an insoluble complex with calcium to develop a calcium oxalate stone. Excessive intake of food and drink containing oxalate leads to calcium oxalate stones. Also, excessive intake of vitamin C which is metabolized to oxalate may lead to hyperoxaluria and an increase in stone formation.

**B. Calcium phosphate (Brushite, Apatite, Whitlockite, Octacalcium-phosphate)**

Calcium phosphate (CaP) develops in alkaline urine with pH greater than 7.2. Calcium phosphate stones typically occur in patients with metabolic or hormonal disorders such as hyperparathyroidism and renal tubular acidosis. These stones are of two types, Apatite \([\text{Ca}_3(\text{PO}_4)_2\cdot\text{OH}]\) and Brushite \([\text{CaHPO}_4\cdot\text{H}_2\text{O}]\). Apatite is a very frequent component with 33%. Brushite constitutes 1-2% of the calcium stones. The other calcium phosphates, such as whitlockite and octacalcium phosphate are very rare.

**C. Struvite**

Struvite crystals are also known as magnesium ammonium phosphate, triple phosphate or "infection stones" with a frequency of 10-15%. Unlike other calculi, struvite crystals are caused by a urinary tract infection. The bacteria that cause the urinary tract infection affect urine chemistry and neutralize urine acids. This allows the bacteria to grow even more, resulting in struvite masses. They are the only types of calculi that are treated medically as if they were infected foreign particles. The basis of struvite stones formation is the presence of urea-splitting bacteria. *Proteus mirabilis* is one of the most common urea splitting bacteria. Apart from *Proteus mirabilis*, there are other bacteria such as *Klebsiella*, *Serratia*, *Providencia* species that can split urea into ammonia. This process of splitting urea into ammonia decreases the overall acidity of the urine and thus resulting in the favorable conditions that help in the formation of struvite kidney stones. Struvite formations are more common in women than men, mainly because the female urinary tract system is more susceptible to infections.
D. Uric acid stones

Uric acid is an end product of purine metabolism. It is the same crystal that causes gout, an arthritic condition. If the acid level in the urine is high or too much acid is excreted, the uric acid may not dissolve and uric acid stones may form. Genetics may play a role in the development of uric acid stones, which are more common in men. Approximately 10% of patients with kidney stone disease develop this type of stone. Foods high in purines like red meat, fish, and chicken are the main causes behind uric acid stone formation. The solubility of uric acid depends on the acidity or alkalinity of the urine. In acidic urine, pH less than 5.5, uric acid crystals precipitate leading to stone formation. If urine is alkaline, uric acid remains soluble and do not precipitate out. Knowledge of this fact is the basis of the medical treatment of uric acid stones. Factors that increase the risk for uric acid stones are given below.

1. Low urine output.
2. A diet high in animal protein, such as red meat.
3. Increased consumption of alcohol.
4. Gout
5. Inflammatory bowel disease

E. Cystine stones

Cystine stones are rare since less than 1% of kidney stones are made of them. Cystine can build up in the urine to form a stone. It runs in families who are more likely to develop a metabolic condition that produces excess cystine in the urine (cystinuria). Cystine stones may be prevented or dissolved with medication but this may be difficult and not very effective. If the stones cause blockage or are too large, then removal of the stone will be needed. Appropriate prevention measures will reduce the risk of recurrence. Drugs such as penicillamine can be administered to make cystine more soluble and drug captopril can be used to make cystine less likely a reason to cause stones.
1.1.4. Organic matrix of stones

Morphologically, the organic matrix exists as either amorphous or fibrous forms. The organic matrix is investigated to be a mucoprotein in nature and has been designated as uromucoid [13]. The matrix is found to be formed of 64% protein, 9.6% non-amino sugars, 5% glycosamine, 10% bound water and traces of lipids with organic ash as remainder [14]. The proteins are generally characterized by high glutamic acid and aspartic acid contents and the frequent occurrence of gamma-carboxyl glutamic acid. In matrices of calcium phosphate crystals, the principal proteins are Tamm-Horsfall protein followed by albumin, prothrombin-related proteins and osteopontin. However, when crystals were induced in the urine of stone formers, albumin was the major component of the organic matrix of both CaOx and CaP crystals [15]. Moreover, nine of the 13 proteins were found in all types of stone: human serum albumin, alpha 1-acid glycoprotein, alpha 1-microglobulin, immunoglobulins, apolipoprotein A1, transferrin, alpha 1-antitrypsin, retinol-binding protein and renal lithostathine. The beta 2-microglobulin was present only in calcium oxalate and uric acid stones [16]. An in vitro study shows that the canine renal distal tubular cell line Madin-Darby canine kidney (MDCK) forms calcium phosphate stones during a long-term culture which is found to contain osteopontin (OPN) and calprotectin [17]. In another study an extracellular protein, produced from Pseudomonas fluorescens strain D with molecular mass of 41.5 kDa was partially purified from stones [18]. The organic matrix of calcific stones is also found to contain significantly more acidic and complexed phospholipids than uric acid and struvite stones. Osteopontin was undetectable in calcium oxalate monohydrate (COM) extracts, but clearly visible in calcium oxalate dehydrate (COD). Prothrombin fragment 1 was abundant in COM, but present in COD in lesser amounts than osteopontin [19].

1.1.5. Inhibitors of renal stones

Although the kidney is supersaturated with calcium and oxalate, the basic components of kidney stones, only three to five percent of people in the world form them. Most people pass microscopic calcium oxalate crystals with their urine before
they can grow into dangerous masses. It is the natural fate of supersaturated solutions to grow crystals, however, something must be working to prevent this in the kidneys and these are inhibitory biomolecules. These inhibitors are also present in soft tissues like tendons, aorta etc. An in vitro study demonstrates that flexor tendons of rabbit contain an acidic polypeptide which inhibits the mineralization [20].

In an another study the inhibitory activity of the aorta extract was found to be primarily due to the presence of three biomolecules having molecular weights of 66, 45, and 27-29 kDa. These inhibitory biomolecules loosely associated with aorta may be involved in the control of calcification associated with arteriosclerosis [21]. The crystallization inhibitors help in avoiding or delaying calculi development [22].

An ELISA based assay system study shows that a potent inhibitor having molecular weight between 14.2 and 16.2 kDa was found to be primarily responsible for the differences observed in the urinary inhibitory activity between normal persons and kidney stone patients. This assay system can be used to screen human beings for potential stone formers [23]. Among the most prominent inhibitors is citrate, which, by forming a soluble complex with calcium, reduces the amount of available calcium to form an insoluble complex with oxalate [24].

High molecular weight inhibitors are also investigated which inhibit one or more phases of stone formation in vitro, includes several urinary proteins viz. Tamm-Horsfall protein, uropontin, prothrombin F1 peptide [19], uronic acid rich protein (Bikunin), nephrocalcintc and glycosami glycans [25] viz. chondroitin sulfate and heparan sulfate. The bone matrix protein like Osteocalcin, Osteonectin and γ-Carboxylglutamic acid (Gla) protein are also known to inhibit stone formation process. An anti-inflammatory protein called calgranulin, play a key role in the prevention of kidney stones. Calgranulin present, even in minute amounts stop the growth of calcium oxalate crystals, which is the major component of kidney stones. It is made up of two distinct subunits, which are often defective in stone-formers [26].

Another potent inhibitor is inter-alpha-trypsin trimer which could be a mechanism contributing to the differences in CaOx urolithiasis between sexes [27]. Recently, it is investigated that some oxalate binding protein like, histone H (1B)
(27.5kDa), nuclear membrane protein (68 kDa) and nuclear pore complex protein (205 kDa) present in nucleus were having the oxalate binding properties.

The oxalate binding proteins were thought to modulate the crystallization process in a hyperoxaluric condition similar to calcium specific binding protein modulators [28]. It has been observed that cations as well as anions also inhibit in vitro mineralization which are Mg$^{2+}$, P$_2$O$_7^{4-}$, CrO$_4^{2-}$, SrO$_4^{2-}$ etc [29]. Studies showed that these inhibitors act by getting absorbed on the surface of microcrystalline calcium salts.

