 Chapter I

Introduction

Urolithiasis is the formation of stones in the urinary tract. The urinary system is the organ system that produces, stores, and eliminates urine. The urinary tract consists of two kidneys, two ureters, one urinary bladder, and one urethra. Kidney stones are common in industrialized nations with an annual incidence of 0.5 to 1.9% (Lieske et al., 2006). Studies report that the prevalence rate varies from 2-13% in developed countries to 0.5-1% in developing countries (Lee et al., 2002). The overall probability of forming stones differ in various parts of the world and is estimated as 1.5% in Asia, 5.9% in Europe, 3% in North America and 20% in Saudi Arabia (Trinchieri, 2008). Areas with higher incidence of kidney stones are Scandinavian countries, Mediterranean countries, British Isles, Northern Australia, Central Europe, portions of the Malayan Peninsula, China, Pakistan and northern India whereas the incidence of kidney stone formation is lower in areas like Central and South America and some parts of Africa. In Asia, stone-forming belt has been reported to stretch across Sudan, Saudi Arabia, the United Arab Emirates, the Islamic Republic of Iran, Pakistan, India, Myanmar, Thailand, Indonesia and Philippines (Abbagani et al., 2010). India has higher incidence of urinary calculi especially in Gujarat, Rajasthan, Punjab and Madhya Pradesh (Shah, 2003). Countries in tropical and subtropical areas have also reported a high incidence of urolithiasis (Rizvi et al., 2002). Furthermore, urolithiasis is largely a recurrent disease with a relapse rate of 50% in 5–10 years and 75% in 20 years (Trinchieri, 2008). Thus, urolithiasis imposes substantial economic consequences and a great public health importance.
CLASSIFICATION OF RENAL STONES

The renal stones or uroliths which are formed in the kidneys are of various forms based on their chemical composition and structures. Based on the predominant chemical composition, uroliths are classified as the following types:

1. Calcium containing stones
   I. Calcium oxalate stones (CaC₂O₄)
   II. Calcium phosphate stones [Ca₃(PO₄)₂]

2. Struvite stones

3. Uric acid stones

4. Cystine stones and

5. Dihydroxyadenine stones

Calcium oxalate (CaOx) urolithiasis accounts for approximately 75% of urinary stone disease in the United States (Coe et al., 1992). Many studies from India have also documented that CaOx forms the major constituent of renal calculi disease (Ansari et al., 2005). Calcium in combination with phosphate is also present in urinary stones as either apatite (the principal constituent of bones and teeth) or brushite (calcium monohydrogen phosphate). Brushite, but not apatite, stones are physically resistant to extracorporeal shock wave lithotripsy, so repeated treatments may be needed (Klee, 1991). The major difference between oxalate and phosphate type of stones is that the CaOx stone formers do not have intratubular crystals, whereas all brushite stone formers exhibited collecting duct plugging. About 10–15% of urinary calculi are composed of struvite (ammonium magnesium phosphate, NH₄MgPO₄·6H₂O) (Worcester and Coe, 2008). Struvite stones are also known as "infection stones", urease or triple-phosphate stones which form most often in the
presence of infection by urea-splitting bacteria. Stones develop if urine is alkaline, has a raised concentration of ammonium, contains trivalent phosphate, and contains urease produced by bacteria. Urease cleaves each mole of (soluble) urea into two moles of (relatively insoluble) ammonium and one bicarbonate, thereby converting urinary divalent phosphate to the trivalent form. About 5–10% of all stones are formed from uric acid (Worcester and Coe, 2008). Uric acid stones are smooth, round, yellow-orange and nearly radiographically transparent—unless mixed with calcium crystals or struvite. Uric acid is a product of purine metabolism. Uric acid becomes insoluble at low urinary pH and urate stones may form secondary to hyperuricaemia or acidic urine alone. The other rare types include cystine stones or dihydroxyadenine stones. Cystine stones are produced in patients with a homozygous recessive gene for cystine transport, producing excess urinary cystine. Dihydroxyadenine stones are rare inherited form of renal stone disease, secondary to adenine phosphoribosyl-transferase deficiency (Miller et al., 2007).

