3. REVIEW OF LITERATURE

3.1 Investigational drug profile

3.1.1 Scientific classification of *Hordeum vulgare* Linn. (Ross, 2005)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Liliopsida</td>
</tr>
<tr>
<td>Subclass</td>
<td>Commelinida</td>
</tr>
<tr>
<td>Order</td>
<td>Cyperales</td>
</tr>
<tr>
<td>Family</td>
<td>Gramineae; Poaceae.</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Hordeum L.</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>Vulgare</em></td>
</tr>
</tbody>
</table>

**Vernacular names**

The various vernacular names of the plant are as follows:

- **English**: Barley
- **Ayurvedic**: Yava, Hayeshtha, Hayapriya, Shuka-dhaanya, Tikshnashuka.
- **Unani**: Barley, Jao Shaeer.
- **Siddha**: Yavam, Saambaluppu (ash)
- **Hindi**: Jav, Jau
- **Malayalam**: Yavam, Soochigotambu

3.1.2 Geographical distribution

The *Hordeum vulgare* Seeds cultivated as food crop in Uttar Pradesh, West Bengal, Bihar, Chattisgarh, Madhya Pradesh, Maharashtra, Rajasthan, Uttarakhand, Haryana, Punjab, Himachal Pradesh and Jammu and Kashmir (Fertilizer Association of India, 2006).
3.1.3 Plant description
An erect annual herb grows up to 1 meter in height. Leaves linear, flaccid and few. Spike flattened, 30 cm long and terminal. Spikelets sessile, arranged in 3 on two sides of rachis. Fruits are elliptic short pointed caryopsis.

Barley seeds is a ‘caryopsis’ consisting of lemma, palea, and rachilla. The caryopsis is composed of pericarp, endosperms and empryo. Barley seeds are a caryopsis measuring about 8-12 mm long, 3-4 mm wide and 2-3 mm thick. The seeds normally consist of hull expect that of naked cultivar which becomes free after threshing. Spikelets may be awnless or hooded, awned or beared all these species of barley are under cultivation (Ahlawat, 2004)

Part used: Seeds

3.1.4 Chemical constituents
The nutritional quality of the barley depends on beta-glucan fraction of the grain. Barley contains arabinoxylans and ß-glucan; fibers also found in oat bran and reported to reduce cholesterol levels (McIntosh et al., 1991). It also contains the oil tocotrienol. Barley is the source of a natural sweetener known as malt sugar or barley jelly sugar, which is high in maltose. The root of the germinating seeds contains the alkaloid like
hordenine, gramine and aminophenol (Kremer and Ben-Hammouda, 2009). Enzymes located in the seed for mobilization of carbohydrate reserves are well studied in many plants, particularly amylases, which are responsible for the breakdown of starch. Amylases are by far the most abundant enzymes in the seed. (Juge et al., 2002). There are variety of other enzymes, such as pectinases, hemicellulases, galactosidases and mannanases, which are all crucial to the function of the seed. The enzymes mentioned above all target structural polysaccharides such as starch, cellulose, hemicellulose, glucomannans, galactomannans and other structural compounds in the cell wall (Buckeridge et al., 2000). It also contains abscisic acid, alkaline resorcinol, benzoquinone, calmoduline, carnitine and endo-ß mannanase, etc. (Hrmova et al., 2006).

The seeds are the affluent source of phenolic compounds like oleic, lenoleic, palmitic, caffeic, chlorogenic, protocatechuic, syrignic acids and catechins. It also contain phenolic compounds like o-coumaric acid, vellinic acid, scopolentin, ferulic acid, p-hydroxybenzoic acid, syringic acid (Kremer and Ben-Hammouda, 2009). It also contains other constituents like vallinic acid, gibberellin acid, p-hydroxycinnamic acid, 4-hydroxy-3-methoxy cinnamic acid, and caffeic acid. An oxalate oxidase that has commercial applications in monitoring oxalate levels in patients with hyperoxaluria has been obtained from barley seedling plants. The seeds also contain thiamin, Riboflavin, Niacin and little Ascorbic acid. It also contain lipid including carotenoids and tocopherol. The seeds are the rich source of saponins, starch and cathecin (McIntosh et al., 1991). Juice of young barley seeds- 7 times richer in vitamin E than oranges, 5 times richer in iron than spinach, 25 times richer in potassium than wheat; high in SOD (superoxide dismutase), also contain Vitamin K. the seeds contains 2-3% lipids, including several health promoting activities ausch as carotenoids and tocopherol. The barley also contain high amount of protein like albumin, globulins, prolamins and glutelins. (Newman and Newman, 1992).

3.1.5. Pharmacological review
Shah et al. (2009) reported Hepatoprotective activity of Hordeum vulgare Linn. Seeds against different chemicals like ethanol, acetaminophen and CCl4 induced liver damage.
Jeong et al. (2009) reported that 3,4-Dihydroxybenzaldehyde purified from the barley seeds (*Hordeum vulgare*) inhibits oxidative DNA damage and apoptosis via its antioxidant activity.

Gilroy and Jones, (1994) reported that the Perception of gibberellin and abscisic acid at the external face of the plasma membrane of barley (*Hordeum vulgare* L.) aleurone protoplasts.

### 3.1.6 Traditional uses
The Seeds of *Hordeum vulgare* have been used in ethnomedicine for several medicinal properties: nutritive and demulcent during convalescence and in cases of bowel inflammation, diarrhea and improve immunity. *The Ayurvedic Pharmacopoeia of India* (1999) and Evan Ross (2005) recommends that barley is distinctly used in urinary disorders like urinary retention, urinary tract infection and urolithiasis (kidney stone), muscular rigidity, chronic sinusitis, lipid disorder, cough, asthma, and obesity. It is also used as anticoagulant, anti-atherogenic, antidiabetic, antiallergent, demulcent, digestive, embolic, emollient, expectorant, and stomachic agent (Duke and Wain, 1981). It is a folk remedy for bronchitis, burns, debility, fever, inflammation and urogenital ailments. It also exhibits anti-inflammatory and anti-hepatotoxic effect. The *Hordeum vulgare* is very potent about its antihypercholesterolemic, anti-hyperglycemic as well as anti-oxidant activities (Ross, 2005).
3.2 Chronic Kidney Disease

3.2.1 Overview of Chronic kidney Disease

Chronic kidney disease is a worldwide public health problem, with increasing incidence and prevalence, high costs, and poor outcomes. More than 400,000 Americans have end-stage renal disease (ESRD), and over 300,000 of these patients require maintenance dialysis and kidney transplantation (United States Renal Data System, 2000). There is even a substantially higher prevalence of the earlier stages of chronic kidney disease (CDK), with adverse outcomes, including decreased or loss of kidney function, cardiovascular disease (CVD) and premature death. Strategies to improve outcomes will require a global effort directed at the earlier stages of CDK (Remuzzi et al., 2002).

Chronic kidney disease (CKD), also known as chronic renal disease, is a progressive loss in renal function over a period of months or years. In other words Chronic kidney disease is present if any of the following criteria is present for three months or more (National Kidney Foundation, 2002):

1. Structural or functional abnormalities of the kidney (with or without decreased GFR). These may manifest as either:
   a. Pathologic abnormalities
   b. Markers of kidney damage
      i. Proteinurea (albumin-to-creatinine ratio >30 mg/g)
      ii. Abnormalities in the urinary sediment - cells, casts, crystals
      iii. Abnormalities on imaging studies - collecting system, cysts, stones
      iv. Tubular syndromes
   c. Kidney transplant recipients

2. GFR less than 60 ml/min/1.73 m$^2$, with or without kidney damage.
3.2.2 Causes of Chronic Kidney Disease

Clinical evaluation for CKD should include elucidation of the cause of disease. Table 3.1 represents the causes of chronic kidney disease.

**Table 3.1: Causes of kidney disease**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Major Types (Examples)*</th>
<th>Approximate Prevalence Among Patients with Kidney Failure**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic kidney disease</td>
<td>Type 1 and type 2 diabetes mellitus</td>
<td>33%</td>
</tr>
<tr>
<td>Non-diabetic kidney disease</td>
<td>Glomerular disease (autoimmune disease, systemic infection, drugs, neoplasia)</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>Vascular disease (hypertension, renal artery disease, micro-angiography)</td>
<td>21%</td>
</tr>
<tr>
<td></td>
<td>Tubulo-interstitial disease (urinary tract infection, stone, obstruction, drug toxicity)</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>Cystic disease (polycystic kidney disease)</td>
<td>6%</td>
</tr>
<tr>
<td>Disease in kidney transplant</td>
<td>Allograft nephropathy (chronic rejection)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Drug toxicity (Cyclosporine or Gentamicin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrent disease (glomerular disease)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transplant glomerulopathy</td>
<td></td>
</tr>
</tbody>
</table>

*Examples of some causes for specific pathologic types. Italics indicate types of kidney disease that may be associated with large amounts of proteinuria (for example, spot urine total protein-to-creatinine ratio >500-1000 mg/g).

3.2.3 Risk factors and CKD
There are several factors which cause the initiation and the progression of CKD. These can be classified into two distinct categories: those proven to be causal (risk factors) and those that are associated with CKD in the absence of established causal relations (risk markers).

3.2.3.1 Susceptibility factors
CKD commonly clusters within families, which implies genetic or familial predisposition (Bergman et al., 1996). Genetic studies have suggested links between CKD and various alterations or polymorphisms of candidate genes encoding putative mediators, including the Rennin-Angiotensine System (RAS). Racial factors also have a role in the susceptibility to CKD as shown by the high prevalence of CKD related to hypertension, diabetes or both among African and Native Americans in the USA, as well as Afro-Caribbean and Asian individuals in the UK (Buck et al., 1999). Low birth weight and infant malnutrition in some ethnic minorities might be associated with a reduction in the number of nephrons, predisposing to hypertension and renal disease in later life (Brenner et al., 1994). Male and elderly people might also be more susceptible to CKD, which would explain the high proportions of these population groups in renal-replacement-therapy programmes (The Renal Association, 2004; United States Renal Data System, 2003).