1.1.6. Epidemiology of kidney stones: its causes and distribution

Kidney stone is a clinical disorder which is known to be caused by multiple etiologic factors. People with kidney stones may first have dysfunction or damage to some of the collecting tubes in the kidney. The epidemiology of renal stones includes preurinary and urinary risk factors.

1.1.6.1. Urinary factors

Urinary factors include the condition and composition of urine which could result in formation of kidney stones. These factors can be concentration of ions in urine, volume of urine, pH of urine, enzymes concentration and level of various stone formation inhibitors in urine.

A. Concentration of salts in urine

The key process in the development of kidney stones is supersaturation. This process involves salts that are carried in urine such as calcium oxalate, uric acid, cystine, or xanthine. These salts can become extremely concentrated under certain circumstances. If the volume of urine is significantly reduced or abnormally high amounts of crystal-forming salts are present, the concentration levels reaches the point at which the salts no longer dissolve, they precipitate out and form crystals.

An in vitro study has shown that oxalate, either in crystalline or in soluble form triggers a spectrum of responses in renal cells that favor stone formation, including alterations in membrane surface properties that promote crystal attachment and alterations in cell viability that provide debris for crystal nucleation. Activation of
cytosolic phospholipase A2 appears to play an important role in oxalate actions, triggering a signaling cascade that generates several lipid mediators by (arachidonic acid, lysophosphatidyl choline, ceramide) acting as key intracellular targets (mitochondria, nucleus).

The net effect is increased production of reactive oxygen molecules (that in turn affect other cellular processes), an increase in cell death and an induction of a number of genes in surviving cells, some of which may promote proliferation for replacement of damaged cells or may promote secretion of urinary macromolecules that serve to modulate crystal formation. [30]. Further, it was found that calcium oxalate monohydrate gets precipitated at membrane lipid rafts [31].

B. Volume of urine

Increased urinary volume is an important tool in the prevention of calcium renal stones. Urine dilution considerably reduces crystallization phenomena induced in vitro by an oxalate load in both calcium stone-formers and normal subjects [32].

C. pH of urine

Uric acid stones occur especially in patients with very low urine pH (below pH 5.0) and in those with hyperuricosuria. Uric acid is very insoluble in urine at pH 5.0, but becomes significantly more soluble in urine at pH 7.0. Any combination of low urine pH, concentrated urine, and increased urinary uric acid excretion, make one at risk for uric acid stone disease. In some patients this very low urine pH is caused by a defect in renal ammonia secretion that results in less buffering of secreted hydrogen ion and lower urine pH [33] suggested that the very low urine pH is in some way related to the insulin resistance

D. Enzymes in urine

The initial step in the pathogenesis of urolithiasis must be the precipitation of an organic matrix of mucoprotein. An important factor in this process may be the activity and/or concentration of the urinary enzyme urokinase which would affect the level of urinary mucoprotein. A decrease has been observed in urinary urokinase concentration of renal stone patients which, once again, underlines the possible involvement of urokinase in renal stone formation [34]. Increased excretion of urinary
enzymes like lactate dehydrogenase, alkaline phosphatase, $\gamma$-glutamyl transpeptidase and beta glucuronidase in calculogenic rats indicates membranuria and damage to proximal tubules during stone formation [35].

1.1.6.2. Preunary factors

The preunary factors could be both intrinsic and extrinsic. Intrinsic factors include the heredity, age and sex. Extrinsic factors, on which the incidence of renal stones occurrence depends are geographical distribution, climatic factors and dietary factors which includes water intake.

A. Age and sex

The inhibition of calcium oxalate crystal growth is influenced by a complex combination of gender and age. With age the vigorous ability to inhibit crystallization is reduced. Men are at higher risk for kidney stones than women. Urinary citrate and magnesium excretion were lower, and glycosaminoglycan and zinc excretion were higher, in stone formers than in controls. The citrate:creatinine excretion ratio was significantly higher in women than men. The higher citrate excretion in women may explain the lower incidence of calcium stones in women [36]. Another known reason attributing to the higher incidence of kidney stones in men then women is inter-alpha-trypsin trimer. It was shown that the inter-alpha-trypsin trimer is a CaOx binding inhibitor which is a function of age and sex-hormone status in males and females. In males a decrease in inter-alpha-trypsin trimer was associated with the onset of adulthood and entry into the 'stone-forming years'. Females did not show this decrease, and neither sex showed an increase in inter-alpha-trypsin trimer in the above 60 group [27].

B. Geographical distribution

The overall probability of stone forming people differs in various parts of the world: 1-5% in Asia, 5-9% in Europe, 13% in North America, 20% in Saudi Arabia. The composition of stones and their location in the urinary tract, bladder or kidneys may also significantly differ in different countries. Stones in the upper urinary tract appear to be related to the life-style, being more frequent among affluent people, living in developed countries, with high animal protein consumption. Bladder stones
are nowadays mainly seen in the Third World, on account of very poor socio-economic conditions [37]. A high frequency of stone formation among hypertensive patients has been reported, and among those with high body mass as well [37]. In India, two high incidence stone belts have been reported. The first belt starts from Amritsar in North and while passing through Delhi and Agra ends up in Uttar Pradesh. The other belt which starts from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been in West Bengal and coastal areas of Maharashtra, Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh.

C. Climatic factors

While determining the geographic variability in rates of kidney stones in the United States, authors found that ambient temperature and sunlight levels are important risk factors for stones. The differences in exposure to temperature and sunlight may contribute to geographic variability. Reasons for higher incidence in summers could be an increased conversion of vitamin D3 to its active metabolites resulting in increased calcium absorption from intestines and decrease in urine production due to loss of water as sweat causes supersaturation of urine with stone constituents [38].

D. Dietary factors

Diet plays an important role in the pathogenesis of kidney stones. As the metabolism of many dietary factors, may change with age, the relation between diet and kidney stones may be different in older adults. Uncertainty also remains about the association between many dietary factors, such as vitamin C, magnesium, and animal protein, and the risk of kidney stone formation.

The relative risk for men who consumed 1000 mg or greater of vitamin C showed higher risk of kidney stone formation whereas magnesium at dose 1000mg or more per day showed decreased risk of kidney stone formation, thus indicating that magnesium intake decreases and total vitamin C intake increases the risk of symptomatic nephrolithiasis [39]. Currently, the recommended upper limit for ascorbic acid intake is 2000 mg/d. However, because vitamin C is endogenously converted to oxalate and appears to increase the absorption of dietary oxalate, supplementation may increase the risk of kidney stones. The 1000 mg vitamin C
twice each day increased urinary oxalate and calcium oxalate crystals as kidney stones in 40% of participants when both stone formers and non-stone formers given oxalate and vitamin C for 24 hr [40]. Vitamins have been associated with the kidney stones disease in various references. The potential benefits of dietary or supplemental calcium and vitamin D in reducing the risk of recurrence kidney stones have been documented [41]. It was also suggested that idiopathic renal stone genesis could be generated by vitamin A deficiency [42].

Vitamin K deficiency has also been associated with stones of renal origin. Vitamin K has been known to promote the formation of gamma carboxyglutamic acid which has high affinity for calcium. A reduced carboxylase activity was observed in the urolithic patients, this suggests its important role in the course of renal calcium oxalate urolithiasis [43]. A number of investigators have shown that increased water intake and increased urinary output decreases the incidence of urinary calculi. Recently, it was shown that the "stone clinic effect" which encourages a high intake of fluid and recommended diet, significantly decreased urinary supersaturation for calcium oxalate and the formation of new kidney stones in 80% of patients during first year of follow-up [44].