All calcium stones are radio-opaque, and most stones contain calcium combined with oxalate or phosphate. The urinary oxalate and calcium are equally important in raising urinary CaOx supersaturation (Worcester and Coe, 2008). The underlying mechanisms of hyperoxaluria can be either due to oxalate overproduction as a result of an inborn error in metabolism or due to increased dietary intake and increased intestinal oxalate absorption. The calcium oxalate stones form on the surfaces of the renal papillae over collections of interstitial suburothelial calcium phosphate particles (Evan et al., 2003) named Randall plaque. The number of CaOx stones formed, adjusted for duration of stone formation, varies directly with plaque surface coverage (Kim et al., 2005), as would be expected if plaque were a surface that promotes CaOx overgrowth. The plaque begins in the basement membranes of
the thin Henle loops. The basement membrane plaque comprises a myriad of particles in which crystal and organic layers are present in alternate form. Outside the basement membrane, in the interstitium, plaque particles coalesce and it is this coalescent material that extends to the suburothelial region and over which CaOx stones grow. The identities of the organic molecules surrounding apatite in plaque are unknown except for osteopontin, which coats the surface of apatite and positions itself precisely at the apatite organic layer interface (Evan et al., 2005). The driving force for CaOx overgrowth on plaque is urinary supersaturation. But the forces that create the plaque are not so clear. The fraction of papillary surface covered by plaque in CaOx stone formers correlates directly with urinary calcium level and inversely with urine volume and pH (Kuo et al., 2003). It was observed that the initial formation occurs in the basement membrane of the thin Henle loops followed by the efficient water extraction in the collecting duct combined with the high deliveries of calcium as a result of hypercalciuria may increase tubule and interstitial calcium concentrations and thereby stimulate apatite deposition in the basement membrane of the thin-limb.

The other sources of oxalate are endogenous in nature and are derived from the metabolism of glycine, ethanolamine, glycolaldehyde and ascorbic acid which leads to increased excretion of oxalic acid (Holmes and Assimos, 1998). Another type of hyperoxaluric syndrome has been recognized in patients with a variety of malabsorptive states in which the gastro-intestinal absorption of oxalate is increased and they came under the category of enteric hyperoxaluria (Seftal and Resnick, 1990). In those cases, the urinary glycollate and glyoxylate concentrations were within the normal range. Patients with ileal diseases have increased absorption of dietary oxalate hyperoxaluria and an increased incidence of nephrolithiasis (Smith et al., 1980). The malabsorption of fatty acids and bile salts is an important pathogenic factor in
hyperoxaluria (Marangella et al., 1982). The main cause of diarrhoea in hyperoxaluric patients is malabsorption of bile salts (Stauffer et al., 1973). Gregory (1981) has reported that small bowel bypass surgery in patients leads to hyperoxaluria. Hyperoxaluria may be also due to malabsorption of citrate, ascorbate and possibly other hydroxycarboxylic acids which act as crystal inhibitors (Cowley et al., 1987).

**PATHOPHYSIOLOGY OF RENAL STONE FORMATION**

Kidney stone formation is a complex process that results from succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the renal tubules.

1. **Supersaturation and Nucleation**

   The supersaturation of urine is the driving force behind crystal formation in the kidney. Supersaturation actually refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. These results in nucleation which is defined as the formation of solid crystal phase in a solution (Finlayson and Reid, 1978; Khan and Byer, 1999). The process of nucleation in a pure solution i.e., formation of initial crystal phase, is known as homogenous nucleation (Finlayson and Reid, 1978).

   In secondary nucleation, the newly formed crystals deposit on pre-existing crystal surfaces of similar type which then results in the mass production of the crystals. However, in another process that is “epitaxy”, material of one crystal type is precipitated upon the surface of another type whose lattice dimensions are almost identical (Lonsdale, 1968). Both these processes referred as “heterogenous nucleation”.

   Since urine is not a pure solution, nucleation in urine often occurs over an existing surface. The most heterogenous frequent nucleation sites in urine are
epithelial cells, cell debris, urinary casts, other crystals and bacteria. The renal tubular cell injury can promote crystallization of calcium oxalate crystals by providing substances which are the sources for heterogenous nucleation. The renal tubular cell injury followed by the cellular degradation produces numerous membrane vesicles, which have been shown to be potent nucleators of calcium crystals. The crystals observed in the renal tubules of hyperoxaluric rats are reported to be associated with cellular degradation products (Fasano and Khan, 2001).

2. Crystal growth

The growth of the crystal phase is determined by the molecular size and shape of the molecules, the physical properties of the material, urinary supersaturation level, pH and defects that may form in the crystal structure. The crystal growth is the driving force for the particle formation and thus for stone formation (Masao, 2008).

3. Crystal aggregation

In this process, crystals in solution stick to each other to form larger particles. The smaller the inter-particle distance, the larger will be the attractive forces and this favors particle aggregation. Crystal aggregation is promoted by viscous binding i.e., foreign crystalline compounds with multiple binding sites, such as abnormally self aggregated macromolecules attach to the crystal surfaces (Masao, 2008). Thus the macromolecules secreted by the brush border of proximal tubular cells in the urine results in crystallization on the interaction between tubular cells and crystals (Verkoelen et al., 1998).

Experimental studies have shown that the injury of renal epithelial cells due to free radicals result in sloughed membrane fragments in the tubular lumen which provides a suitable surface for nucleation of calcium phosphate and oxalate (Khan et al., 1999). It is now widely accepted that the process of calcium stone formation in
supersaturated urine initiates as a precipitation of calcium phosphate in the loop of Henle or the distal part of the distal tubule (Kok, 1997). Whereas in normal physiochemical conditions, repulsion occurs between the calcium phosphate crystals and tubular cells and thus result in elimination of small calcium phosphate crystals by dissolution in spontaneous urine.