3.2.3.2 Initiation factors
Many cohort studies in the USA (Klag et al., 1997; Schaeffner et al., 2003; Haroun et al., 2003) and Japan (Iseki, 2003) have identified hypertension, diabetes, hyperlipidemia, obesity and smoking as risk factors or markers in the general population for the development of CKD. Common risk factors and markers seem to be linked to both renal and cardiovascular diseases in more developed countries. Also, albuminuria itself is a predictor not only of CKD but also of cardiovascular morbidity and mortality (Hillege et al., 2002). The risk factors, particular diabetes and hypertension, are also likely to affect individuals in less developed countries, where “western” lifestyle is adopted. Diabetic nephropathy is now one of the leading causes of ESRD (exceeding 30-40%) worldwide (United States Renal Data System, 2003).
3.2.3.3 Progression factors

The progression of established CKD is variable and depends on several risk factors or markers. Non-modifiable factors include genetics, race, age and sex. For instance, there is much evidence that the rate of progression of CKD is faster among patients who are elderly (Jungers et al., 1996), male (Hannedouche et al., 1993) or African-American (Hsu et al., 2003). Most notable among the modifiable progression factors is systemic hypertension (Klahr et al., 1994; Jafar et al., 2003). Proteinuria is a reliable marker of the severity of CKD and a powerful and independent predictor of its progression (Remuzzi et al., 1998).

Controversy prevails as to whether proteinuria is a risk factor for the progression of clinical nephropathies. Patients with persistently high rates of urinary protein excretion (> 3-5 g in 24 hr) in general have a much faster rate of progression than those with mild or moderate proteinuria (< 1-3 g in 24 hr). Metabolic factors have been implicated in the progression of CKD. The Diabetes Control and Complications Trial (The Diabetes Control and Complications Trial, 1995) and the UK Prospective Diabetes Study (Adler et al., 2003) established that poor diabetes control accelerates the progression of diabetic nephropathy in both type 1 and type 2 diabetes. Experimental evidence has also shown a link between hyperlipidemia and the progression of diabetic and non-diabetic nephropathies (Keane et al., 1991). A link between hyperuricaemia and the development of systemic hypertension, cardiovascular disease and renal disease has been postulated (Johnson et al., 1999). The worldwide pandemic of obesity could also affect the progression of CKD.

Obesity has been associated with the initiation and progression of glomerulonephritides, (de Jong et al., 2002; Kambham et al., 2001) the incidence of focal and segmental glomerulosclerosis is higher in obese than in lean individuals (Verani et al., 1992), and the progression of IgA nephropathy is thought to be faster in overweight patients (Bonnet et al., 2001). Whether these links are causal or simply associated with CKD remains unclear; obesity is associated with hypertension, albuminuria and dyslipidaemia, all of which are potential modifiers of the progression of CKD. Cigarette smoking has been implicated in the initiation and progression of CKD. A graded increased risk of ESRD was noted in non-diabetic nephropathies with
increasing cigarette smoking; the incidence of ESRD was increased by 5-9 times among heavy smokers (Orth et al., 1998). Regular and heavy (more than two drinks daily) consumption of alcohol might also increase the risk of ESRD according to a survey undertaken in the USA (Perneger et al., 1999). In USA, one study suggested that individuals who use opioid drugs recreationally have a risk of developing ESRD of up to 19 times that in nonusers (Perneger et al., 2001). Some studies have linked the consumption of analgesics, especially paracetamol and non-steroidal anti-inflammatory agents (NSAIDs) with a higher risk of developing CKD (Klag et al., 1996; Perneger et al., 1994).

3.3 KIDNEY STONE
3.3.1 Epidemiology
A kidney stone/Urolithiasis is a solid lump (from as small as a grain of sand to as large as the size of a golf ball) made up of crystals that separate from urine and build up on the inner surfaces of the kidney. Kidney stone or nephrolithiasis represents a significant burden of illness for worldwide. The yearly incidence of nephrolithiasis is estimated to be about 0.5% in North America and Europe (Pak, 1998). In USA, the prevalence (frequency in population) has risen from 3.2% to 5.2% in just over two decades from the mid-1970s to the mid-1990 (Stamatelou et al., 1994). The incidence of nephrolithiasis for children aged 18 years was found to be 18.5 per 100,000 children in 2007, an increase from 7.9 per 100,000 in 1996 (David S, 2011). Nephrolithiasis is largely a recurrent disease with a relapse rate of 50% in 5-10 years and 75% in 20 years. Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened (Strauss et al., 1982). Features associated with recurrence include a young age of onset, positive family history and infection stones and those secondary to underlying medical conditions - e.g., hyperparathyroidism (Marshall et al., 1975; Heller et al., 2002; Johnson et al., 1979). The 'metabolic syndrome' includes all the diseases, e.g. hypertension, lipid imbalances, type 2 diabetes mellitus, gout and cardiovascular disease, which are concomitant in the majority of stone formers.
3.3.2 Kidney Stone (Urolithiasis) (Ross and Wilson, 2001)
Calculi (stones) form in the kidneys and bladder when urinary constituents normally in solution are precipitated. The solutes involved are oxalates, phosphates, urates and uric acid, and stones usually consist of more than one substance, deposited in layers. They are more originating in collecting tubules or in renal papillae. They then pass into the renal pelvis where they may increase in size.

Urolithiasis denotes stones originating anywhere in the urinary tract, including the kidneys and bladder. Some become too large to pass through the ureter and may obstruct the outflow of urine causing renal failure. Others pass to the bladder and are either excreted or increase in size and obstruct the urethra. Sometimes stones originate in the bladder, usually in developing countries and often in children. Predisposing factors include:
• Dehydration. This leads to increased reabsorption of water from the tubules but does not change solute reabsorption, resulting in a reduced volume of highly concentrated filtrate in the collecting tubules.
• pH of urine. When the normally acid filtrate becomes alkaline some substances may be precipitated, e.g. phosphates. This occurs when the kidney buffering system is defective, and in some infections.
• Infection. Necrotic material and pus provide foci upon which solutes in the filtrate may be deposited and the products of infection may alter the pH of the urine. Infection sometimes leads to alkaline urine.
• Metabolic conditions. These include hyperparathyroidism and gout.
Review of literature

Figure 3.2: The stone in kidney

Table 3.2: Composition of kidney stone (Ramakumar et al., 1999)

<table>
<thead>
<tr>
<th>Stone composition</th>
<th>% of all renal calculi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium oxalate-monohydrate</td>
<td>- 40-60%</td>
</tr>
<tr>
<td>Calcium oxalate-dehydrate</td>
<td>- 40-60%</td>
</tr>
<tr>
<td>Calcium phosphate + Calcium oxalate</td>
<td>- 10%</td>
</tr>
<tr>
<td>Pure calcium phosphate</td>
<td>- 2-4%</td>
</tr>
<tr>
<td>Struvite (magnesium ammonium phosphate)</td>
<td>- 2-20%</td>
</tr>
<tr>
<td>Uric acid</td>
<td>- 5-10%</td>
</tr>
<tr>
<td>Cystine</td>
<td>- 1-2.5%</td>
</tr>
<tr>
<td>Ammonium urate</td>
<td>- 0.5-1%</td>
</tr>
</tbody>
</table>

3.3.3 Classification of renal stone (Barbas et al., 2002)

There are several types of stones that differ in composition and pathogenesis.

1. calcium containing stones
   a. Calcium oxalate stone (CaOx)
   b. Calcium phosphate stones (CaPh)
2. Uric acid stones
3. Struvite stones
4. Cystine stone
5. Miscellaneous stones.

**Calcium containing stones**

Most stones contain calcium combined with oxalate, phosphate, or occasionally uric acid. All calcium stones are radio-opaque, calcium oxalate and calcium phosphate stones are black, grey or white and small (1 cm in diameter) dense and sharply circumscribed on radiographs. Different conditions contribute to calcium stones. Hypercalciuria (defined as 0.1 mmol/kg body weight of patient per day, calculated for ideal bodyweight) can be idiopathic or result from any disorder that induces even mild hypercalcemia. Such disorders include: (i) primary hyperparathyroidism; (ii) other disorders that induce hypercalcemia such as malignancies, granulomatous diseases, sarcoidosis, thyrotoxicosis, and immobilisation; (iii) idiopathic hypercalciuria, a familial disorder affecting both sexes equally, in which urinary calcium concentration is raised despite normal concentrations of blood calcium; (iv) mutations in the CLCN5 chloride channel in Japanese patients which resulted in low molecular weight proteinuria, hypercalciuria and calcium stone formation and (v) other causes of hypercalciuria are inherited syndromes of familial benign and autosomal dominant hypercalciuria.

It has been proposed that there are three forms of idiopathic hypercalciuria: (i) Absorptive hypercalciuria in which there is an increase in intestinal calcium absorption; this is the most common form; (ii) fasting hypercalciuria in which the excess calcium may be coming from bone; and (iii) renal hypercalciuria in which there is a defect in renal tubular calcium reabsorption. The clinical and pathogenic significance of this classification is uncertain. Another condition associated with renal stone formation is hyperoxaluria.

Urinary oxalate is an end-product of metabolism primarily derived from the metabolism of glycine (40%) and ascorbic acid (40%), with dietary oxalate accounting for 10%. The role played by diet is related to the limited intestinal
absorption of oxalate (10% of intake) due to the formation of insoluble calcium oxalate salts in the intestinal lumen. Foods rich in oxalate: rhubarb, standard teas, nuts, beans, spinach, coffee, and chocolates can increase concentrations in urine to 670 mmol/day (normal value 440 mmol/day). However, concentrations of more than 890 mmol/day indicate enteric oxaluria (associated with malabsorptive small-bowel diseases), mild metabolic hyperoxaluria, or primary hyperoxaluria. Mild metabolic hyperoxaluria does not seem to represent a substantial fraction of hyperoxaluria. Although the exact pathogenic mechanisms have not been identified; cytosolic enzyme perturbations are thought to result in mild hyperoxaluria and recurrent calcium oxalate stones. Primary hyperoxaluria type 1 (PH I) disease is caused by lack of the liver enzyme Alanine glyoxylate aminotransferase and type 2 disease (PH II) by lack of D-glycerate dehydrogenase. The defective genes in that cause these diseases and their abnormal liver enzyme products cause excessive oxalate metabolism, which results in systemic calcium oxalate deposition from a young age. Finally, hypocitraturia is also associated with renal lithogenesis. Citrate acts in the tubular lumen by combining with calcium to form a non-dissociable but soluble complex. As a result, there is less free calcium available to combine with oxalate. In addition, citrate also appears to inhibit the important process of crystal agglomeration, in which individual calcium oxalate crystals combine to form a stone. Hypocitraturia could result from causes of intracellular acidosis such as renal failure, potassium deficiency, distal renal tubular acidosis, chronic diarrhea states and drugs such as acetazolamide. Many patients with stones have unexplained low urinary citrate and dysfunction of the renal sodium citrate co transporter has been proposed as a possible mechanism.