E. Hereditary factors

Nephrolithiasis is a complex phenotype that is influenced by both genetic and environmental factors. There are several rare, heritable causes of nephrolithiasis that result in the onset of oxalate stone disease early in childhood and frequently lead to renal failure. A study done by Goldfarb et al [45] showed that kidney stones are genetically linked. Their study done on dizygotic and monozygotic twins showed that the heritability of the risk for stones was 56%. Many instances have also showed that nephrolithiasis disproportionately affects white patients. Whites have a higher prevalence of hypercalciuria compared with nonwhites [46]. Previous studies suggest a familial incidence in a subset of persons who have recurrent urinary tract stone disease. Identification and characterization of families of recurrent stone formers is essential for the identification of unique genetic, environmental and metabolic factors that predispose individuals to recurrent calcium oxalate stone formation. As oxaluria and calciuria have a prominent role in calcium stone formation, any genes that
influence their excretion can be considered prime candidates in calcium nephrolithiasis. Among the determinants of urine calcium salt saturation, the rate of urine oxalate excretion is an important risk factor for the development of renal calcium lithiases. Genetic studies have identified a small group of individuals with known inherited metabolic disorders who develop recurrent calcium oxalate stones at a very early age. Recently a new suggestive gene locus for autosomal dominant nephrolithiasis, have been discovered. It is localized on chromosome 9q33.2 q34.2. The responsible gene will provide new insights into the molecular basis of nephrolithiasis [47]. Low serum phosphate concentrations due to decrease in renal phosphate reabsorption have been reported in some urolithiatic patients with defect in gene coding for the type 2a sodium-phosphate co-transporter [48].

As it is known that there exist many inhibitors in our urine which prevent stone formation. These inhibitors are encoded by genes, so a genetic abnormality of these genes can increase the risk of stone formation. Such proteaceous inhibitors are bikunin, a glycoprotein crystal adhesion inhibitor, heparin, a recently found anti-inflammatory protein called calgranulin, made up of two distinct subunits, and many more. The genes encoding them are often defective in stone-formers.

The majority of urinary tract stones are composed of calcium oxalate. The genetic contribution to development of this, is more prevalent, calcium oxalate stone diseases of adult can be due to increased calcium and oxalate absorption. Since vitamin D3 plays a central role in calcium metabolism in the intestine, kidneys and bone, and its plasma levels are usually increased in stone patients [49], a genetic disorder in its pathway was a most attractive hypothesis to explain this trait; the candidate genes contributing to hypercalciuria and, hence, calcium stone formation, could involve some steps of the vitamin D pathway, or anomalies in its receptors, or in its synthesis or activation. Formation of kidney stones is hypothesized to be associated with the vitamin D receptor gene (VDR). On evaluating the association between calcium stone disease and the polymorphism VDR gene in a North Indian population, it was found that the VDR FokI polymorphism may be a good candidate for a marker for calcium oxalate-stone disease [50].
Oxalate is transported into the cells through chloride bicarbonate exchanger band 3 protein ‘AE1’. Oxalate competes with chloride for the same transporter [51]. Oxalate is also found to be a substrate of sulfate transport system due to its negative charge and structure. Oxalate is transported across the mitochondrial membrane by a phosphate linked, carrier-mediated system similar to or identical to the dicarboxylate transporter [52]. The affinity of oxalate to these transporters is much less as compared to their respective substrate but due to some mutations in their respective genes, an increase may occur in their transportation.

APRT (Adenine phosphoribosyl transferase) deficiency, is known to cause dihydroxyadenine (2, 8-DHA) urolithiasis. APRT catalyses the synthesis of AMP from adenine and 5'-phosphoribosyl-1-pyrophosphate in the presence of Mg$^{2+}$. In APRT deficiency, adenine is oxidized to 2,8-dihydroxyadenine (2,8-DHA) by xanthine dehydrogenase (XDH). This defect is inherited as a recessive autosomal trait. The gene is located on chromosome 16q24. Caucasian species predominantly show this defect [53].

The genes responsible for several uncommon but important kidney stone diseases have been cloned, including those for cystinuria, primary hyperoxaluria, hereditary distal renal tubular acidosis, X-linked nephrolithiasis (Dent's disease), and hereditary hypomagnesemia-hypercalciuria. Each of these diseases is inherited as a single Mendelian trait and has clinical features that distinguish it from other causes of kidney stones. Primary Hyperoxaluria is inherited as an autosomal recessive trait and is caused by a mutation in the gene for alanine-glyoxalate aminotransferase. Inactivation or impairment of this enzyme's activity increases the risk of calcium oxalate stones and nephrocalcinosis leading to renal failure.

Cystinuria is inherited autosomal recessive traits that impair renal reabsorption of cystine. It is of two types viz type I and non-type I. Type I disease is caused by a mutation in the solute carrier family 3 gene, SLC3A1 encoding heavy subunit (rBAT) of the heterodimeric transporter. Cystinuria non-type I, is caused by mutations in the SLC7A9 gene. Cystinuria is a heterogeneous disorder at the molecular level as a patient is a compound heterozygote for one SLC3A1 and one SLC7A9 mutation [54]. The inherited autosomal dominant form of distal renal tubular acidosis (dRTA) is
caused by mutations in a gene for the basolateral anion exchanger (AE1) responsible for bicarbonate transport. AE1 mutations can result in both recessive and dominant dRTA, possibly depending on the position of the amino acid change in the protein [55]. The first molecular defect associated with hypercalciuric stone formation was in the voltage-gated chloride channel protein, CIC-5. The product of a gene on the X chromosome, CIC-5 is predominantly expressed in the kidney, primarily in the subapical endosomes of proximal tubule cells.

In Dent’s disease, defects in the CIC-5 channel inhibit chloride entry into the endosomes. This prevents the acidification needed for post-endocytic degradation of low-molecular-weight proteins [56]. The gene responsible for hereditary hypomagnesemia-hypercalciuria, a syndrome characterized by magnesium and calcium wasting in the urine, nephrolithiasis, nephrocalcinosis, and muscle weakness, encodes a protein, paracellin-1, that may function either as a component or a regulator of a cation channel for paracellular reabsorption of magnesium and calcium in the loop of Henle and distal tubule. Homozygous mutations of PCLN 1 result in a selective defect of paracellular Mg and Ca reabsorption in the thick ascending loop [57].

Up to 40% of patients with idiopathic hypercalciuria have a family history of kidney stones. Patients have excessive intestinal calcium absorption, or renal calcium leak, some may also have secondary hyperparathyroidism. This shows involvement of genes such as those for the calcium-sensing receptor [58] renal sodium-phosphate co-transporter, vitamin D-receptor, renal 1-alpha-hydroxylase (vitamin D-activating enzyme), or factors affecting bone mineralization.

1.2. Treatments of kidney stones

Kidney stones that do not occur as a result of a genetic or metabolic disorder are considered to be due to diet-related condition. Proper nutrition can support healthy kidney function and may discourage stone formation, and natural therapies may help ease the pain and spasm that accompanies stone passage. Initial treatment for symptomatic kidney stones is similar for all patients. However, measures to prevent future stones vary depending upon a person’s risk of recurrence. During the initial
phase of kidney stone symptoms, many patients require only pain medication and fluids until the stone is passed. Nonsteroidal anti-inflammatory drugs (NSAIDs, such as ibuprofen or naproxen) may be prescribed for pain and can be taken in pill form. The treatments available for kidney stones include: drinking lots of water, surgical treatments and herbal Medicine.

1.2.1. Drinking of water

Water intake is useful in preventing stone disease [59]. Drinking lots of water (two and a half to three pints per day) and staying physically active are often enough to move a stone out of the body. With increased water intake the kidney stones, of small size and appropriate shape can be passed out with urine. Increased water intake between meals to prevent renal stone recurrence should preferably be achieved with relatively low calcium water and calcium-rich mineral water should be avoided [60].