This is then followed by formation of masses of crystals by growth and aggregation leading to the adherence of calcium phosphate crystal aggregates to the tubular surface.

4. Crystal retention

Crystal retention is the actual association of crystals with the epithelial cells lying in the renal tubules. The initial formation of the crystals in the urine depends on the composition of the tubular fluid, whereas the retention of crystals depends on the composition of the renal tubular epithelial cell surface (Schepers et al., 2002). Crystal retention can be caused by the association of crystals with the epithelial cells lining the renal tubules. The process of attachment of endocytosis of crystals to renal tubular cells is generally accomplished by crystal – cell interaction. A non-adherent surface of the distal tubules, collecting ducts, ureters, bladder, and the urethra may provide a natural defence mechanism against crystal retention, and may become defective when the anti-adherence properties are compromised (Basavaraj et al., 2007).

MANAGEMENT OF STONE DISEASE

The management of stone disease depends on the size and location of the stones. The stones which are smaller than 5 mm have a higher probability of spontaneous passage. In contrast, stones larger than 5 mm, stones in patients with a higher risk of developing renal insufficiency (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), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL) as well as other therapeutic treatments.

**Extracorporeal shock wave lithotripsy (ESWL)**

The extracorporeal shock wave lithotripsy is a non-invasive procedure which uses high-intensity acoustic pulse shock waves to fragment calculi (Fellstrom et al., 1984). This technique is the most widely used method for managing renal and ureteral stones. However, treatment success rates depend on stone composition, size, properties and location of the stone as well as the instrumentation type and shock frequency (Knoll, 2007). It also needs to be considered that the same forces that are directed at the stones have deleterious effects on surrounding tissues (Fellstrom et al., 1984). The damage to almost every abdominal organ system has been reported (Rashid et al., 1996; Hassan and Zietlow, 2002), but by far the most common injury is acute renal hemorrhage although its true incidence is unclear and poorly defined (Fellstrom et al., 1984). The most often renal hemorrhage can be managed conservatively; however, in rare instances the complications are fatal (Fellstrom et al., 1984). The reports of post-ESWL perirenal hematoma range from less than 1% to greater than 30% (Rubin et al., 1987). Furthermore, ESWL has also been associated with long-term medical effects such as diabetes mellitus and hypertension (Krambeck et al., 2006).

**Ureteroscopy (URS)**

In addition to ESWL, other procedures such as ureteroscopy have also been developed for removal of ureteral stones. Ureteroscopy is usually performed with an endoscope that is “ureterscopes” which is passed through the urethra, bladder, and then directly into the ureter. The new generations of ureterscopes are flexible, smaller in diameter, stiffer and more durable, and have an improved tip deflection (Knoll, 2007).
However, there are many drawbacks also associated with this technique. The major drawback of URS is that it is more invasive than ESWL and the rate of ureteric perforation and stricture formation remains around 2 to 4% (Pearle et al., 2001). In contrast, the major advantage of URS is that it is cheaper and results in higher and faster stone free rates (Knoll, 2007).

**Percutaneous nephrolithotomy (PCNL)**

Percutaneous nephrolithotomy is a surgical procedure to remove stones from the kidney by a small puncture wound through the skin. It is most suitable to remove stones of more than 2 cm in size and which are present near the pelvic region. However, this also involves several complications like parenchymal bleeding, septicaemia and colonic or pleural injury (Michel et al., 2007).

**THERAPEUTIC TREATMENTS**

Along with the other interventional procedures as described earlier, therapeutic agents are also used routinely. The most effective hypocalciuric agents are thiazide diuretics which hypocalciuric action enhances calcium reabsorption in the distal renal tubules (Laerum and Larsen, 1984). However, long-term use in up to 50% of patients is limited because of side-effects including fatigue, dizziness, impotence, musculoskeletal symptoms, or gastrointestinal complaints (Park and Pearle, 2007). Another reported complication is thiazide-induced potassium depletion, which causes intracellular acidosis and can lead to hypokalemia and hypocitraturia (Moe, 2006). Potassium citrate is effective in the treatment of patients who have calcium stones and normal urinary calcium. By providing an alkali load, potassium citrate increases urinary pH and citrate, therefore mediating the inhibitory effects of macromolecular modulators of calcium oxalate crystallization (Mattle and Hess, 2005). The main limitation for a more widespread use of alkali citrate preparations is
the relatively low tolerability of available alkali citrate preparations. Adverse effects that reduce treatment compliance have been noted mainly in the gastrointestinal tract and include eructation, bloating, and diarrhea (Mattle and Hess, 2005). In conclusion, none of the listed treatment modalities is without any side-effects. Thus, the focus should be on the development of novel strategies for the prevention and treatment of kidney stone disease. Herbal medicines could close a gap in this regard.