**Uric acid stones**

Uric acid is an end product of purine metabolism. It’s the same crystal that causes gout, an arthritic condition. Uric acid stones are smooth, round, yellow-orange and nearly radiographically transparent unless mixed with calcium crystals or struvite. Diets high in purine, especially those containing meats, chicken and fish, result in hyperuricosuria. The solubility of uric acid depends on the acidity or alkalify of the urine. In acid urine, pH less than 5.5, uric acid crystals precipitate leading to stone formation, if urine is alkaline; uric acid remains soluble and doesn’t precipitate out.
Furthermore, hyperuricaemia disorders including gout (20% of patients with gout are hyperuricosuric), myeloproliferative disorders, tumor lyses syndrome and inborn errors of metabolism (such as glucose-6-phosphatase deficiency), result in an increased filtered load of uric acid and thus, hyperuricosuria. As with all stones, certain drugs may enhance stone formation and in the case of uric acid stones, hyperuricosuric agents include low dose salicylates, probenecid, and thiazides.

**Struvite stone or triple phosphate stone**

The majority of stones are composed of oxalates, calcium salts, and phosphates. Among phosphates, magnesium ammonium phosphate hexahydrate (MAPH; MgNH$_4$PO$_4$·6H$_2$O), known as struvite, is the predominant crystalline component. Struvite crystallization is related to substantial bleeding, obstruction and urinary tract infections by microorganisms producing urease. They are mainly the microorganisms from species of *Proteus*, which are isolated in the case of 70% of the so-called infectious stones (Jolanta et al., 2012). Urine of a healthy person is under saturated with regard to struvite formation. Sign of struvite stones including urinary pH greater than 7, stag horn calculi and urease. However, struvite stones are formed in the case of urinary tract infection by urease-producing bacteria.

**Cystine stones**

Formation of cystine stones is the only clinical expression of cystinuria, an autosomal recessive disorder. People who are homozygous for cystinuria excrete more than 600 mg per day of insoluble cystine, so cystine stones should be suspected in patients with a history of childhood stones or a family history. Cystinuria is the cause of 1-2% of stones observed in adults and 10% of those occurring in children. The stones are greenish yellow, flecked with shiny crystallites and are moderately radio-opaque with a rounded appearance. More than half the stones in cystinuria are of mixed composition and many patients have associated physiological problems such as hypercalciuria (19% of patients), hyperuricosuria (22%), and hypocitraturia (44%).

**Protease-related stones**

This is the newest type of stone described. The increasing incidence of HIV-positive patients has led to widespread use of the protease inhibitor indinavir sulphate.
Although the drug is generally well tolerated, it can be associated with urolithiasis in 4-12% of patients. Thus, calcium oxalate stones may coexist or form a nidus for indinavir stones or vice versa.

Miscellaneous stones (Moe, 2006)

Crixivan stones: One of the most common protease inhibitors used to treat HIV disease is crixivan or indinavir sulfate. Urinary stones have been associated with the use of crixivan. Other type of stones can be of sulphamethoxazole, guaphenesin, aminophylline, ciprofloxacin, triamterene, phenytoin, oxypurinol, etc.

![Different types of stones](image)

**Figure 3.3: different types of stones**

3.3.4  Mechanism of stone formation

Physical concept

Urine is said to be saturated with, for example, calcium and oxalate, when the product of the concentrations of calcium and oxalate exceeds the solubility product (Ksp). Below the solubility product, crystals of calcium and oxalate will not form and the urine is under saturated. Above the solubility product, crystals of calcium and oxalate should form. But they do not because of the presence of inhibitors of crystal formation. However, above a certain concentration of calcium and oxalate, inhibitors of crystallization become ineffective and crystals of calcium oxalate start to form. The concentration of calcium and oxalate at which this is reached (i.e., at which
crystallization starts) is known as the formation product (Kf), and the urine is said to be supersaturated with the substance or substances in question at concentrations above this level. Urine is described as being metastable for calcium and oxalate at concentrations between the solubility product of calcium and oxalate and the formation product.

The ability of urine to hold more solute in solution than can pure water is due partly to the presence of various inhibitors of crystallization (e.g. citrate forms a soluble complex with calcium, preventing it from combining with oxalate or phosphate to form calcium oxalate or calcium phosphate stones). Other inhibitors of crystallization include magnesium, citrate, glycosaminoglycans, and Tamm-Horsfall protein. Periods of intermittent supersaturation of urine with various substances can occur as a consequence of dehydration and following meals. The earliest phase of crystal formation is known as nucleation. Crystal nuclei usually form on the surfaces of epithelial cells or on other crystals. Crystal nuclei form into clumps - a process known as aggregation. The urolithiasis inhibitors like citrate and magnesium inhibit not only crystallization but also aggregation.

Steps leading to stone formation

- Calcium and oxalate concentration < solubility product no stone formation
- Metastable calcium and oxalate concentrations no stone formation
- Calcium and oxalate concentrations > formation product no stone formation

3.3.5 Pathogenesis of stone formation (Johnson et al., 1993; Purohit et al., 2003; Balaji and Menon, 1997)

Renal stone formation requires that stone forming crystalloids in urine come out of solution. Because crystalloids in solution are in equilibrium with crystalloids in the solid phase, a minimum condition is that urine be supersaturated with relevant crystalloids. This condition is often met: many healthy persons, probably the majority have concentrations of calcium and oxalate in urine such that their activity product exceeds the solubility threshold (i.e. urine is supersaturated with these crystalloids). But urine has a strong inhibitory action that prevents crystallization and other stone forming processes. Hence, although urine is supersaturated, crystalloids remain in solution.
Three process promote the stone formation

- **Nucleation**
- **Aggregation**
- **Crystal growth**

**Nucleation**

Nucleation involves the association of crystalloids in solution (e.g. calcium and oxalate) to form a submicroscopic particle of about 100 atoms. The process requires energy and is facilitated when an external surface can serve as a lattice or anchor, thereby lowering the free energy requirement. Such a surface is provided by microscopic uric acid moieties, which function as promoters of CaOx stone formation.

- **Homogeneous nucleation:**
  The process by which the earliest crystal nuclei form in pure solutions is called homogeneous nucleation. It occurs in the absence of a surface or lattice.

- **Heterogeneous nucleation:**
  In this process the nuclei usually form on existing surfaces. Epithelial cells, urinary casts, red blood cells and other crystals can act as nucleating foci in urine. The saturation needed for heterogeneous nucleation is much less than for homogeneous nucleation. Once the nucleus is formed, particularly if it is anchored, crystallization can occur at lower chemical pressures than for the formation of the initial nucleus.

Stone formation probably begins on the surface of a papilla or in the renal tubule; CaOx is bound rapidly to the surfaces of renal tubular cells in culture and undergoes endocytosis. Such mechanism is necessary for stone formation. Without an anchor the newly formed solid phase would simply wash into the urine while still microscopic, with no clinical consequences.

**Aggregation**

Aggregation is the process by which nuclei or larger structures adhere to one another. The initial nuclei can grow by the precipitation of additional salt on the lattice framework. It takes between 5-7 min for urine to flow from the glomerulus to the collecting duct. The earliest site of stone formation in human is the papillary duct or the collecting tubule, where the diameter is 50 to 200 µm. This is time depending
upon the state of supersaturation of the urine. Clearly, free-floating nuclei simply pass innocuously into the renal pelvis given these constraints. Once nuclei are formed they bounce apart from each other, float freely and become kinetically active. If they remain independent and float freely, they are washed away by urine flow. However, under certain circumstances, these nuclei come in close contact and due to chemical or electrical forces can bind to each other, a process called crystal aggregation. Although it is impossible for crystal growth alone to give rise to a crystal large enough to occlude the lumen of the collecting duct, aggregates of crystals easily can attain such size.

**Crystal growth**
A third process is crystal growth, in which crystalloids come out of solution to associate with the solid phase of growing crystal in a geometrically precise arrangement. The combination of crystal aggregation and crystal growth can explain the genesis of urinary calculi. Another process that may lead to CaOx stone formation is crystal retention. In most instances, crystal aggregates are too fragile to occlude the collecting duct long enough to give rise to a stone. Many kidney stones have a layered structure suggesting intermittent growth most likely during the periods of supersaturation.

**Role of Oxidative stress in Calcium oxalate stone formation**
Membrane injury facilitated the fixation of calcium oxalate crystals and subsequent growth into kidney stones. Oxalate-induced membrane injury is mediated by lipid peroxidation reaction through the generation of oxygen free radicals. In urolithic rat kidney or oxalate exposed cultured cells; both superoxide anion and hydroxyl radicals were generated in excess that causing cellular injury. The lipid peroxidation products, thiobarbituric acid-reactive substances (TBARS), hydroperoxides and Diane conjugates were excessively released in tissues of urolithic rats and in plasma of rats as well as stone patients. The accumulation of these products was concomitant with the decrease in the antioxidant enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glucose-6 phosphate dehydrogenase (G6PD) as well as radical scavengers, vitamin E, ascorbic acid, reduced glutathione (GSH) and protein thiol. All the above parameters were decreased in urolithic condition,
irrespective of the agents used for the induction of urolithiasis. Oxalate binding activity and calcium oxalate crystal deposition were markedly renounced, along with decreased Adenosine tri-phosphatase (ATPase) activity. Lipid peroxidation positively correlated with cellular oxalate, oxalate binding, c-glutamyl carboxylase calcium level and negatively correlated with GSH, vitamin E, ascorbic acid and total protein thiol. Antioxidant therapy to urolithic rats with vitamin E, glutathione mono-ester, methionine, lipoic acid or fish oil normalized the cellular antioxidant system, enzymes and scavengers and interrupted membrane lipid and protein peroxidation reaction, ATPase inactivation and its associated calcium accumulation.

Antioxidant therapy prevented calcium oxalate precipitation in the rat kidney and reduced oxalate excretion in stone patients. Similarly, calcium oxalate crystal deposition in vitro to urothelium was prevented by free radical scavengers such as phytic acid and mannitol by protecting the membrane from free radical-mediated damage. These observations were suggestive of the active involvement of free radical-mediated lipid peroxidation in the pathogenesis of calcium oxalate crystal deposition and retention (Selvam., 2002).