1.2.2. Surgical treatment

Surgery is only an option when the stone attains a size or shape that will prevent its passage and blocks the flow of urine or when it causes damage to the kidney or another part of the urinary tract. Recovery time is longest with open surgery. Today, treatment for these stones is greatly improved and in many cases do not require major surgery and the recovery time is also reduced. Such treatments include, Ureteroscopic, Percutaneous Nephrolithotomy and Extracorporeal Shockwave Lithotripsy.

A. Ureteroscopic

It is usually needed for mid- and lower-ureter stones. No incision is made in this procedure. Instead, the surgeon passes a small fiberoptic instrument called an ureteroscope through the urethra and bladder into the ureter as shown in figure 1.4. The surgeon then locates the stone and either removes it with a cage-like device or shatters it with a special instrument that produces a form of shock wave.
A small tube or stent may be left in the ureter for a few days to help the lining of the ureter heal. Before fiber optics made ureteroscopy possible, physicians used a similar "blind basket" extraction method. But this outdated technique should not be used because it may damage the ureter. Simultaneous combined use of flexible ureteroscopy and percutaneous nephrolithotomy to reduce the number of access tracts in the management of complex renal calculi.

**B. Extracorporeal shock wave lithotripsy (ESWL)**

Extracorporeal shock wave lithotripsy was first applied successfully in a patient with gallbladder stones in January 1985 and revolutionized for treatment of stones throughout the urinary tract. More than 1 million patients are treated annually with ESWL in USA alone. In recent years extracorporeal shock wave therapy is also used in veterinary medicine especially in equine orthopedics [61]. ESWL produces high pressure shock waves which pass through the body. When wave encounters the calculus, the pressure causes the stone to be stressed, then fractured and eventually disintegrated (Figure 1.5). A fluoroscopic x-ray system is used to direct the focus of the waves precisely on the stone. A lithotriptor is a medical device used in the non-invasive treatment of kidney stones (urinary calculosis) and gallstones. It works best with stone between 4 mm and 2 cm in diameter located in the kidney. Lithotripsy offers many advantages over kidney stone removal through surgery, with lithotripsy, there is a sizable reduction in complications and pain. The trauma, inconvenience and pain of a surgical incision are avoided. Post-treatment complications are minimized and recuperation time after treatment is greatly reduced. Recuperation with lithotripsy usually takes only a few days compared to the average of three to six weeks following surgery.
Figure 1.5. Treatment of kidney stones by extracorporeal shock wave lithotripsy

Lithotripsy is of two types, shock wave and laser. A shock wave is transmitted through the patient’s skin and passes harmlessly through the patient’s soft tissue. The shock wave passes through the kidney and strikes the stone. At the stone boundary, energy is lost and this causes small cracks to form on the edge of the stone. Laser lithotripsy disintegrates the kidney stones using a pulsed laser. An endoscope (small tube) is inserted in the urinary tract next to the stone. With the endoscope in place, the laser fiber is inserted to touch the stone. The laser uses short rapid pulses of energy to break up the kidney stone into smaller particles allowing the body to flush the stone naturally. Sometimes, when kidney and urinary tract stone fragments are being passed, urine flow from the kidney can be blocked. If this causes severe pain or blockage of the kidney, a tube called stent may be placed through the back and into the kidney to keep the kidney drained until all the fragments pass out.

C. Percutaneous nephrolithotomy

Sometimes a procedure called percutaneous nephrolithotomy is recommended to remove a stone. This treatment is often used when the stone is quite large or in a location that does not allow effective use of ESWL.

In this procedure, the surgeon makes a tiny incision in the back and creates a tunnel directly into the kidney as shown in figure 1.6.
Using an instrument called a nephroscope, the surgeon locates and removes the stone. For large stones, some type of energy probe (ultrasonic or electrohydraulic) may be needed to break the stone into small pieces. One advantage of percutaneous nephrolithotomy over ESWL is that the surgeon removes the stone fragments instead of relying on their natural passage from the kidney.

1.3. Drawbacks of current treatments

The extracorporeal shock waves lithotripsy is fundamental in the treatment of lithiasis. However, there are evidences that it can produce renal damage [62]. High-energy shock waves (HESW) when applied to rat did not inhibit the animal growth but caused transitory histological lesion in spleen (proliferative changes in the red pulp) and in liver (cloudy swelling of hepatocytes) [63]. Shockwaves can enhance metastasis of tumors and this effect is attributable to cavitations. It has been reported that extracorporeal shock wave lithotripsy also leads to reduced sperm concentration and motility in men [64]. The effects of ESWL, on patiente undergoing renal stone treatment have been studied using activities of glucose-6-phosphate dehydrogenase, superoxide dismutase, catalase and levels of malondialdehyde in the erythrocyte haemolysate. Recent study revealed that ESWL can induce erythrocyte lipid peroxidation and antioxidative defense mechanism may be transiently impaired by it.
[65]. A case study also shows an unusual complication like rupture of the kidney observed after extracorporeal shock wave lithotripsy [66].

1.4. Crystals of calcium oxalate in plants

Calcium oxalate crystals are widespread minerals in higher plants [67, 68, 69, 70, 71]. These crystals exhibit specific morphological patterns suggesting a genetically controlled formation [72,73,74,75] induced by the plant. These crystals are typically developed within a crystal chamber in the vacuole of specialized cells called idioblasts [76,77]. The microenvironment into this chamber is believed to play an important role in the crystal nucleation and morphology. For instance, the size and shape of the chamber in which crystals form determine the macromolecules and ions that are present in solution into the chamber [72,73,78]

The specific biological function of calcium oxalate crystals in plants has neither been well characterized nor fully understood. In recent studies, it has been proposed that these crystals can perform diverse biological functions in plants such as storage of calcium, deposit of secondary metabolites, and molecular container of metallic ions that are found in toxic levels inside tissues or cells [71,79]. Additionally, these crystals seem to have specific functions in different plants such as to promote the formation of air chambers in aquatic plants [80], provide structural support [81] or protect against herbivores by their association with irritating chemicals or with proteolytic toxins [82,83,84].

The precise patterns exhibited by plants that produce calcium oxalate reflect multiple levels of organism and cellular control over the crystallization process [77] Extensive observations indicate that calcium oxalate does not result from random precipitations wherever appropriate levels of calcium and oxalate happen to meet but that certain cells within the plant become specialized to accumulate calcium and crystallize calcium oxalate in a controlled and defined manner. The features of calcium oxalate crystals, their functions, and the plant cells that produce them have interested plant biologists for more than a century [85,86]
1.4.1. Calcium oxalate crystallization and calcium regulation

Why do plants sequester calcium oxalate? Calcium is very abundant in the natural environment in which most plants grow. A required element for plant growth and development, calcium plays many important roles, for example, as a structural component of cell walls [87], a signal in various physiological and developmental pathways [88] and an osmoticum [89]. Nonetheless, cytosolic free calcium must be restricted to levels of ~10⁻⁷ M or less [90], because higher concentrations interfere with a variety of crucial cell processes, including calcium-dependent signaling [88], phosphate-based energy metabolism [90], and microskeletal dynamics [91].

Although the mechanisms controlling calcium absorption at the root are controversial, plants accumulate calcium in excess of cytosolic requirements and limits [89]. In addition, most plants, unlike animals, do not have well-developed excretory systems to dispose of excess calcium. Instead, higher plants appear to modulate differences between the natural abundance of environmental calcium and the very low levels required for cytosolic free calcium by controlling the distribution of calcium and its compartmentation within the cell [92]. The cell wall and the vacuole provide major sinks for calcium in plants [89].