SCREENING MODELS FOR INDUCING UROLITHIASIS

Several animal models have been developed to investigate hyperoxaluria and its consequences. Calcium oxalate type of kidney stones are produced in rats by the induction of acute or chronic hyperoxaluria (Khan and Hackett, 1987; Khan, 1991) using a variety of agents such as sodium oxalate, ammonium oxalate, hydroxy-L-proline, ethylene glycol, and glycolic acid. These lithogenic agents are generally administered either orally in food or water or by gavage but have also been injected intraperitoneally.

The acute hyperoxaluria can be induced by intraperitoneal administration of sodium oxalate (30, 70, or 100 mg/kg body weight of rat) resulted in increased urinary excretion of oxalate and an almost instant appearance of calcium oxalate crystals in lumina of the renal proximal tubules (Khan et al., 1992). The crystals were later seen in collecting ducts of the cortex and papilla. The amount and duration of urinary excretion of excess oxalate and the size, number, and location of crystals within the kidneys depended on the amount of sodium oxalate given. However, the largest amount of oxalate was excreted within the first 6 h of the challenge. At the lower dose of sodium oxalate, crystals were restricted to the tubular lumens and cleared the kidneys within a few days. At higher doses, crystals were initially located in tubular lumina and then they were later seen in the interstitium. Apparently, some crystals
and crystal aggregates remained small and therefore, did not adhere to the renal epithelium and moved with the urine and finally were flushed out. The larger crystals and their aggregates also moved but at a slower rate.

Renal CaOx deposition induced by ethylene glycol is most appropriate model which is frequently used to mimic the urinary stone formation in human beings (Atmani et al., 2003). In the most common model, ethylene glycol, a precursor of oxalate, is given to rats in their drinking water, with or without additional ammonium chloride or vitamin D. Today it is used as an experimental model in many in vivo systems. It is without doubt, the most intensively studied lithogen with dose ranges from 0.5% to 1.5%.

Ethylene glycol (CAS No. 107-21-1; Table 1.1) is a colourless, odourless, sweet-tasting and relatively non-volatile liquid. It has a low vapour pressure and is completely miscible in water. Ethylene glycol is used in the manufacture of polyethylene terephthalate, in natural gas processing, and as an antifreeze agent.

The step-wise metabolism of ethylene glycol in liver, proceeds in a nicotinamide adenine dinucleotide (NAD) - dependent fashion (Fig. 1.1). The first step is oxidation of ethylene glycol to glycoaldehyde by alcohol dehydrogenase. Subsequently, glycoaldehyde is oxidised to glycolic acid, to glyoxylic acid and finally to oxalic acid. Because the conversion of glycolic to glyoxylic acid is the rate-limiting step in this process, the accumulation of glycolic acid is largely responsible for the metabolic acidosis seen in this poisoning (Clay and Murphy, 1977). Approximately 80% of an absorbed dose of ethylene glycol is hepatically metabolized, with the remainder excreted unchanged by the renal system.

Renal CaOx deposition induced by ethylene glycol is associated with proximal tubule cell necrosis leading to production of several metabolites (glycolaldehyde,
glycolate, glyoxylate and oxalate, in order) and accumulation of large CaOx crystals in tubular lumen (Tsai et al., 2008). Khan et al. (1997) have reported the induction of severe calcium oxalate crystalluria, both biochemically and histopathologically in rats treated with ethylene glycol in drinking water. In rats fed diets containing ethylene glycol for 16 weeks (Cruzan et al., 2004), the development of nephropathy correlated highly with the kidney oxalate crystal accumulation in response to chronic treatment. The chronic hyperoxaluria can be induced by the administration of 0.75% ethylene glycol alone in drinking water which shows persistent crystalluria. Initially, small dipyramidal crystals were seen in the urine. Later, most of the urinary crystals were develop into large aggregates of dumbbell-shaped CaOx monohydrate crystals and twinned CaOx dihydrate crystals. The crystals were shown to be located in both the cortex and the medulla (Pak, 1991). During chronic hyperoxaluria, crystals were initially distributed randomly in the renal medulla. Eventually, collecting ducts at the renal papillary tip and papillary base were the preferred sites of crystal deposition (Khan and Glenton, 1995; Khan, 1996). Most crystals were found to be intraluminal aggregates. After 4-6 weeks of ethylene glycol administration, crystals were seen to be developing between the tubular epithelial cells as well as inside the epithelial cells and the interstitium. These stones contained both CaOx mono- and dihydrate crystals and reached a size of over 1000 µm, occupying and calcifying the entire papillary tip. The light microscopy and scanning and transmission electron microscopic studies of the papillary stones revealed that they originated in the lumina of collecting ducts near the renal papillary surface (Khan, 1996).

The acute and chronic hyperoxaluria induced by ethylene glycol resulted in increased crystal deposition which was also associated with increased urinary excretion of the membrane marker enzymes alkaline phosphatase, and gamma-
glutamyl transpeptidase. The renal tubular epithelium of nephrolithic kidneys was damaged with the crystals (Khan and Hackett, 1987).