3.3.6 Sign and symptoms of kidney stone (Johnson et al., 1999)
Kidney stones on accumulation in the urinary tract exhibit specific symptoms and the most specific one is severe pain in the locale. The signs and symptoms of all the forms of urolithiasis are same with little differences. The common symptoms include Flank pain or back pain on one or both sides, progressive severe colicky (spasm-like) may radiate or move to lower in flank, pelvis, groin, genitals, nausea, vomiting, urinary frequency/urgency, increased (persistent urge to urinate), blood in the urine, abdominal pain, testicle pain , groin pain, fever, chills and abnormal urine color.

The pain associated with passing a kidney stone is referred to as renal colic. It begins suddenly and quickly becomes an unbearable pain that may cause nausea and vomiting. The distribution of the pain resembles that of the path of the stone to the bladder, beginning in the flank and curving anteriorly toward the groin. Urinary frequency and dysuria occur as the stone reaches the uretero-vesical junction. When the stone passes into the bladder or moves in the ureter to decompress the urinary
system, the pain vanishes. A unique symptom of the disease develops when the stone passes into the urethra.

**Calcium oxalate urolithiasis:** Patients often present with episodes of flank pain that radiates to the anterior abdomen or even to the genitalia. The pain is often severe and comes in waves. Often there is microscopic or gross hematuria. Calcium oxalate crystals may be seen with urine microscopy, but this finding is not diagnostic. Since calcium oxalate crystals may be seen in the urine of non-stone-forming patients. In some patients the renal stones are completely asymptomatic or may produce painless hematuria.

**Uric acid stones:** Patients suffer from flank pain that radiates to the anterior abdomen or even to the genitalia, as in calcium stone disease. The pain is often severe and comes in waves. Often there is microscopic or gross hematuria.

**Struvite stones:** These stones may cause the typical symptoms of renal colic, but often they are discovered in the course of investigating a patient with recurrent urinary infections or in a patient with asymptomatic bacteriuria. Since these stones can grow to significant size, they are often found in the renal pelvis and in fundibula of the kidneys.

**Cystine stones:** The patient presents with symptoms of nephrolithiasis, often at a younger age than a person with calcium stone disease. The stones are radiopaque (ground-glass appearance) and amber. Family history is often helpful (i.e. K.I, siblings may have the disorder).

**3.3.7 Complications of Urolithiasis (Curhan et al., 1994; Bruce et al., 1997)**

Urinary stones often remain asymptomatic for long periods. As the time surpass the stones develop into macroscopic sizes and if not treated for long time they lead to severe stones develop into macroscopic sizes and if not treated for long time they lead to severe tract infection, Obstruction of the ureter, acute unilateral obstructive uropathy, Kidney damage, scarring, Decrease or loss of function of the affected kidney, Stag horn calculus, Nephrocal Cyanosis, Sludge and Osteoporosis.
3.3.8 Risk Factors: responsible for renal stone

1) Gender and Age

Kidney stones affect about 12% of men and 5% of women by the time they are 70 years old. About 80% of kidney stone sufferers are men between the ages of 20 and 50 years. While kidney stones are still a rare occurrence during pregnancy. Children with low birth weight who need to be fed intravenously are also at risk for stones.

2) Family History

People with a family history of kidney stones are at higher risk than those without relatives with stones. A US cohort study found that man who had developed a kidney stone was three times more likely to have a family history of kidney stone than other man. A family history also increased the likelihood of developing a stone in men who had had never had one previously. Researchers are looking into markers or other factors that might predict the onset of stones in relatives, though none has yet been clearly identified.

3) Geographic Differences

Geography plays a part with more stones noted in the southeast, also known as the stone belt. Water properties in different localities are different which increase the risk of stone formation when contains high amount of minerals. The overall probability of forming stones 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. Moreover, in the same region, the clinical and metabolic patterns of stone disease can change over time (Ramello et al., 2000).

4) Life Style Factors

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 (Ramello et al., 2000). High protein and salt intake increase the risk of calcium stone formation. High purine diets (meat, fish, chicken) lower urinary pH and cause increased excretion of uric acid resulting in uric acid stones. Magnesium intake decreases and total vitamin C intake seems to increase the risk of
symptomatic nephrolithiasis (Taylor et al., 2004). In older women and men, greater intakes of dietary calcium, potassium, and total fluid reduce the risk of kidney stone formation, while supplemental calcium, sodium, animal protein, and sucrose may increase the risk (Curhan et al., 2005; Curhan et al., 1997). Vitamin B₆ deficiency leads to increased formation and excretion of oxalate. Dehydration, excessive vitamin C intake, calcium supplementation and calcium containing antacids may also lead to stone formation.

5) Associated diseases:
• **Gout:** The prevalence of kidney stones in patients with gout was 13% and 2.7% of people with kidney stones had been diagnosed with gout, with the occurrence of gout being 8.6% in patients with two or more episodes of kidney stones.
• **High Blood Pressure:** Hypertensive people are up to three times more likely to develop kidney stones.
• **Bowel Diseases:** Crohn's disease and ulcerative colitis (known as inflammatory bowel diseases) cause problems in intestinal absorption that significantly increase the risk for kidney stones. Surgeries that remove parts of the small intestine to correct bowel conditions pose a particular risk for short bowel syndrome. This is a major risk factor for both calcium oxalate and uric acid stones in these patients.
• **Urinary Tract Infections (UTIs):** Struvite stones are almost always caused by urinary tract infections.
• **Hyperparathyroidism:** The parathyroid glands regulate calcium levels in the body through parathyroid hormone. In hyperparathyroidism, one or more of these glands makes too much parathyroid hormone. Some people with hyperparathyroidism develop kidney stones. Surgery to remove the hyperactive parathyroid gland in such patients reduces the risk for stone formation, but the risk still remains high for some time after surgery. Many other medical conditions, including but not limited to kidney disease, chronic diarrhea, certain cancers and sarcoidosis, put people at higher risk for stones.

6) Medications
Many drugs, including thyroid hormones and loop diuretics (drugs that increase urination), can increase calcium concentration in urine. Stones are an uncommon side
effect of these medications; however diuretics are also used to prevent calcium stones. Certain cancer chemotherapies can cause kidney stones. Taking medications for long periods that change the acidic content of urine, such as antacids, may increase susceptibility for kidney stones.

3.3.9 Factors affecting kidney stone formation

**Hypercalciuria**, or excessive urinary calcium excretion, occurs in about 5-10% of the population and is the most common identifiable cause of calcium kidney stone disease. The most common types of clinically significant hypercalciuria are absorptive, renal leak, resorptive and renal phosphate leak. Other causes of hypercalciuria include numbers of disease like hyperthyroidism, renal tubular acidosis and other granulomatous diseases, vitamin D intoxication, glucocorticoid excess, tubular acidosis, various paraneoplastic syndromes, prolonged immobilization, induced hypophosphatemic states, multiple myeloma, lymphoma, leukemia, metastatic tumors especially to bone, Addison disease, and milk-alkali syndrome among others (Vezzoli et al., 2008).

**Hyperoxaluria** may be due to overproduction, from hereditary disorders of metabolism or be acquired from intestinal disease or diet. It occurs in patients with short bowel syndrome or malabsorption. Acquired hyperoxaluria is common in children with a variety of intestinal disorder (Vidya and Varalakshmi, 2000).

**Hyperuricosuria** may be secondary to uricosuric medications, myeloproliferative disorders, primary gout or congenital disorders. A high animal protein (especially purines) may increase uric acid excretion and decrease urinary pH. Uric acid supersaturation is strongly controlled by urinary pH. Uric acid may provide heterogeneous nuclei for calcium oxalate stone formation. The lower frequency of pure uric acid stones, around 5%, may be ascribed to the usual urine acidity, which favors calcium oxalate crystallization instead. However, recent findings provided by metabolic studies have indicated an association between pure uric acid nephrolithiasis and insulin resistance (Abate et al., 2004; Maalouf et al., 2004).

**Hypocitriuria** is defined as urinary citrate excretion less than 250 mg in 24 hours. Urinary citrate forms a soluble complex with calcium that inhibits the formation and
propagation of crystals. It is a common correctable cause of recurrent pure calcium phosphate or brushite stones. Women excrete more citrate and have lower incidence of stone formation than men. Urinary citrate is mainly derived endogenously through the tri-carboxylic acid cycle and is excreted by renal tubular cells. Intracellular acidosis, acidic diets (diets rich in animal proteins) and hypokalaemia decrease urinary citrate excretion. Fruits such as oranges and grapefruits are the main exogenous sources of urinary citrate. Hormonal replacement therapy in postmenopausal women results in higher urinary calcium excretion, but it also increases urinary excretion of citrate and leads to net inhibition of crystal precipitation, thereby decreasing the risk of calcium stones (Day et al., 2002).

**Hypomagnesuria** is one of the major factors in kidney stone. Many experimental studies have suggested that administration of magnesium salts prevents stone disease. Urinary magnesium is known as the inhibitor of calcium oxalate stone formation (Yuji Kato et al., 2004). Thus, magnesium deficiency may lead to increased chances of urolithiasis. Elevated levels of **arachidonic acid** in cell membranes may promote the hypercalciuria and hyperoxaluria that are characteristic of idiopathic calcium nephrolithiasis. The intake of n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may decrease the arachidonic acid content of cell membranes and reduce urinary excretion of calcium and oxalate. It has been proposed that greater intake of EPA and DHA (through dietary sources or fish oil supplementation) may reduce the risk for kidney stone formation. However, after studies now it has been proved that fatty acid intake is not consistently associated with the development of kidney stones. Greater levels of arachidonic and linoleic acid intake do not increase the risk for developing a kidney stone, and greater intake of n-3 fatty acids does not reduce the risk (Taylor et al., 2005).

**Uromodulin** may promote aggregation of calcium oxalate crystals (Carvalho et al., 2002).

**Hyaluronic acid (HA)**, an extremely large glycosaminoglycan is a major constituent of the extra cellular matrix in the renal medullary interstitium and the pericellular matrix of mitogen/stress-activated renal tubular cells. HA is an excellent crystal-
binding molecule because of its size, negative ionic charge and ability to form hydrated gel-like matrices. Crystal binding to HA leads to crystal retention in the renal tubules (nephrocalcinosis) and to the formation of calcified plaques in the renal interstitium (Randall's plaques).