Many plants accumulate crystalline calcium oxalate in response to surplus calcium [93]. With a solubility product of 1.3 X 10⁻⁹ in water, calcium oxalate provides a relatively insoluble, metabolically inactive salt for calcium sequestration [89]. Calcium oxalate thus provides a high-capacity repository for calcium, and plants may accumulate this salt in substantial amounts, up to 80% of their dry weight [94] or 90% of total calcium [93]. The extent of calcium partitioning into calcium oxalate varies among different taxonomic groups of plants. In many species examined so far, calcium oxalate content was found to be about 6.3% of plant dry weight [94]. Numerous studies [95] also indicate that crystals do not only form an inert, non-retrievable pool but they can also be re-dissolved. However, despite the significance of calcium oxalate in sequestering and storing calcium, little is known about factors that direct calcium to this pool.
1.4.2. **Variation of distribution and morphology of CaOx crystals**

Calcium oxalate crystals may form in any organ or tissue within plants. For example, crystals occur in roots, stems, leaves, flowers, fruits, and seeds [69] and within epidermal [96], ground, and vascular [97] tissues. Calcium oxalate often forms in idioblasts cells that develop in isolation, with distinct structure or content from surrounding cells [98]. In other instances, crystals may develop in defined groups of cells, as in files of bundle sheath cells [99], for example, or in a single layer of the seed coat [100]. Less often, entire tissues such as endosperm [101] or leaf epidermis [102] accumulate calcium oxalate in every cell or in a majority of cells.

![Figure 1.7](image.png)

**Figure 1.7.** Characteristic morphologies of calcium oxalate crystals isolated from plants.

Plant crystals display an astonishing variety of morphologies, most of which confirm to one of the following categories defined by botanists [69]: (1) prisms, consisting of simple regular prismatic shapes (Figure 1.7 A) (2) druses, which are spherical aggregates of crystals (Figure 1.7 B) (3) styloids, acicular crystals that form singly (4) raphides, acicular crystals that form in bundles (Figure 1.7 C) (5) crystal sand, small tetrahedral crystals that form in clusters. Some of the characteristic morphological features of calcium oxalate in plants are shown in Figure 1.7. Calcium oxalate exists in two chemical forms, monohydrate and dihydrate, and both of these occur in plants [103,104]. The observed morphologies represent elaborations and modifications of basic crystal structure for either the monohydrate or dihydrate form. The monohydrate is more stable and is more commonly found in plants than is the dihydrate.
1.4.3. Matrix of calcium oxalate crystals

Webb and Arnott [78] showed that grape druse crystals have a nonmineralized core material of unknown but presumably organic composition. Webb et al. [73] demonstrated that a complex organic matrix was present within the vacuole of grape raphide idioblasts and this matrix could facilitate crystal formation. On treatment of isolated calcium oxalate crystals from plants with EDTA, the calcium oxalate is partially or completely dissolved and examination of the samples with TEM reveals a non mineral matrix. This non mineral matrix retains the shape of the original crystal, thus, it is referred as “crystal matrix ghost.” The crystal matrix ghost even remains intact during processing of the sample for TEM but it is flexible and can be bent at a 90° angle, unlike the crystal, indicating that it is made of an interconnected macromolecular complex. Often the central region of the raphide crystals do not de-mineralize so that a small block of crystal remains. Partial dissolution with EDTA can also leave behind small “plates” of Ca Ox along the matrix. It is also observed that if a crystal matrix ghost is incubated with calcium and oxalate, a crystal forms with essentially the same shape as the ghost, although often the surfaces are rough or have micro-crystals projecting from them. The druse crystals also have a central core of material [105], and after dissolution of the mineral with EDTA, this core material can also initiate crystallization, although the crystal morphology is very irregular.

Microautoradiography of crystals or crystal matrix exposed to radioactive calcium or oxalate further demonstrates the ability of the matrix to bind these ions. Recently, Bouropoulos et al. [106] found that crystals of tomato and tobacco contain macromolecules that can promote CaOx nucleation. Macromolecular matrix materials can hold important implications with respect to crystal morphology and, as pointed out by Arnott and Webb [83], crystal stability.

Most calcified tissues in animal systems undergoing controlled mineralization have been found to have an organic matrix associated with them [107], which includes various classes of proteins shown in vitro to be able to control crystal growth and morphology. Such proteins have been found to be integrated into the structure of biominerals of invertebrate organisms such as sponge spicules [108], mollusk shells [109], sea urchin spines [110], and also in CaOx renal stones that form in humans
Although macromolecules appear to be involved in nucleation and modifying growth patterns [112,113], it is also possible that they may have inhibitory effects in the case of CaOx in the urinary tract [114,115].

It is interesting to note that the acidic proteins from animal matrix have some physical properties similar to the matrix protein of plant crystals, such as poor solubility of some of the animal matrix proteins, a tendency to aggregate, and poor staining on SDS-PAGE [112]. It is hypothesized by Li et al [116] that the protein isolated from plant CaOx crystals have a similar function to some of the animal matrix proteins in terms of affecting crystal growth. They suggested that crystal matrix protein has calcium binding properties, which would be important to their integration into the crystalline matrix. More recently [117] four proteins from the organic matrix of CaOx crystals present in the seeds of Phaseolus vulgaris, have been isolated which inhibited the nucleation of CaOx crystallization in solutions. They have also shown that the isolated proteins modified the morphology of CaOx crystal mainly at {120} face (factect growing face).

1.5. Hyperoxaluria induced oxidative stress

Oxalate is a natural byproduct of metabolism and in normal individuals it is harmlessly excreted. However, increased urinary excretion of oxalate, hyperoxaluria, can be toxic largely because of its propensity to crystallize at physiological pH and form calcium oxalate (CaOx) crystal deposits in the kidneys [118]. In the kidneys, CaOx crystals can block the renal tubules, disrupt cellular functions and kill nearby cells. Hyperoxaluria is a result of either genetic (primary hyperoxaluria) or environmental factors (secondary hyperoxaluria). Enhanced absorption of oxalate, secondary to many gastrointestinal diseases, ileal resection, or jejuno-ileal bypass is called enteric hyperoxaluria [119]. Even though various hyperoxalurias have distinct origins, the pathologies they induce can often be indistinguishable, encompassing urolithiasis, nephrocalcinosis, metabolic acidosis, hematuria, pyelonephritis, hydronephrosis and renal failure. In case of primary hyperoxaluria, there is a systemic deposition of CaOx in almost all of the body tissues including kidneys, heart, bone, cartilage, teeth, vasculature and brain. Patients with primary hyperoxaluria eventually
develop end-stage renal failure, usually in childhood. Patients with enteric hyperoxaluria may also develop renal inflammation and end stage renal disease.

Recent research has shown that the response of renal epithelial cells to oxalate and CaOx crystals is biphasic and concentration dependent [120,121,122]. Oxalate by itself is mitogenic at low concentrations and toxic at higher concentrations as well as in association with CaOx crystals. Injury to the renal epithelial cells results in cellular degradation and the production of membranous vesicles [123]. The crystals are either passed as crystalluria particles or are endocytosed by the epithelial cells to be processed by their lysosomal system or transported to the interstitium. CaOx crystal deposition in the kidneys upregulates the expression and/or synthesis of macromolecules, which can promote inflammation and lead to fibrosis [124,125]. In animals and renal epithelial cells in culture, reaction to high oxalate and CaOx crystals is associated with the generation of free radicals [126,127]. Antioxidants reduce hyperoxaluria and CaOx crystal induced toxicity [128,129].

1.5.1. Oxidative stress and CaOx nephrolithiasis

High concentrations of oxalate and CaOx crystals also provoke renal cells to increase the synthesis of various mediators of the inflammatory processes and extracellular matrix production, and modulators of crystallization [124,125,130]. Reactive oxygen species are involved in the activation of signaling molecules such as protein kinase C, c-Jun N-terminal kinase and p38 mitogen activated protein kinase (MAPK), with influence over transcription factors such as NF-κB and activated protein-1 (AP-1). Activation of these transcription factors leads to upregulation of genes and production of crystallization modulators such as osteopontin, bikunin, and microglobulin [124,125,130], which affect all aspects of nephrolithiasis including crystal formation, growth, aggregation as well as their retention within the kidneys.