The other degenerative changes in epithelial cells included an increase in the number of lysosomes, swelling of mitochondria, dilatation of endoplasmic reticulum, cytoplasmic edema, and vacuolization. Some cells appeared to burst open and release their contents into the tubular lumen, whereas others sheared away from the basal lamina (Hackett et al., 1990). The renal papillary surfaces were shown to be badly damaged. The intercellular spaces between the intact epithelial cells also reported to be enlarged. In addition, the injury caused by the exposure to elevated levels of oxalate and CaOx crystals may reduce the crystallization-inhibitory activity of the urine (Hackett et al., 1994). The CaOx crystallization was also seen in all the rats administered with 0.5% ethylene glycol, but to a lower extent (Lee et al., 1992).

Oxidative stress imposed by reactive oxygen species (ROS) plays a crucial role in the pathophysiology associated with kidney stone disease. The ethylene glycol-induced hyperoxaluria and crystal deposition was reported to be associated with renal cell damage along with lipid peroxidation suggesting that cell injury due to the production of free radicals is augmented by crystal deposition in the renal tubules (Thamilselvan et al., 1997). A study conducted in Sprague Dawley rats, has reported that ethylene glycol-induced hyperoxaluria is accompanied by enzymuria, which is suggestive of renal tubular damage. Furthermore, levels of antioxidative enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione transferase (GST) in the renal cortex were significantly decreased with the concomitant increase in renal cortical content of lipid hydroperoxide (Green et al., 2005).
A previous study performed to ascertain the effect on oxidative injury in response to treatment with ethylene glycol for 28 days have indicated that ethylene glycol caused a variety of changes like alterations in the activities/levels of renal tissue enzymic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and glucose-6-phosphate dehydrogenase) and non-enzymic (reduced glutathione, ascorbate and α-tocopherol) antioxidants, along with high malondialdehyde levels in the male hyperoxaluric rats (Coothan et al., 2007).

INTRODUCTION TO HERBAL DRUGS

Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. Interest in natural products research is strong and can be attributed to several factors, including unmet therapeutic needs, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify and structurally characterize these active constituents and advances in solving the demand for supply of complex natural products. The use of herbal remedies for prevention and cure of ailments is of increasing interest due to the superiority and efficiency of activity provided by phytoconstituents in herbs and undesirable effects of modern medicine. Modern medicines are proved to target only one aspect of urolithiatic pathophysiology whereas herbal remedies have been shown to exert effectiveness at different stages of stone pathophysiology (Butterweck and Khan, 2009).
Herbal remedies produce multiple mechanism of action such as diuretic activity (beneficial in increasing the urinary volume that allows the easy passage of small calculi out of the body in urine), crystallization inhibition activity (helps to inhibit the different stages of stone formation by maintaining the balance between inhibitors and promoters of stone formation), lithotriptic activity (avoid binding mucin of calculi to prevent crystal aggregation to form a large stone) and antioxidant activity (prevent renal tissue injury). The traditional herbs also improve the renal function and regulate oxalate metabolism which help in reducing the re-occurrence of renal calculi.

The vast Ayurvedic literature claims a number of plants to be useful in urinary stones. It is now widely accepted that most herbs exhibit their effects by a variety of chemical constituents present therein and the idea of synergy within and between them is also gaining acceptance. Renal stone was well known in Ayurveda. Aqueous extract of Costus spiralis reduced the growth of calcium oxalate calculi in the urinary bladder of rats (Viel et al., 1994). Fourteen patients with renal calculi and sixteen patients with ureteric calculi have been treated with the herbomineral combination containing Bergenia ligulata and Tribulus terrestris; 28.57% of patients with renal calculi and 75% patients with ureteric calculi passed their calculi completely and in other patients there was a marked or partial expulsion of calculi along with changes in the shapes and sizes of calculi (Sannidi et al., 1997). Crataeva nurvala was reported to be effective in the prophylaxis of oxalate antiurolithiasis induced by simultaneous administration of sodium oxalate and methionine in guinea pigs (Singh et al., 1992). Lupeol, a triterpene compound has been isolated from Crataeva 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 (Anand et al., 1994).
Tamarindus indicus intake at the dose of 10 gm per patient showed significant beneficial effect in inhibiting spontaneous crystallization in both normal subjects and in stone formers (Rathore et al., 1993). The ethanolic extract of Ammania baccifera was found to be effective as prophylactic and curative against phosphate type of stones (Prasad et al., 1994). This has given rise to stimulation in the search for investigating natural resources showing antiurolithiatic activity. It is necessary to explore extensively the potential usage of medicinal plants with traditional claims to be having activity against urolithiasis and subject them to systematic phytochemical and pharmacological study. It is under this context lies the relevance of the present study which intends to study the antiurolithiatic properties of two indigenous and widely distributed medicinal plants namely: Bergenia ciliata (Fig. 1.2) and Dolichos biflorus (Fig. 1.3).