**Tamm-Horsfall protein (THP)** may act on nucleation and inhibit crystal aggregation (Carvalho et al., 2002). But structurally abnormal THPs from recurrent calcium stone formers may promote crystal aggregation. Therefore, increased urinary excretion of abnormal THP is of relevance in nephrolithiasis. Severely recurrent calcium stone formers with a positive family history excrete more THP than healthy controls. Such increasingly aggregated THP molecules predispose to exaggerated calcium oxalate crystal aggregation, an important prerequisite for urinary stone formation (Jaggi et al., 2007). THP has a dual role in modifying crystal aggregation: at high pH and low ionic strength (IS), THP is a powerful crystal aggregation inhibitor. Upon lowering pH and rising IS, THP viscosity increases, leading to reduced crystal aggregation inhibition. In the presence of additional calcium ions, some THPs even become strong promoters of crystal aggregation. This phenomenon seems to be more pronounced in THPs isolated from recurrent calcium stone formers whose proteins exhibit an abnormally high tendency of polymerization (Hess, 1992).

### 3.3.10 Diagnoses (Albala et al., 2011; Pak et al., 2003)

The goals of imaging or diagnosis are to determine the presence of stones within the urinary tract, evaluate for complications, estimate the likelihood of stone passage, confirm stone passage, assess the stone burden and evaluate disease activity. At a first episode of nephrolithiasis, a reasonable laboratory evaluation. Children with stones should probably be referred to an urologist or nephrologists for further evaluation. Adult patients with a solitary kidney, struvite stones, abnormal renal function or renal tubular acidosis probably also require further evaluation. The first time stone former carries 50% risk of forming a new stone within 5 years. The history and physical examination is an integral part of the evaluation. History of urinary tract infections, medical diseases (such as sarcoidosis, hyperthyroidism or hypothyroidism, myeloproliferative disorders or malignancy), surgery and dietary history (including dairy products, protein intake and fluid intake) should be considered while evaluating
a patient with urolithiasis. Patients with recurrent stones should undergo a more detailed evaluation, including a 24 hour urine collection. Accurate diagnosis depends on the methods used to determine the relevant urinary electrolytes. The urine collection may be performed while patients follow their usual diet. Optimally, more than one collection should be made to account for day-to-day variability. The laboratory tests include,

• **Complete blood count**
  This test is used to screen for occult malignancies. Serum chemistry survey including sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, calcium, phosphorus, uric acid, total protein and albumin levels, this will screen for a variety of metabolic disorders.

• **Urine analysis**
  The urine analysis to assess urinary pH, specific gravity, glucose, protein, ketones, white blood cells, red blood cells, casts and crystals.

• **Renal ultrasound scans**
  Ultrasound scanning is a fast and painless way to obtain images of the urinary system. Based on Navy sonar, this technology uses a special device, called a transducer, to beam high-frequency sound waves through the body. As they hit the stones, the waves create echoes that turn into real-time images processed by a computer. Ultrasound can detect when the upper urinary tract and kidneys are dilated or stretched by a stone lodged in the ureter.

• **Radioisotope imaging**
  A variety of organic compounds can be labeled with a radioactive isotope that emits gamma rays, allowing the radiation to penetrate through tissues and reach a gamma camera placed adjacent to the patient. The most commonly used radioisotope is technetium-99mTc (half-life 6 hours, gamma-ray emission energy 0.14 MeV). The excretion characteristics of the organic compound to which the 99mTc is bound determine the clinical use.
• **The radiographic studies**

That include Intravenous urogram (IVU) or intravenous Pyelogram (IVP) is used to scan urinary System; it is a special X-ray that reveals the urinary tract’s structure and function. The technician injects an iodine-based dye into a vein before snapping a series of X-rays. The dye collects in the urinary system, producing white contrast where a stone is present. Because the agent yields high-definition images, most kidney stones can be located precisely using IVP.

• **Abdominal CT scan**

Computerized tomography (CT) scans do not require contrast, eliminating the risk of allergic reactions or renal failure. Stones, including relatively radiolucent uric acid calculi, cystine, matrix and xanthenes, show up as bright white spots, making identification easy. While they do not contain calcium, the stones are still much denser than the surrounding soft tissue. The only exception is stones made of various metabolites of medications, such as indinavir (Crixivan), which is a protease inhibitor used in the treatment of HIV disease; these stones are not be visible on CT scans.

• **Renal nuclear scans**

This study is of value in specific instances. A nuclear renal scan can be used to objectively measure differential renal function, especially in a dilated system for which the degree of obstruction is in question. This is also a reasonable study in pregnant patients, in whom radiation exposure must be limited. Technetium Tc 99m (diethylene triamine penta-acetic acid) is the radioisotope used. If the stone is
available, a commercial analysis is more comprehensive and less expensive than the hospital analysis.

When acute flank pain suggests the passage of a urinary stone, many methods of examination can be used. Often, conventional radiography is initially used to screen for stones, bowel abnormalities, or free intra-abdominal air. Radiographs can also be used to monitor the passage of visible stones. IVU (excretory urography or intravenous urography) provides important physiologic information regarding the degree of obstruction. Ultrasonography is useful in young or pregnant patients and in patients allergic to iodinated contrast material. CT scanning is now considered the examination of choice for the detection and localization of urinary stones.

3.3.11 Management of kidney stone (Lewandowski et al., 2004)
Fortunately, most stones will pass through the urinary system on their own, but need to drink sufficient fluids (two to three quarts-a-day) to move them along. Patient may be given a pain medication for a reasonable period of time. Calcium oxalate kidney stone patients are advised to increase their fluid intake to achieve a urine volume of 2 L or more daily to reduce the risk of subsequent calcium stone (Lukas kairaitis. 2007); the recommended calcium intake is 800-1200 mg/day; high oxalate foods should be restricted; daily protein intake should be between 0.8 and 1 g/kg body weight/day; essential fats should be included; vegetable and fruit (except oxalate-rich vegetables) intake should be increased.

3.3.11.1 Preventive treatment of kidney stone (Nabi et al., 2007)
A) Patients with calcium stone

Drinking recommendations
An inverse relationship between high fluid intake and stone formation has been demonstrated. The general recommendation for calcium stone formers is to maintain a high urine flow with a generous intake of fluids. The aim should be to obtain a 24-hour urine volume of at least 2 L.

Dietary recommendations
Diet should be of a ‘commonsense’ type, i.e. a mixed balanced diet with contributions from all food groups, but without excesses of any kind.
• **Fruits, vegetables and fibres**
Fruit and vegetable intake should be encouraged because of the beneficial effects of fibre. The alkaline content of a vegetarian diet also gives rise to a desirable increase in urinary pH.

• **Oxalate**
An excessive intake of oxalate-rich products should be limited or avoided to prevent an oxalate load. This includes fruit and vegetable rich in oxalate such as wheat bran. This is particularly important in patients in whom a high oxalate excretion has been demonstrated. The following products have a high content of oxalate.

  - Rhubarb, 530 mg oxalate/100 g
  - Spinach, 570 mg oxalate/100 g
  - Cocoa, 625 mg oxalate/100 g
  - Tea leaves 375-1450 mg oxalate/100 g
  - Nuts, 200-600 mg oxalate/100 g.

**Vitamin C**
Vitamin C is a precursor of oxalate, but its role as a risk factor in calcium oxalate stone formation remains controversial. Some studies have shown that a daily intake of up to 4 g might be allowed without risk. However, a recent study demonstrated a significantly increased risk in stone formation for men taking 1 g/day or more of vitamin C compared to men taking less than 90 mg. It therefore seems justified to advise calcium oxalate stone formers to avoid excessive intake of vitamin C. The allowed amount is not obvious, but a daily intake of more than 500 mg to 1 g should probably be avoided.

**Animal protein**
Animal protein should not be ingested in excessive amounts. It is recommended that animal protein intake is limited to 0.8-1 g/kg body weight. An excessive consumption of animal protein gives rise to several unfavorable effects on stone formation, such as hypocitraturia, low pH, hyperoxaluria and hyperuricosuria. Moreover, an increased resorption of bone increases urinary calcium.
Calcium

Calcium intake should not be restricted unless there are very strong reasons because of the inverse relationship between dietary calcium and calcium stone formation. The minimum daily requirement for calcium is 800 mg and the general recommendation is 1000 mg/day. Calcium supplements are not recommended except in cases of enteric hyperoxaluria, when additional calcium should be ingested with meals to bind intestinal oxalate.

Sodium

A high consumption of sodium brings about several changes in urine composition. Calcium excretion is increased by reduced tubular reabsorption. Urinary citrate is reduced due to loss of bicarbonate. The risk of forming sodium urate crystals is increased and the effect of thiazides in reducing urinary calcium is counteracted by a high sodium intake. The combined restriction of sodium and animal protein in a randomized study resulted in a reduced rate of calcium stone formation. The daily sodium intake should not exceed 5 g.

3.3.11.2 Pharmacological Therapy

I) Allopurinol (Walsh et al., 2002)

For people with hyperuricosuria and calcium stones, Allopurinol is one of the few treatments that have been shown to reduce kidney stone recurrences. Allopurinol interferes with the production of uric acid in the liver. The drug is also used in people with gout or hyperuricemia (high serum uric acid levels) (Cameron et al., 1987). Dosage is adjusted to maintain a reduced urinary excretion of uric acid. Serum uric acid level at or below 6 mg/100 ml) is often a therapeutic goal. Hyperuricemia is not necessary for the formation of uric acid stones; hyperuricosuria can occur in the presence of normal or even low serum uric acid. Some practitioners advocate adding allopurinol only in people in whom hyperuricosuria and hyperuricemia persist, despite the use of a urine-alkalinizing agent such as sodium bicarbonate or potassium citrate. (Knudsen et al., 2007) The side effects of allopurinol are mainly drug reactions. Severe allopurinol hypersensitivity has been seen in association with thiazides in patients with prior renal compromise (Young et al., 1977).
II) Thiazides and thiazides like Diuretics (Tiselius, 2003)
One of the recognized medical therapies for prevention of stones is the thiazides and thiazides like diuretics, such as Hydrochlorothiazide, bendroflumethiazide, trichlorthiazide and the non-thiazide indapamide have been used for recurrence prevention in patients with calcium stone disease. These drugs inhibit the formation of calcium-containing stones by reducing urinary calcium excretion and by increased reabsorption of calcium in the proximal as well as in the distal parts of the nephron (Preminger, 2007). Sodium restriction is necessary for clinical effect of thiazides, as sodium excess promotes calcium excretion. Thiazides work best for renal leak hypercalciuria (high urine calcium levels), a condition in which high urinary calcium levels are caused by a primary kidney defect. Thiazides are useful for treating absorptive hypercalciuria, a condition in which high urinary calcium is a result of excess absorption from the gastrointestinal tract (Moe, 2006).