Oxidative stress is injurious to all components of the cells. Figure 18 outlines the lithogenic effects of hyperoxaluria induced generation of reactive oxygen species (ROS). Previous studies from many laboratories indicate that damage to the urothelium may predispose to de novo crystallization and crystal retention in the kidneys.
It has been demonstrated that experimentally induced hyperoxaluria in rats results in renal tubular cell damage and CaOx crystal deposition [123]. Both apoptotic and necrotic injuries have been detected [131,132]. Crystals always deposit at sites of tubular injury in association with membrane vesicles and are also seen attached to the exposed basement membrane [123,133,134]. Our in vitro studies have shown that membranes and lipids of cellular degradation products are excellent nucleators of CaOx crystals at supersaturation normally found in the renal tubular fluids [135]. Tissue culture studies in which renal epithelial cells were exposed to oxalate or CaOx crystals have shown that epithelial injury promotes attachment of CaOx crystals [136, 137]. This attachment is mediated by oxalate induced exposure of phosphatidylserine
on cell surfaces [138,139]. Interestingly, apoptosis involves the exposure of phosphatidylserine on cell surfaces [131].

1.5.2. Antioxidants for renal protection

Figure 1.9 illustrates the stages in which specific antioxidants and other protective detoxification treatments have been shown to impede the development of hyperoxaluria-induced oxidative stress. Pretreatment with vitamin E along with mannitol abolished the deposition of CaOx crystals in the kidneys of rats injected with sodium oxalate [140]. Alanine-induced deposition of CaOx crystals in rat kidneys was blocked by dietary supplementation with vitamin E plus selenium [141]. Interestingly, vitamin E alone caused only a decrease in crystal deposition, while combined treatments totally abolished it. Treatment with methionine [142] or glutathione monoester [143] also reduced renal CaOx crystal deposits in the kidneys of hyperoxaluric rats. The reduction or total elimination of crystal deposition was associated with restoration of the anti-oxidation defenses of the kidneys by increasing activities of the enzymes SOD, catalase, glutathione peroxidase (GPx) and/or free radical scavengers, reduced glutathione (GSH), ascorbic acid, vitamin E and protein thiol groups. Vitamin E is the major lipid soluble antioxidant present in the cell membranes and acts synergistically with the other antioxidants. It can react with lipid radicals and stop the propagation of lipid peroxidation. Selenium is normally incorporated in GPx. Mannitol is a scavenger of hydroxyl radicals while methionine is a thiol generating compound.

Green tea has recently been shown to reduce CaOx crystal deposits in the kidneys of rats made hyperoxaluric by the administration of ethylene glycol [144]. Reduced crystal deposition was coupled with improved SOD activity. In addition, green tea consumption caused a decrease in apoptotic activity in the kidneys. It was concluded that antioxidants, catechins, present in green tea were mainly responsible for these improvements. Reduction of angiotensin production by inhibiting ACE or blocking angiotensin receptors has been shown to significantly reduce renal CaOx crystal deposition as well as the development of interstitial inflammation [145]. These treatments also resulted in a reduction in the oxidative stress measured as products of lipid peroxidation. Angiotensin II is implicated in causing oxidative stress by
activating membrane associated NADPH oxidase, which leads to the production of superoxide [146]. ROS generated through the activation of NADPH oxidase are also involved in the production of MCP-1, for the recruitment of monocytes/macrophages to the interstitium. Exposure of renal epithelial cells in culture to oxalate caused ROS dependent up regulation of the MCP-1 gene, and the production and secretion of the protein [147]. Antioxidant treatment for stone disease has not been clinically tested.

![Figure 1.9. Antioxidant and other detoxification treatments to impede the development of hyperoxaluria induced oxidative stress](image)

However, some well-known antioxidants such as citrate and allopurinol, have been evaluated in a number of prospectively randomized trials, and their efficacy has been established [148] in populations of recurrent stone formers. Citrate increases cellular nicotinamide adenine dinucleotide phosphate and reduced glutathione while allopurinol is a xanthine oxidase inhibitor.

1.6. Herbal medicine and urolithiasis

Many remedies have been employed during the ages to treat urinary stones. In the traditional systems of medicine, most of the remedies were taken from plants and they were proved to be useful, though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some
Composite herbal drugs and plants. The recent treatment procedures like surgical removal, percutaneous techniques and extracorporeal shock wave lithotripsy (ESWL) are prohibitively costly for the common man. With these procedures recurrence is quite common and the patient has to be subjected to careful follow up for a number of years. Pharmacotherapy has a potential to reduce the recurrence rate. The use of plant products with claimed uses in the traditional systems of medicine assumes importance.

1.6.1. Medical plants and Goethe

Due to the adverse effects of the present day treatment strategies for kidney stone, alternative treatment modalities composed of herbal remedies have been the mainstay of medical therapy for thousands of years, especially in Eastern civilizations. Although it is believed that the resurgence of interest in phytotherapy became popular in the second half of the 19th century in Western countries, this complementary medical therapy was widely used in Europe much before that date. Johann Wolfgang von Goethe (1749 to 1832), the famous German poet, novelist, playwright, courtier and natural philosopher, was one of the greatest figures in western literature. Besides this, Goethe experienced urolithiasis all his life. The first renal colic episode was reported in 1795 and the most dramatic period was in February 1805. He experienced severe fever and was almost dying of urosepsis. Goethe performed a cure in Lauchstäd in July under the supervision of Professor Johann Christien Reil, who prescribed a treatment composed of thermae carolinae, aqua calcis, Hyoscyamus niger, Arum maculatum, soda crystallisata, herbae subastringentes and uva-ursi. Hyoscyamus niger is a plant that grows in Europe and has relaxing, antispasmodic, anesthetic features that share the pharmacological effect of drugs prescribed for renal colic treatment even today. Uva-ursi was prescribed to Goethe because of its diuretic and antilithogenic effects. After this therapy colic disappeared but stone passage was not mentioned [149].

1.6.2. Social and economic impact of phytotherapy

Only a decade ago, alternative medicine in America was still a distinctly counterculture phenomenon. It has now become an established presence in mainstream culture. In fact, a national survey was conducted in 1990 in US about the
use of unconventional therapies for health problems [150]. The survey recorded 1539 adults by telephone interview. One of three (34%) of respondents reported using, at least one unconventional therapy in the past year and 10.2% of them had seen providers for unconventional therapy. In 1998, this percentage has increased to 15.1% [151]. The frequency of use such therapy varied somewhat among socio-demographic groups with the highest use reported by non black persons who had relatively more education and higher incomes. The majority used unconventional therapy for chronic and serious conditions. The interesting thing in this study is the revelation that 70% of the respondents who used unconventional therapy did not inform their medical doctor that they had done so.

Clearly, the use of plant products as medicines is widespread and growing. In 1996, Brevoort estimated the size of the 1994 US herbal market at $1.6 billion [152]. By 1998, her estimate had increased to $3.9 billion. A 1996 survey by prevention magazine and ABC News reported that 1 in 3 Americans use herbal medicines, estimating the size of the annual herbal market at $3.2 billion [153]. Several national polls in 1997 and 1998 corroborated these estimates, reporting that 32% to 37% of Americans use medicinal botanicals in a given year. One of the among unconventional therapy that we will focus on is phytotherapy or the use of plants in the treatment of diseases especially kidney stone formation. Indeed, herbal medicine is as ancient as the history of mankind. From the very beginning, herbal treatment has been a favorite tool of naturopathically inspired practitioners. Of course, conventional medicine has also derived many of its drugs from plant sources. According to the World Health Organization, approximately 75% of the global population, most of the developing world, depends on botanical medicines for their basic healthcare needs [154]. Substances first isolated from plants account for approximately 25% of the western pharmacopoeia, with another 25% derived from modification of chemicals first found in natural products [155].