**Bergenia ciliata**

**A) CLASSIFICATION**

Kingdom: Plantae; Division: Magnoliophyta; Class: Dicotyledoneae; Subclass: Rosidae; Order: Rosales; Family: Saxifragaceae; Genus: Bergenia; Species: Bergenia ciliata

**B) VERNACULAR NAMES**

Bengali: Patharkuchi, Patrankur; Gujarati: Pashanbheda Hindi: Pakhanabheda, Sulpbha, Silphara, Dakarhru; Kannada: Alepgaya, Pahanbhedi; Malayalam: Kallurvanchi, Kalluvanni, Kallorvanchi; Marathi: Pashanbheda; Sanskrit: Asmabhedaka, Silabheda; Tamil: Sirupilai; Telugu: Kondapindi.
C) MORPHOLOGICAL CHARACTERISTICS AND DISTRIBUTION

This is a rhizomatic herb with fleshy leaves, growing up to 30 cm tall, having a stout creeping rhizomatous rootstock with scars and intermittent axillary buds. Plant is quite hardy and able to survive frost during winter turning reddish in colour. It is evergreen and flowers in April to June. Its flowers are white, pink and purple in colour. Stem is short. The rhizome comes out from the cervices of rocks and hangs in the air in sloppy areas. Leaves are 5-30 cm long, glabrous, sparsely hairy in margins, broadly obovate or elliptic, finely or sparsely denticulate or shallowly sinuate-denate.

The plant is endemic to Northern and Eastern temperate Himalayan region in Himachal Pradesh, Jammu and Kashmir, Uttarakhand, Nepal and North Eastern hilly states between altitudes of 2100-3000 m in the cold or glacial mountain rocky slopes in stone crevices. It is also found in adjoining countries like Pakistan, Afghanistan upto Tibet and China in higher altitudes.

D) PHYTOCHEMICAL CONSTITUENTS

The major chemical constituents reported from *B. ciliata* are gallic acid, bergenin, (+)-afzelechin (Reddy *et al.*, 1999), 11-O-galloyl bergenin (Vaishali *et al.*, 2008), paashaanolactone (Reddy *et al.*, 1999), β-Sitosterol (Kirtikar and Basu, 1975) and β-Sitosterol-D-glucoside (Bahl *et al.*, 1974).

E) PHARMACOLOGICAL PROPERTIES

The root is well known in traditional medicine for protection against diarrhea; cough, in uric acid diathesis and in pulmonary infections (Kirtikar and Basu, 2006). The juice of the root is also used to treat coughs and colds, hemorrhoids, asthma and urinary problems (Manandhar, 2002). The paste of the root powder is applied to treat boils and ophthalmia; it is also considered helpful in relieving backache (Manandhar, 2002). The powder of the roots along with honey is applied to the gums in teething of
children to allay irritation (Kirtikar and Basu, 2006). The juice of the leaves is used as drops to relieve earaches (Manandhar, 2002). Various Ayurvedic classical drugs such as Pashanabhedadi kwath, Pashanabhedadi ghrit, Pashanabhedadi Churan etc. are prepared from Pashanbhed rhizome.

Bhattaraj (1994) in his studies reported that in Nepal, one teaspoonful of the juice of dried rhizome of *B. ciliata* along with equal volume of honey has been taken orally 2-3 times a day by post-partum women, against the digestive disorders as carminative, and tonic as well. In the same study, Bhattaraj (1994) has also cited that in Nepal, juice of the rhizome of *B. ciliata* has been taken orally by human adults to treat low fever (hypothermia) and intermittent fever. Its decoction is also taken orally by the human adults, as antipyretic (Bhattaraj, 1993).

All the extracts of roots and leaves of *Bergenia ciliata* were found to possess significant antihyperglycaemic activity in streptozotocin - treated rats (Islam et al., 2002a). Moreover, (+)-afzelechin, a phytochemical isolated from rhizomes of *Bergenia ciliata*, showed significant alpha-glucosidase inhibitory activity with IC$_{50}$ value as low as 0.13 mM (Saijyo et al., 2008). Furthermore, Bhandari et al. (2008) isolated another active compounds (−)-3-O-galloylepicatechin and (−)-3-O-galloyl catechin for the first time from *Bergenia ciliata*. These compounds again demonstrated significant dose - dependent enzyme inhibitory activities against rat intestinal α-glucosidase and porcine pancreatic α-amylase (Bhandari et al., 2008).

The methanolic and aqueous extracts of *Bergenia ciliata* rhizome were found to have promising potential towards the development of drug that might be used to target tumours for chemoprevention/chemotherapy to check neoplastic growth and malignancy. The extracts tested for their cytotoxicity, showed significant inhibition on MDA-MB-435S (human breast carcinoma), Hep3B (human hepatocellular carcinoma)
and PC-3 (human prostate cancer) cell lines by the XTT assay. Thus, B. ciliata bears potent antineoplastic activities that may have prospective clinical use as precursor for preventive medicine (Venkatadri et al., 2011).