Moreover, been suggested that thiazides might decrease oxalate excretion, possibly via a reduced intestinal absorption of calcium but recent studies have shown that such an effect is unlikely to occur. The major drawback of thiazides treatment is the occurrence of side-effects. The unmasking of normocalcaemic hyperparathyroidism and the development of diabetes, gout and erectile dysfunction contribute to a limited tolerance and a high patient drop-out rate.

Compliance is usually in the range of only 50-70%. Hydrochlorothiazide is usually administered at a dosage of 25-50 mg once or twice daily adjusted stepwise according to its effect on blood pressure. The thiazides-induced loss of potassium should be substituted by giving either potassium citrate 3.5-7 mmol twice daily or Amliorode (Midamor) potassium sparing diuretics that may be used if thiazide is not sufficient. It has been shown; however, that potassium citrate was superior to potassium chloride in this regard.

III) Antibiotics (Walsh et al., 2002)
Antibiotic therapy can sterilize the urine, reduce urinary pH, and thus render urine under saturated with respect to struvite. This can result in complete or partial dissolution of the stone. Long-term, culture-specific antimicrobials often reduce the
bacterial burden, even if they do not completely sterilize the urine. The reduction of bacterial colony count from $10^7$ to $10^5$ per mL reduces urease production by 99% (Griffith et al., 1988). Bacteria are still located on the surface or within the lattice of the stones. However, re-infection is common with cessation of antibiotic therapy. The majority of urease-producing infections are caused by \textit{P. mirabilis}, more than 90% of which are sensitive to penicillin or ampicillin (Feit and Fair, 1979). Tetracyclines or fluoroquinolones such as ciprofloxacin or norfloxacin can be used for the treatment of patients with \textit{Pseudomonas} or \textit{Ureaplasma} urinary tract infections (Musher et al., 1975; Hedelin et al., 1984). Long-term antibiotic therapy is not recommended as prima facie treatment for patients with infection stones. Antibiotics are an adjunct to procedural therapy and should be used to prevent stone recurrences or growth after operative procedures.

**IV) Acetohydroxamic Acid (Walsh et al., 2002)**

Acetohydroxamic acid, a chemical that has structural similarity to urea, is a potent, irreversible inhibitor of urease (Fishbein and Carbone, 1965). Acetohydroxamic acid is rapidly absorbed from the gastrointestinal tract and reaches peak plasma levels after about 1 hour (Putcha et al., 1985). The half-life is 3.5 to 10 hours in normal subjects and 15 to 24 hours in patients with diminished renal function (Summerskill et al., 1967; Feldman et al., 1978). The effectiveness of acetohydroxamic acid depends on its concentration in the urine. At doses of 250 mg every 8 hours, urinary concentrations of 15 to 30 mg/dL are usually achieved in patients with normal serum creatinine (Griffith et al., 1988).

The side effects of therapy included deep vein thrombosis, tremor, headache, palpitations, edema, nausea, vomiting, and loss of taste, hallucinations, rash, diarrhea, alopecia, abdominal pain, and anemia. Other inhibitors of urease include hydroxyurea, propionohydroxamic acid, acetohydroxamic acid, hydroxycarbamide, nicotinohydroxamic acid, and fluoroamide. These agents have not been studied extensively in humans. Details of their usage are given by (Gleeson and Griffith, 1990).
V) Citrates (Walsh et al., 2002)
Treatment with alkaline citrate is commonly used as a way of increasing urinary citrate in patients with hypocitraturia. A low citrate excretion is a well-recognized and common finding in patients with calcium stone disease. The role of citrate is important because of its complex formation with calcium. This chelation reduces the ion-activity products of both calcium oxalate and calcium phosphate. Two agents have been used for the treatment of hypocitraturia: sodium potassium citrate commonly used in Europe, and potassium citrate - in liquid form or as a wax matrix tablet-used in the United States. (Sakhaee et al., 1991) Moreover, citrate is an inhibitor of growth and aggregation/agglomeration of these crystals. Administration of an alkaline salt brings about an increased pH and an increased excretion of citrate. Sodium citrate does not lower urinary calcium excretion, perhaps as a result of the increased sodium load associated with therapy (Sakhaee et al., 1993).

The alkalinizing agents used to prevent recurrent calcium stone formation are sodium potassium citrate, potassium citrate, sodium citrate, potassium magnesium citrate, potassium bicarbonate and sodium bicarbonate. The usefulness of alkaline citrate as a way of increasing stone clearance after SWL has been studied by several groups. It was accordingly shown that sodium potassium citrate as well as potassium citrate increased the clearance of stone fragments.

VI) Sodium cellulose phosphate (Goldman et al., 2004)
It is calcium-binding resin, reduces calcium absorption when taken with meals. This approach has not had a high success rate, possibly because of reflex hyperoxaluria. A negative calcium balance may lead to additional bone mineral loss.

In patients with absorptive hypercalciuria, 1500mg of neutral potassium phosphate per day in three to four divided doses lowered urinary calcium excretion in some trials as effectively as thiazides diuretics. However, compliance is more difficult to achieve because of the frequency of dosing and intestinal side effects such as diarrhea and bloating. Studies estimating the efficacy of oral phosphate treatment reported relapses of 9% and 25%. A new slow-release formulation has been developed that avoids many of the side effects and the dosing frequency required with earlier preparations. This new agent reached clinical trials, but it is no longer under development.
VII) Magnesium (Walsh et al., 2002)
An increased excretion of magnesium might reduce the ion-activity product of calcium oxalate and inhibit the growth of calcium phosphate crystals. There are also observations of an increased excretion of citrate following administration of magnesium. Magnesium is also considered important for the transformation between various calcium phosphate crystal phases. A high urinary concentration of magnesium is thus thought to decrease the risk of brushite formation. Magnesium oxide, magnesium hydroxide, potassium magnesium citrate and magnesium aspartate have been used. The effect of potassium magnesium citrate is discussed above regarding alkaline citrate.

VIII) Potassium-Magnesium Citrate (Tiselius, 2003)
Citrate inhibits the crystallization of calcium oxalate, calcium phosphate and chelates calcium. Citrate reduces calcium excretion, increases intestinal loss of calcium and restores a positive calcium balance so that bone loss is prevented. Potassium-magnesium citrate is available over the counter. The potassium citrate products mentioned here are available only by prescription but serve the same purpose as potassium-magnesium citrate, which can be given as the sole treatment for those with normal serum calcium. Major side effects are gastrointestinal intolerance, Magnesium citrate may be used in patients who develop stones secondary to impaired absorption of calcium as a result of small bowel disease.

Pyridoxine
Theoretically, administration of pyridoxine (vitamin B6) might favorably influence the endogenous production of oxalate. This may be explained by an increased transamination of glyoxylate due to the action of the co-enzyme pyridoxal phosphate.
B) Medical treatment of patients with uric acid stone disease (Nabi et al., 2007)
Uric acid stones form in urine highly supersaturated with uric acid. The most common abnormality is a low urine pH often occurring with a small urine volume. These two abnormalities provide the basis for precipitation of uric acid, even in patients with a normal urate excretion. A typical example is the patient with ileostomy with loss of both alkali and fluid. The high excretion of urate seen in patients with disturbed purine metabolism can result in a critical supersaturation with reasonably normal pH and volume.

1. Drinking and dietary recommendations:
Fluid intake should be adjusted to allow for a 24-hour urine flow of approximately 2-2.5 L. The intake of animal protein should not exceed 0.8 g/kg/day.

2. Pharmacological treatment:
Alkalisation of urine is mandatory and should preferably be carried out using alkaline citrate. The pH should be increased to obtain a pH in the range 6.2-6.8 aiming to prevent recurrence. In case chemolitholysis is planned for, the pH should be adjusted to between 7.0 and 7.2 there might be a risk of calcium phosphate stone formation if the pH is raised to higher levels, although such a complication seems to be less common than expected. A reduced excretion of urate is accomplished with allopurinol. This agent should be used when 24-hour urate excretion exceeds 4 mmol. A combination of alkali, allopurinol and a high fluid intake can be used to dissolve uric acid stones. For this purpose urine pH levels should be adjusted to between 7.0 and 7.2.

C) Medical treatment of cystine stone disease (Nabi et al., 2007)
1. Dietary recommendations:
Although theoretically a diet low in methionine might help to reduce urinary excretion of cystine, the patient is unlikely to comply with such a diet and so this regimen is not usually used or recommended. However, a restricted intake of sodium is probably
more effective in reducing urinary cystine. The recommendation given is to avoid a daily consumption of sodium above 2 g.

2. Drinking advice:
A high diuresis is of fundamental importance. The aim is to dilute the urine so that supersaturation with cystine is decreased below the solubility product of cystine, or at least below its formation product. In general, the goal is a 24-hour urine volume of at least 3 L. To reach this goal, a considerable fluid intake evenly distributed during the day is necessary. A more accurate recommendation of the size of urine volume needed can be obtained by knowing the ion-activity product of cystine, which can be calculated from the cystine concentration and the pH.

3. Pharmacological treatments:
The solubility of cystine increases in alkaline urine, but a substantial increment in solubility does not occur unless the pH is above 7.5. The rule of thumb is that the solubility of cystine is approximately 250 mg/L (1 mmol/L) at pH 7, 500 mg (2 mmol/L) at pH 7.5 and 750 mg (3 mmol/L) at pH 8. Potassium citrate is the best option for alkalinising the urine. Sodium bicarbonate, sodium citrate or sodium potassium citrate should not be given because of the undesirable effect of sodium on cystine excretion. A typical dose of potassium citrate is 20-25 mmol per day given three times a day, but the required dose has to be determined by the effect this regimen has on urinary pH. Urine Alkalinisation with azetazolamide is currently no longer recommended.