1.6.3. Scientific evidences of phytotherapy for urolithiasis

As far as urolithiasis is concerned, acupuncture, herbal medicine, natural products and homeopathy have been used to treat and/or to alleviate symptoms of kidney stone patients. In case of herbal medicines, there is a large number of species
Review of Literature

described in many pharmacopoeias of several countries in the world as remedies for urolithiasis. However, few investigators have devoted their efforts to study these plants by using objective and scientific methods. Such studies are needed to understand the mechanism by which these plants exert their effects and identify their active principles. The efficacy of rice-bran therapy was evaluated in patients with idiopathic hypercalciuria [156]. During the treatment, a reduction of urinary calcium excretion was noticed. These results were confirmed experimentally and clinically [157]. In another study conducted on hypercalciuric patients with calcium-containing urinary stones, the frequency of stone episodes was reduced dramatically from 0.462 to 0.101 per patient per year [158]. Urinary calcium excretion was considerably reduced while urinary phosphate and oxalate were slightly increased. Interestingly, the treatment was well tolerated and no side effects were observed even after conducting the therapy for up to 43 months for certain patients.

Grases and colleagues have studied the efficacy of a medicinal plant, *Rosa canina* on the urinary risk factors of calcium oxalate [159,160,161]. Except some little effect on calciuria and citraturia, no significant changes have been observed during the treatment on urinary chemistries. However, the important findings are that beneficial effect of this plant depends on diet.

To ascertain the beneficial effect of banana stem extract on urinary risk factors, a prospective study showed that the plant extract reduced significantly urinary oxalate in experimentally hyperoxaluric rats. Such effect could be beneficial in the treatment of patients with hyperoxaluria urolithiasis.

Another Chinese medicinal plant that has attracted more attention is Kampou medicine known to be used in the treatment of various diseases for hundreds of years. It had been also used for prevention and treatment of urinary calculus. An experimental study suggested a direct inhibitory effect of Kampou extracts of calcium oxalate crystallization *in vitro* and *in vivo* [162]. In this study, two species from Kampou, Takusya (*Alisma orientale*) and Kagosou (*Prunella vulgaris*), were employed to evaluate their stone prophylactic effect in an animal model. In the same study, Chorey-to which is a Chinese medicine that contains five Kampou plants including Takusya, has been evaluated *in vivo*. The low dose of this preparation
exhibited apparent anti-stone effect despite the disadvantage of decreasing citrate excretion. Takusya had been also used to examine the inhibitory effect of the formation of calcium oxalate renal stones induced by ethylene glycol and vitamin D3 in rats as well as on osteopontin expression that is identified as an important constituent of stone matrix [163]. The rate of renal stone formation is lower in the group receiving Takusya extracts than in the control group. The expression of osteopontin in the rat group receiving the plant was smaller than in stone group. This finding suggests that Kampou medicine action include decreasing on calcium oxalate aggregation and growth as well as proliferation.

In Brazil, Phyllanthus niruri has been used to treat several pathological conditions [164]. The plant has been called "break stone" because it has been used for generations as an effective product to eliminate gallstones as well as kidney stones. Such observation has been confirmed in rat model of urolithiasis induced by the introduction of CaOx seed into the bladder [165]. Its effect was noticed particularly on crystal growth. The effect seemed to be independent of changes in the urinary excretion of citrate and magnesium but might be related to the higher incorporation of GAGs into calculi. The plant has been the subject of many phytochemical and pharmacological investigation in which different classes of compounds have been identified like alkaloids, flavonoids, lactones, steroids, terpenoids, lignans, and tannins.

Some researchers have demonstrated an antispasmodic and an analgesic activity in Phyllanthus niruri which could explain the popular use of the plant for kidney and bladder stones [166]. The effect of an aqueous extract of Phyllanthus niruri has also been investigated in vitro on a model of CaOx crystal endocytosis by Madin-Darby Canine Kidney cells (MDCK) in culture. The extract exhibited a potent and effective nonconcentration dependant inhibitory effect on CaOx crystal internalization [167]. The use of 68 medicinal plants belonging to 29 families and 58 genera were documented against the treatment of kidney and urinary disorders in the tribal communities of Ladakh region in India. The most common species were Bergenia ligulata, Emblica officinalis, Mangifera indica, Punica granatum, Terminalia bellerica, Terminalia chebula, Zingiber officinale [168]. The fresh juice of Coleus
*aromaticus* was found to reduce the deposition of calcium and oxalate in the kidney of experimental rats [169]. Certain enzyme systems implicated in the process of calcification like ATPases and phosphohydrolases were affected by the juice proving its regulatory influence on calcium oxalate stone formation. Studies on various fractions of *Tribulus terrestris* have indicated that the aqueous methanolic fraction is more effective against experimentally induced urolithiasis by foreign body insertion method using glass beads in albino rats [170]. Investigation of the effect of aqueous extract of *Tribulus terrestris* on the oxalate metabolism in male rats fed with sodium glycolate, revealed a decrease in urinary oxalate excretion and a significant increase in urinary glyoxylate excretion and also a decrease in liver galactose oxidase and glutamic acid decarboxylase activities [171].

Studies on the stem juice of *Musa paradisiaca* were found to be effective in dissolving the phosphate type of stones in albino rats induced by foreign body insertion method using zinc discs [172]. In another experimental study, stem juice of *Musa* significantly reduced the incidence of oxalate urolithiasis by lowering the activity of the enzyme glycolic acid oxidase [173]. The stem juice of *Musa paradisiaca* reduced urinary oxalate, glycolic acid, glyoxylic acid and phosphorus excretion in hyperoxaluric rats [174]. *Agropyron repens* is another widely consumed extract for nephritis, urethritis and urinary calculi [161]. However, *Agropyron repens* infusion has not been assigned to any positive effect on urolithiasis risk factors. The decreased citriuria, increased calciuria and decreased magnesium, were observed by this treatment. This indicates contradiction towards consumption of this remedy for urolithiasis [161]. Some most commonly used plants for kidney stone treatment are given in table 1.1. Another antilithogenic effect of some herbal remedies is antimicrobial properties. It must be emphasized that a deficit in the crystallization inhibitory effect of urine and the presence of promotors are considered the most important risk factors in the process of urinary stone disease [175] when these conditions favor stone formation, the anti-adherent layer of glycosaminoglycans acts as a protective barrier against urinary stone disease. If this layer is damaged, i.e. as a consequence of bacterial attack, a stone nucleus might develop, leading to a full stone in the urinary tract.
### Table 1.1. Currently consumed phytotherapeutic agents and their mechanisms of action

<table>
<thead>
<tr>
<th>Agent</th>
<th>Evaluated</th>
<th>Potential Beneficial Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herniaria hirsuta</td>
<td>Urine or cell culture <em>in vitro</em></td>
<td>Removes crystals already attached to cell surface, results in higher COD vs COM excretion</td>
</tr>
<tr>
<td>Cranberry juice</td>
<td>Humans <em>in vivo</em></td>
<td>Increases urinary citrate excretion, decreases urinary oxalate and calcium ion excretion</td>
</tr>
<tr>
<td>Grapefruit juice</td>
<td>Humans <em>in vivo</em></td>
<td>Increases urinary citrate excretion</td>
</tr>
<tr>
<td>Lemonade juice</td>
<td>Humans <em>in vivo</em></td>
<td>Increases urinary citrate excretion</td>
</tr>
<tr>
<td>Dolichos biflorus</td>
<td>Urine or cell culture <em>in vivo</em></td>
<td>Decreases calcium phosphate precipitation</td>
</tr>
<tr>
<td>Bergenia liguata</td>
<td>Urine or cell culture <em>in vivo</em></td>
<td>Decreases calcium phosphate precipitation</td>
</tr>
<tr>
<td>Vigna unguiculata</td>
<td>Humans <em>in vivo</em></td>
<td>Increases urinary magnesium</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Animals <em>in vivo</em></td>
<td>Diuretic</td>
</tr>
<tr>
<td>Ammi visnaga</td>
<td>Animals <em>in vivo</em></td>
<td>Diuretic</td>
</tr>
<tr>
<td>Aerva lanata</td>
<td>Animals <em>in vivo</em></td>
<td>Decreases urinary calcium, oxalate, uric acid &amp; phosphorus excretion</td>
</tr>
<tr>
<td>Costus spiralis</td>
<td>Animals <em>in vivo</em></td>
<td>Decreases stone size with unknown mechanism, no diuretic effect</td>
</tr>
</tbody>
</table>
At this point, some extracts that show antimicrobial properties can be considered antilithogenic by protecting the anti-adherent glycosaminoglycan layer covering the epithelium of the collecting system. In regard to this aspect Muangman et al evaluated *Andrographis paniculata*, an Eastern herb, for its bacteriostatic activity [176]. They prescribed this drug in patients who underwent SWL and noted a significant decrease in pyuria and hematuria. *Arctium lappa*, *Equisetum arvense* and *Arctostaphylos uva ursi* exerted the same antiseptic activity, which prevented the retention of microcalculi on the kidney epithelium as the antilithogenic action [177]. Also, *Agropyron repens* has been thought to exert antimicrobial features, which might be responsible for its protective effect [161]. In addition, Melzig evaluated herbal remedies based on goldenrod (*Solidago virgaurea* L.) and found new mechanisms responsible for the antilithogenic activities of these remedies [178]. He reported that such remedies exert a rather complex action spectrum, such as antimicrobial and anti-inflammatory properties. It is highly recommended for infections and inflammations to prevent kidney stone formation.