The aqueous and methanolic extracts of rhizomes of Bergenia ciliata has been reported to show significant gastroprotective activity. Both the extracts were found to be potent in reducing the ulcer lesion in all models including ethanol/HCl, indomethacin and pylorus ligation-induced gastric ulcers in rats. The antiulcer activity is mediated via cytoprotective effects conferred by enhancement of the mucosal barrier, rather than by prevention of gastric acid secretion or the lowering of pH and acidity (Kakub and Gulfraz, 2007).

In another study, the methanolic extracts of 41 plant species belonging to 27 families used in the traditional medicine in Nepal were investigated for in vitro antiviral activity against Herpes simplex virus type 1 (HSV-1) and influenza virus A by dye uptake assay in the systems HSV-1/Vero cells and influenza virus A/MDCK cells. Only the extract of B. ciliata reported to demonstrate remarkable activity against both viruses (Bhandari et al., 2009).

The methanolic extract has also been evaluated for antiinflammatory potential using two acute (carrageenan- and serotonin (5-HT) - induced rat paw oedema) and a chronic (cotton pouch-induced granuloma) rat models. The extract exhibited more potent inhibition of rat models of paw oedema as compared with the standard phenylbutazone. This confirms the antiinflammatory potential of the plant (Sinha et al., 2001a).

The methanolic extract of Bergenia ciliata also exhibited significant antitussive activity in a dose-dependent manner, as compared with control, which was comparable to that of codeine phosphate, a standard anti-tussive agent. The extract
showed significant inhibition of cough, within 90 min of the experiment (Sinha et al., 2001b).

The methanolic extract of *Bergenia ciliata* showed wide spectrum of concentration-dependent antibacterial activity (Sinha et al., 2001c). The ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts of the roots and leaves of *Bergenia ciliata* exhibited various degrees of inhibition activity against gram-positive and gram-negative bacteria *viz.*, *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas aeruginosa* (Islam et al., 2002b).

**Dolichos biflorus**

A) CLASSIFICATION

**Kingdom:** Plantae; **Division:** Magnoliophyta; **Class:** Dicotyledoneae; **Subclass:** Rosidae; **Order:** Fabales; **Family:** Fabaceae; **Genus:** Dolichos; **Species:** Dolichos biflorus

B) VERNACULAR NAMES

**Bengali:** Kurli-kalai; **Hindi:** Kutthi; **Kannada:** Hurali; **Malayalam:** Muthiva; **Marathi:** Nagakrijon; **Sanskrit:** Kulattha; **Tamil:** Kollu; **Telugu:** Ulavalu.

C) MORPHOLOGICAL CHARACTERISTICS AND DISTRIBUTION

The plant is sub-erect annual with cylindrical, slightly hairy to tomentose stems. The leaves are trifoliolate; leaflets ovate, rounded at the base, acute or slightly acuminate, terminal leaflet symmetrical, softly tomentose on both surfaces, fimbriolate and paler beneath. The flowers are yellow or greenish yellow, single or in short, sessile or subsessile, 2- to 4-flowered, calyx tomentose, standard oblong with two linear appendages of about 5 mm long. The pods are shortly stipitate, slightly curved, smooth or tomentose. Its seeds are ovoid, 5–8 per pod, pale fawn, light red,
brown, or black sometimes with faint mottles or with small, scattered black spots, (or both) and hilum is central.

It is an herbaceous plant with annual branches, sub-erect or twining, leaflets 2.5-5 cm, seeds are ovoid, 5-8 per pod, brown or black with scattered spots and widely distributed in Africa, Asia and Australia. It is also widely distributed in India, ascending up to 1000 m in Sikkim; cultivated mainly in Andhra Pradesh, Tamil Nadu and Karnataka.

D) PHYTOCHEMICAL CONSTITUENTS

Analysis of seeds showed moisture 11.8%, crude protein 22.0%, fat 0.5%, mineral matter 3.1%, fibre 5.3%, carbohydrate 57.35%, calcium 0.28% and phosphorous 0.39%; iron 7.6 mg, nicotinic acid 1.5 mg, carotene 119 (international vitamin unit A unit) per 100 gm and rich in various enzymes. Other chemical constituents present are quercetin, streptogenin, beta-sitosterol, a phyto-haemagglutinin, beta-N-acetylglucosaminidase, alpha and beta galactosidases, alpha mannosides and beta glucosides (Khare, 2003).

E) PHARMACOLOGICAL PROPERTIES

_Dolichos biflorus_ has been commonly used as a traditional medicine for treating various diseases. It is well known for its medicinal uses because different parts of the plants are used for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary discharges and for treatment of kidney stones (Ghani, 2003). _D. biflorus_, commonly known as kulattha, was also used by Indian physicians in the 16th century for allergic conditions such as urticaria, chronic rhinitis, asthma, bronchitis and for treating flatulence and adiposity. _D. biflorus_ has the greatest potential for further utilization as nutraceuticals, forage, and food for malnourished and drought-prone areas of the world (Morris, 2008).
*Dolichos biflorus* was administered to colic patients who have stones formed due to deposits of calcium phosphates or oxalates. In Unani medicine, the concentrated water extract of kulthi seed and shalgam (*Brassica rapa*) seeds is given for destroying stones in the kidney. The aqueous extracts of seeds of *Dolichos biflorus* inhibited homogenous precipitation of calcium hydrogen phosphate dihydrate crystals (Peshin and Singla, 1994). Moreover, a lectin isolated from *D. biflorus* in combination with a collecting duct-specific antibody was used successfully in detecting collecting duct damage in rat and human kidney (Sourial *et al.*, 2010).