When the combined effects of a high diuresis and alkalinisation are not enough to prevent stone formation, complex formation by chelating agents is necessary. Thiol compounds, such as D-penicillamine and α-mercaptopropionyl glycine (tiopronin), are most commonly used. The latter compound seems to be associated with fewer side-effects than penicillamine. The recommended daily dosage is 10-15 mg/ kg (or 750 mg/day), but the daily required dose might be in the range of 250-2000 mg. For pencillamine, the daily dose is 1-2 g. A third alternative is captopril (an angiotensin-converting enzyme inhibitor). Positive effects on urinary cystine and stone formation
have been reported with a daily dose of 75-100 mg. Administration of thiols always should be accompanied by pyridoxine to avoid vitamin B₆ deficiency at a recommended dose of 50 mg/day.

3.3.11.3 Surgical treatment
Management of stone disease largely depends on the size and location of stones. Stones smaller than 5 mm that were more distal and on the right side have a high probability of spontaneous passage. However, spontaneous stone passage may take up to 40 days. During this watchful waiting period, patients can be treated with hydration and with pain medications to control pain. In contrast, stones larger than 5 mm, stones in patients with a higher risk of developing renal insufficiency (e.g., patients with a single kidney), or stones that fail to pass through should be treated by some interventional procedures including

- Extracorporeal shock wave lithotripsy (ESWL)
- Percutaneous nephrolithotomy (PNL)
- Ureteroscopy/Uteroscopic Stone Removal (USR)
- Open Surgery

Extracorporeal Shock-wave Lithotripsy (ESWL)
This is the most common method of dealing with kidney stones. The kidney stone is located using X-ray imaging or ultrasound scanning. Extracorporeal shockwave lithotripsy is a medical technique for dissolving kidney stones using shock waves to break a kidney stone into small pieces so that they can more easily travel through the ureter and into the bladder.

It uses high-energy shock or sound waves to crush dense calculi into sand-like granules that then pass naturally with urine. With this procedure, high-energy impulses from an outside machine, called a lithotripter, are focused on your ureters, kidney or bladder through a water bath or soft gel cushion. The sound waves are guided by X-ray or ultrasound. Because there is some discomfort with this procedure, you will likely undergo local or general anesthesia. If the stone is not completely shattered in one treatment, which takes about an hour, additional sessions may be necessary.
Complications with ESWL are uncommon. Most patients have blood in their urine for a few days after treatment. Bruising and minor discomfort in the back or abdomen from the shock waves are also common. To reduce the risk of complications, doctors usually tell patients to avoid taking aspirin and other drugs that affect blood clotting for several weeks before treatment. Another complication may occur if the shattered stone particles cause discomfort as they pass through the urinary tract. In some cases, the doctor will insert a small tube called a stent through the bladder into the ureter to help the fragments pass. Sometimes the stone is not completely shattered with one treatment, and additional treatments may be needed. ESWL is not ideal for very large stones. (NIH Publication No. 05-2495 December 2004). Clinical data suggest that the stone-free rate is higher if you treat the patient with a lower shock wave frequency (60–90 shocks per minute) than with a higher frequency (120 shocks per minute), particularly for larger stones (>10 mm).

**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 skin incision in the back to create a narrow tunnel directly into the kidney. After threading a telescopic instrument, called a nephroscope, through the opening, the surgeon locates the stone and insert small tools to dislodge the calculus. The scope is used to transmit high frequency sound waves when a stone fragmented before suctioned. For large stones, some type of energy probe-ultrasonic or electro-
hydraulic may be needed to break the stone into small pieces. Often, patients stay in the hospital for several days. And, may have a small tube called a nephrostomy tube left in the kidney during the healing process.

Figure 3.6: Percutaneous Nephrolithotomy

Percutaneous nephrolithotomy is an advantageous method because the stone is removed directly; you don’t have to wait for it to pass naturally. While patients are hospitalized for two to three days, they can resume normal activity within one to two weeks.

Ureteroscopic Stone Removal
Relies on a special telescopic instrument, called an ureteroscope, to pinpoint stones in the mid- and lower- portion of the ureter. No incision made in this procedure. The stereoscope Inserted through the urethra and bladder, this narrow fiberoptic tube provides the urologist with a clear view up the ureters to locate and retrieve any stone. The urologist either removes it with a cage-like device or shatters it with a special instrument that produces a form of shock wave. If the stone is small, surgeon will use a tiny wire basket to retrieve it. If it is large, the stone will be fragmented, so the pieces can pass naturally. While uteroscopy requires general anesthesia, most patients go home the same day. A small scaffold, called a stent, may be left in the ureter to promote healing. The stent is removed on a follow-up office visit.
Review of literature

Before fiber optics made ureteroscopy possible, physicians used a similar “blind basket” extraction method. But this technique is rarely used now because of the higher risks of damage to the ureters.

Open surgery

Open surgery method is rarely used today, given the other available treatment options. For complicated cases however, it may be the best and even the only option. It involves incisions through the patient's back and ureter or into the kidney. The kidneys are cooled down using ice. X-rays are used during the procedure to locate specific areas and the stone. The arteries in the kidney are identified and isolated away from the surgical region. The surgeon locates the collecting system and retrieves the stone. If the surgeon finds any blockage, this is corrected. Most of the patients require prolonged hospitalization. Recovery takes several weeks.

3.3.11.4 Herbal Drug used in Urolithiasis

The worldwide incidence of urolithiasis is quite high and In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Hence, the search for herbo-mineral preparations is still ongoing. A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they are reported to be effective with no side effects (Nadkarni, 1976). 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. Herbal remedies that have antilithiatic (stone-dissolving) action can assist in dissolving small kidney stones. These drugs are recommended to increase the amount of urine and to relieve pain. Such herbal drugs are gravel root (*Eupatorium purpureum*), hydrangea (*Hydrangea arborescens*), wild carrot (*Daucus carota*), Starfruit (*Averrhoa carambola*), Patharvel (*Bryophyllum pinnatum*) and many more like *Cedrus deodara* (Ramesh et al., 2010), *Paronychia argentea* (Bouanani et al., 2010), *Crataeva nurvala* (Varalakshmi et al., 1990), *Costus spiralis Roscoe* (Viel et al., 1999), *Herniaria hirsute* (Atmani et al., 2004), *Sesbania grandiflora* (Doddola et al., 2008), Lupeol and Betulin (Vidya & Varalakshmi, 2000), *Moringa oleifera* (Karadi et al., 2006), *Eysenhardtia polystachya* (Perez et al., 1998), *Phyllanthhus niruri* (Barros et al., 2006), *Raphanus sativus* (Vargas et al., 1999), *Tribulus terrestris* (Anand et al., 1984), *Hibiscus sabdariffa* (Betanabhatla et al., 2009), *Rubia cadofolias* (Divakar et al., 2010), *Aevra lanata* (Selvam et al., 2001), *Tephrosia purpurea* (Swathi et al., 2008), *Tribulus terrestris* (Poonguzhali and Chegu, 1994) are widely used for the treating urolithaisis.

Studies have shown that Lupeol, a triterpene compound has been isolated from *C. nurvala* and was shown to have dose related prophylactic and curative activities in albino rats when studied by foreign body insertion method using glass beads (Viel et al., 1994). An excellent account of the ‘Pashanabheda’ group of plants, claimed to be useful in the treatment of urinary stones is given by Narayana Swami and Ali and Bahl and Sheshadri. (Singh et al., 1991; Anand et al., 1989). The freash juice of *Coleus aromaticus* was found to reduce the deposition of calcium and oxalate in kidneys of experimental rats (Prasad et al., 1993). The antiurolithiatic activity of *Z. mays* has been assigned to its diuretic activity (Grases et al., 1995). *R. canina* was found to have significant activity on calcium oxalate urolithiasis as it decreased calciuria and increased citraturia (Schneider et al., 1979). The antiurolithiatic activity of *H. hirsuta* has been assigned to increase in citraturia (Christina et al., 2005). A large number of Indian medicinal plants have been used in the treatment of urolithiasis and nephroprotective usefulness and they are reported to be effective with no side effects (Nadkarni, 1976). Hence, the Indian herbal plants are constantly being evaluated for possible antilithiatic effects in a systematic manner. *Hordeum vulgare*
Linn. (*Gramineae; Poaceae*) locally known as ‘Barley’ or ‘jav’ is distributed throughout India. It is an annual climber with reported to be highly medicinal (Nadkarni, 1976; Khare, 2007). Locally in India, the seeds of *Hordeum vulgare* are being used for the different medicinal purpose.

### 3.4 Drug induced nephrotoxicity

#### 3.4.1 Overview of Nephrotoxicity

Nephrotoxicity is a common event, which can cause significant morbidity and can be easily overlooked. It is a toxic effect of some substances (both toxic chemicals and medication) on the kidneys. Nephrotoxicity should not be confused with the fact that some medications have a predominantly renal excretion and need their dose adjusted for the decreased renal function (e.g. heparin). Several drugs are nephrotoxic (Table 4). Reaction to drugs and other compounds are relatively common and have been described for many substances. They are commonly associated with renal dysfunction although the actual incidence of drug-induced renal failure has not been reported, since incidence is complicated by the complexity of the causes of acute renal failiour (ARF) in seriously ill patients.
Table 3.3: drugs which can cause renal failure and their mechanism of toxic effect

<table>
<thead>
<tr>
<th>Mechanism of toxic effect</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular</strong></td>
<td>General: Diuretics, ß blockers, vasodilator agents</td>
</tr>
<tr>
<td></td>
<td>Local: ACE inhibitors, cyclosporine A</td>
</tr>
<tr>
<td><strong>Direct tubular effect</strong></td>
<td>Proximal tubule: Aminoglycosides, amphotericin B, cisplatin, radiocontrast media, immuno-globulin, mannitol.</td>
</tr>
<tr>
<td></td>
<td>Distal tubule: NSAIDs, ACE inhibitors, cyclosporine A, lithium, cyclophosphamide amphotericine B</td>
</tr>
<tr>
<td><strong>Tubular obstruction</strong></td>
<td>Sulphonamide, acyclovir, polyethylene glycol</td>
</tr>
<tr>
<td><strong>Acute interstitial nephritis</strong></td>
<td>ß-lactam, vancomycin, rifampicin, sulphonamide, ciprofloxacin, NSAIDs, ranitidine, cimetidine, furosemide, thiazides, phenytoin</td>
</tr>
<tr>
<td><strong>Acute glomerulonephritis</strong></td>
<td>Penicillamine</td>
</tr>
</tbody>
</table>

Nephrotoxicity is caused by several xenobiotic substance damaging renal proximal tubules, the portion of nephron with greater sensitivity to nephrotoxic effects. Some of the chemicals which cause damage to proximal tubule are antibacterial agents such as cephaloridine and aminoglycosides, anticancer agents such as cisplatin and industrial chemicals such as cadmium, hexavelent chromium, mercury and palladium. **Nephrotoxicity** is a major side effect of amino glycosides especially **Gentamicin** has increased from 2 to 3% to 1969 to 20% in the past decade. Generation of reactive oxygen species (ROS) has been one of the major contributing factors towards nephrotoxicity. ROS also induces cell injury and necrosis via lipid peroxidation and protein modification (Bibu and Joy, 2010). Despite nephrotoxicity and otoxicity, the aminoglycosides are continuously being used in clinical practice because of their bactericidal efficacy, synergism with ß lactam agents, low cost, limited bacterial resistance, and s post-antibiotic effects.