The vast Ayurvedic literature claims a number of plants to be useful for urinary stones, still many plants need to be exploited for their pharmacological actions. Despite of intensive research to establish the mechanisms of stone formation, dietary management, evaluation of medicinal plants and other agents in the treatment of urinary stones, still to date there is no standard drug available. The main drawbacks in the development of a standard drug may be attributed to multifactorial nature of urolithiasis, different biochemical disorders that lead to urolithiasis and different chemical forms of renal stones.

### 1.6.4. Herbal plants and diuretics

The current role of diuretics in urolithiasis is limited. Although thiazides are commonly used to treat urolithiasis due to their hypocalciuric effects, certain diuretics might even have a negative effect on stone risk, e.g. urine calcium excretion increases in patients on a loop diuretic [179]. In fact, immediate improvement in hemodynamic variables and cardiac performance can be detected in patients with chronic heart failure following diuretic therapy, primarily due to decreases in plasma and extracellular fluid volumes. Plasma renin, angiotensin and aldosterone increase
maximally in week 1 but they attenuate during sustained therapy [180]. These findings suggest that diuretics cause volume contraction other than an increment in urine volume in a short period with a variable clinical outcome in the long term due to diuretic resistance [180]. Although many studies claimed that the diuretic effect of the herbs was responsible for the antilithogenic effect, still there was no study confirming that the long-term diuretic activity has a potential antilithogenic effect. On the other hand, the aim of many folk medicines is based on the increment of urine volume. It is considered that the critical step in idiopathic CaOx nephrolithiasis is the nucleation and growth of crystals on solid particles [181]. High urine volume after volume contraction in the short term might be an important factor because it can prevent nucleation and may be beneficial for the spontaneous passage of small fragments. Thus, extracts leading to increased volume of urine can be a part of the antilithogenic phytotherapy.

1.7. Herbal based commercial formulations for urolithiasis

The marketed composite herbal formulations, Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India), Uriflush (Inti Sumatera Global, Indonesia), Uriflow (Discovery Herbs, USA) and Chandraprabha bati (Baidyanath, India) have been widely used clinically to dissolve urinary calculi in the kidney and urinary bladder. Pharmacological and clinical studies carried out on a composite herbal formulation, Trinapanchamool consisting of five herbal drugs namely Desmostachya bipinnata, Saccharum officinarum, Saccharum munja, Saccharum spontaneum and Imperata cylindrica found it to be effective both as a prophylactic in preventing the formation and as a curative in dissolving the preformed stones in albino rats. The antiurolithiatic activity of this formulation has been attributed to its diuretic activity [182]. Cystone is a product of The Himalaya Drug Co. (Figure 1.10) which is often prescribed by the physicians to the patients suffering from urinary calculi. There are various studies which showed its ability to inhibit calcium phosphate and calcium oxalate mineralization [183]. The main components of Cystone are given in table 1.2.
Table 1.2. The components in one Cystone tablet [183]

<table>
<thead>
<tr>
<th>Herb</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didymocarpus pedicellata</td>
<td>65 mg</td>
</tr>
<tr>
<td>Saxifraga ligulata</td>
<td>49 mg</td>
</tr>
<tr>
<td>Rubia cordifolia</td>
<td>16 mg</td>
</tr>
<tr>
<td>Cyperus scariosus</td>
<td>16 mg</td>
</tr>
<tr>
<td>Achyranthes aspera</td>
<td>16 mg</td>
</tr>
<tr>
<td>Onosma bracteatum</td>
<td>16 mg</td>
</tr>
<tr>
<td>Vernonia cinerea</td>
<td>16 mg</td>
</tr>
<tr>
<td>Shilajit</td>
<td>13 mg</td>
</tr>
<tr>
<td>Hafsrul yahhood bhasma*</td>
<td>16 mg</td>
</tr>
</tbody>
</table>

*Hafsrul yahhood bhasma is prepared with Ocimum basilicum, Tribulus terrestris, Mimosa pudica, Dolichos biflorus, Pavonia odorata, Equisetum arvense and Tectona grandis.

In addition, its efficacy to reduce urolithiasis was also reported in male Wister rats [184]. In various reports, the anticalcifying properties of Cystone are used as a reference for evaluating the antilithiatic properties of other plants.

1.8. Herbal medicines: Aspects untouched

Recent years have seen dramatic advances in phytotherapy for urolithiasis. An unavoidable interest in this, results in an expense of more than $1.5 billion annually in the United States [185]. Although phytotherapeutic extracts are popular in folk culture, review of literature suggests that very few studies have been done on the exact clinical role, efficacy and side effects of these herbs after long-term consumption. Correspondingly potential acceptance of this herbal therapy as an alternative or an adjunct to classic medical therapy remains to be determined. An increased excretion of urinary citrate, decreased excretion of urinary calcium and oxalate, and diuretic and antiseptic features are only some of the known mechanisms
of these extracts. A precise understanding of the mechanism of action of these herbal extracts would have diagnostic value with regard to the nature of this disease, in addition to the potential therapeutic implications in this future field of research. In this respect the absence of this information is a fruitful area for scientific research by willing investigators. Although preclinical research has proved that the efficacy of some of these herbs is truly mythical, all deserve innovative scientific study to clarify the mechanism of action because myths may always become reality in the future.

1.9. *Dolichos biflorus*

*Dolichos biflorus* L. (Leguminosae-Papilionoideae), locally known as “Kulthi” in Himachal Pradesh, India, is a fast growing annual herb. Its stems are nearly erect. Its branches climb and twine while producing clusters of pea-like yellow flowers which are 0.75” inch long. It makes flat, curved 2” long seed pods (Figure 1.11).

It is mainly found in tropical and subtropical regions in south Asian countries. Its immature seeds are widely consumed as food in south Asian countries including rural areas of India.

It is commonly used in folklore to treat urolithiasis. The seeds of *Dolichos biflorus* are also the component of various commercial drug formulations like Cystone. So far, only few investigators have studied its efficacy on calcium mineralization. More recently, the seeds of *Dolichos biflorus* were tested and compared with Cystone (commercial drug) for their *in vitro* antilithiathic and anticalcification activity on calcium phosphate precipitation [186]. The extract of *Dolichos biflorus* showed inhibitory activity almost equivalent to Cystone towards calcium phosphate precipitation. Similar *in vitro* study showed the possibility of more than one biomolecules in *Dolichos biflorus*, possessing the ability to inhibit calcium phosphate precipitation [187]. There are no reports in the literature of the exact clinical role and efficacy of this plant on *in vivo* urolithiasis. The present study is aimed at examining the efficacy of *Dolichos biflorus* and identification of the most potent biomolecule responsible for its antilithiatic property.