3.4.2  Mechanism of drug induced nephrotoxicity

When the kidneys are exposed to a toxic agent like antibiotics (Gentamicin), NSAIDS (Acetaminophen), Anti-cancer drugs (Cisplatin) and contrast agents etc. either
accidentally or intentionally (as in a suicide attempt), damage can occur in a number of different ways, depending upon the agent. These include altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. (Schetz et al., 2005; Zager, 1997)

Aminoglycoside antibiotic like Gentamicin, is widely used against infections by Gram-negative microorganisms. A central aspect of Gentamicin nephrotoxicity is its tubular effect, which may range from a mere loss of the brush border in epithelial cells to an overt tubular necrosis. Tubular cytotoxicity is the consequence of many interconnected actions, triggered by drug accumulation in epithelial tubular cells. Accumulation results from the presence of the endocytic receptor complex formed by megalin and cubulin, which transports proteins and organic cations inside the cells. Gentamicin then accesses and accumulates in the endosomal compartment, the Golgi and endoplasmic reticulum (ER), causes ER stress, and unleashes the unfolded protein response. An excessive concentration of the drug over an undetermined threshold destabilizes intracellular membranes and the drug redistributes through the cytosol. It then acts on mitochondria to unleash the intrinsic pathway of apoptosis. In addition, lysosomal cathepsins lose confinement and, depending on their new cytosolic concentration, they contribute to the activation of apoptosis or produce a massive proteolysis. However, other effects of Gentamicin have also been linked to cell death, such as phospholipids, oxidative stress, extracellular calcium-sensing receptor stimulation, and energetic catastrophe. Besides, indirect effects of Gentamicin, such as reduced renal blood flow and inflammation, may also contribute or amplify its cytotoxicity (Yaremi et al., 2011). One toxin may directly affect the glomerulus or the renal tubules, causing the cells of these structures to die. Another toxin may create other substances or conditions that result in the same cell death. Nephrotoxic injury can lead to acute renal failure, in which the kidneys suddenly lose their ability to function, or chronic renal failure, in which kidney function slowly deteriorates. If unchecked, renal failure can result in death (Khan and Khanum, 2005).

The toxicity of Gentamicin is believed to relate to generation of reactive oxygen species (ROS) in kidney. Several reports have documented the pathogenesis of aminoglycosides induced renal tubular cell injury such as derangement of lysosomal,
mitochondrial and plasma membrane structure. Furthermore results of many studies have been shown that the altered concentrations of various biochemical indicators of oxidative stress in kidney tissue are due to Gentamicin. Because of the obvious mediation of ROS in Gentamicin induced renal damage, several antioxidant agents have been used to block Gentamicin induced nephrotoxicity (Sharma et al., 2011).

3.4.3 Role of herbs in nephrotoxicity
In the recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support kidney function and treat disease of kidney. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanism and mode of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Several hundreds plants have been examined for use in a wide variety of kidney disorders. The aim of the research is to find out new nephroprotective drugs from indigenous plants which are potent and non-toxic agents. Normally herbal plants are free from side effects/adverse effects and they are low cost medicines, which will be beneficial for the people. Keeping in this view, we have selected *Hordeum vulgare* seeds based on ethnopharmacological information traditionally used in various disorders including kidney disease.

3.5 Nephropathy
3.5.1 Overview of Nephropathy
Nephropathy is a leading cause of morbidity and mortality and its prevalence is continuously increasing in industrialized nations. Nephropathy is defined as partial loss of function of kidney associated with nephritic syndrome, varying degrees by nodular glomerulosclerosis, glomerular basement membrane thickness and mesangial expansion, leading to a decline in glomerular filtration rate (GFR), persistent elevated albuminuria, elevated arterial blood pressure and fluid retention. Hyperglycemia, hyperlipidemia and hypertension are considered to be the major risk factors implicated in the progression of nephropathy (Blickle et al., 2007). End-stage renal disease is a growing problem worldwide, and is especially serious in developing countries. The incidence of diabetic nephropathy (DN) is believed to be a major
contributor to the increased end-stage renal disease (Lopes, 2009; Wada and Makino, 2009).

3.5.2 Diabetes induced nephropathy

Diabetes is the major cause of chronic kidney disease which in turn may lead to end-stage renal disease (ESRD) ending up in dialysis. Diabetic nephropathy is one of the diabetic kidney diseases. It is also known as Kimmelstiel Wilson syndrome and it was discovered in 1963 by Clifford Wilson and Paul Kimmelstiel. Diabetic nephropathy occurs as a result of an interaction between hemodynamic and metabolic factor (Cooper, 2001). Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive hormone pathways including the Rennin Angiotensine System and endothelin (Hargrove and Wong, 2000). These hemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC), Mitogen-activated protein (MAP kinase) (Haneda, 1997), nuclear transcription factors such as NF-κB and various growth factors such as the prosclerotic cytokine, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF. Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation and the accumulation of advanced glycation end products (AGEs). In combination, these pathways ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in increasing proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis. At present, diabetic kidney disease affects about 15%-25% of type I diabetes patients and 30%-40% of patients with type II diabetes. Diabetic nephropathy (DN) develops in approximately 40 percent of all patients with type 2 diabetes and has become the leading cause of end-stage renal disease. In Europe, Japan, and the United States, accounting for 25%-42% of cases are affected by diabetic nephropathy. Therefore, the early identification and subsequent renoprotective or nephroprotective treatment of all patients at risk are of greatest importance. The screening of urine for albumin has revealed that patients with type 2 diabetes and so-called microalbuminuria i.e., a urinary albumin excretion rate of 20 to 200 µg per minute-have a risk of diabetic nephropathy that is 10-20 times that of patients with normoalbuminuria. Diabetic nephropathy develops in 5%-10% of patients with type 2
diabetes and microalbuminuria each year. Blockade of the renin–angiotensin system slows the progression to diabetic nephropathy in patients with type 1 diabetes (Hans-Hanrik et al., 2001). Diabetic nephropathy is characterized by specific renal morphological, haemodynamic and functional alterations. ROS play an important role in high glucose induced renal injury.

3.5.3 Mechanism of Diabetic Nephropathy (DN)
Diabetic nephropathy occurs as a result of an interaction between hemodynamic and metabolic factors (Copper, 2001). Hemodynamic factors that contribute to the development of diabetic nephropathy include increased systemic and intraglomerular pressure, as well as activation of vasoactive hormone pathways including the renin angiotensin system (Zats et al., 1986) and endothelin (Hargrove et al., 2000). These haemodynamic pathways activate intracellular second messengers such as protein kinase C (PKC) (Xia et al., 1994), MAP kinase (Haneda et al., 1997), nuclear transcription factors such as NF-κB (Barnes and Karin, 1997) and various growth factors such as the prosclerotic cytokine, TGF-β and the permeability enhancing growth factor, vascular endothelial growth factor, VEGF (Cooper et al., 1999). Glucose dependent pathways are also activated within the diabetic kidney and result in enhanced oxidative stress, renal polyol formation (Dunlop, 2000) and the accumulation of advanced glycation end products (AGEs) (Soulis-Liparota et al., 1991). In combination, these pathways ultimately lead to increased renal albumin permeability and extra cellular matrix accumulation, resulting in increasing proteinurea, glomerulosclerosis and ultimately tubulointerstitial fibrosis. There appears to be a complex interplay between hemodynamic and metabolic pathways which contribute to the development of diabetic nephropathy.
Figure 3.8: Mechanisms involved in streptozotocin-induced diabetic nephropathy. AGEs = advanced glycation end products; eNOS = endothelial nitric oxide synthase; nNOS = neuronal nitric oxide synthase; PARP = poly (ADP-ribose) polymerase; PDGF = platelet derived growth factor; PKC = protein kinase C; ROS = reactive oxygen species; VEGF = vascular endothelial growth factor.
3.5.4 Medical treatment of diabetic nephropathy (Lewis et al., 2001; Brenner et al., 2001; Parving et al., 2001):
Glucose and blood pressure control are most important for the prevention of nephropathy, and blood pressure control is the most important for retarding the progression of established nephropathy. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers, considered first-line recommended treatment modalities, have shown efficacy in preventing the clinical progression of renal disease in patients with type 2 diabetes mellitus. Diuretics frequently are necessary due to the volume-expanded state of the patient and are recommended second-line therapy. Three or more antihypertensive are often needed to treat to goal blood pressures. More novel strategies to influence vasoactive hormone action or inhibit various metabolic pathways such as advanced glycation and specific protein kinase C isoforms provide important promise for the future. It is predicted that a combination of therapies that target different steps in the pathophysiology of the disease will be required to further reduce the rate of progression and ultimately prevent the development of diabetic nephropathy in the future.
Reference


Review of literature


Review of literature


Review of literature


Jeong, J.B., Hong, S.C., Jeong, H.J., 2009. 3,4-Dihydroxybenzaldehyde purified from the barley seeds (Hordeum vulgare) inhibits oxidative DNA damage and apoptosis via its antioxidant activity. Phytomedicine 16, 85-94.


Review of literature


Review of literature

North Carolina, Urology 61, 523-527.


Sharma, R.K., Rajani, G.P., Sharma, V., Komala, N., 2011. Effect of ethanolic extract and aqueous extracts of *Bauhinia variegata* Linn. on Gentamicin-induced...