The diet enriched with seeds of *D. biflorus* has been reported to be effective in detoxification of paracetamol-induced hepatotoxicity. The administration of seeds of *D. biflorus* in diet for 28 days significantly recover the serum aminotransferase activities, bilirubin and blood urea levels in rats (Laskar *et al.*, 1998).

There has been report of *D. biflorus* to be safe and effective for weight management. The supplementation of methanolic extract of *D. biflorus* to high fat diet fed rats showed near to normal levels of the plasma and tissue total cholesterol, triglycerides, free fatty acids, phospholipids, plasma LDL cholesterol and decreased level of plasma HDL cholesterol, thus possesses significant hypolipidemic activity in high fat diet fed rats (Muthu *et al.*, 2005). This was further confirmed by another study in which alcoholic extract of seeds of *D. biflorus* in combination with alcoholic extract of leaves of *Piper betle* have been reported to cause significant reduction in body weight and basal metabolic index in obese subjects. Moreover, there has also been found to be significant increase in serum adiponectin concentration and significant decrease in serum ghrelin concentration in mouse pre-adipocyte fibroblasts cell culture (Sengupta *et al.*, 2012) due to this herbal combination.
Previous reports have also confirmed the ability of this plant in inhibiting 7,12-dimethylbenzanthracene (DMBA) - induced skin carcinogenic effects thus proving its potential as an anti-carcinogenic agent (Nanta and Kale, 2011). The plant is also given credit for its capacity to protect against benzo(a)pyrene [B(a)P] - induced forestomach papillomagenesis model in Swiss albino mice (Nanta and Kale, 2011). In skin and forestomach papillomagenesis model, extract of *D. biflorus* in diet significantly reduced the tumor incidence (up to 25%), tumor multiplicity (up to 59%) and tumor volume per mouse (Nanta and Kale, 2011). In the same study, *D. biflorus* extracts were also found to increase hepatic drug metabolizing enzymes, antioxidant enzymes and reduced glutathione content with concomitant decrease in lactate dehydrogenase (LDH) activity and peroxidative damage in carcinogen - induced damage.

The antioxidant potential of alcoholic extract of *D. biflorus* seeds was also assessed using different tests including total antioxidant activity, hydroxyl radical scavenging, superoxide radical scavenging, nitric oxide radical scavenging, hypochlorous acid scavenging, reducing power and lipid peroxidation. The extract was found to be potential source of natural antioxidant (Hazra *et al*., 2009). Moreover, the ethanolic seed extracts of *D. biflorus* have been reported to have potent antioxidant activity when incubated with nitric oxide and hydroxyl radicals (Ravishankar and Priya, 2012a). In an another experiment, the methanolic extract of seeds and sprouts of *D. biflorus* was investigated for antioxidant activity by adopting various *in vitro* models such as reducing power assay, DPPH assay, total phenolic assay and total antioxidant assays (Ramesh *et al*., 2011). The results showed higher antioxidant abilities in the sprouts as well as in seeds and thus proved to be a beneficial source of food with very high nutritional value.
Phenolic extracts from *D. biflorus* showed significant inhibitions of enzymes that are α-glucosidase, α-amylase and angiotensin-I converting enzyme thus showed to be associated with the protection against hyperglycemia and hypertension (Sreerama *et al*., 2012). An α-amylase inhibitor was purified using ion exchange chromatography on a CMC column from seed extract of *D. biflorus* and then investigated for its anti-diabetic effect against streptozotocin-nicotinamide-induced diabetic mice (Gupta *et al*., 2013). In the same study, *D. biflorus* α-amylase inhibitor inhibited both the mouse pancreatic and human salivary α-amylase in a non-competitive manner. This α-amylase inhibitor was further assessed for insecticidal activity and showed to inhibit the *Sitophilus oryzae* in a noncompetitive manner with significant mortality (Gupta *et al*., 2013). The bioefficacy of the seeds of *D. biflorus* has also been reported against diabetes in another study (Parthsarathi and Saxena, 2013).

An ethanolic extract of seeds of *D. biflorus* was also found to have effective anthelmintic activity when evaluated on adult Indian earthworm *Pheretima posthuma* (Philip *et al*., 2009). This plant was also evaluated for inhibition of microbial growth of selected gram – positive and gram – negative bacteria (Kawsar *et al*., 2008). The ethyl acetate extract of whole plant showed most significant antimicrobial activity against all gram – positive and gram – negative bacteria whereas dichloromethane extract showed moderate activities and the 1-butanol and aqueous extracts did not show any antimicrobial activities. In addition, the antifungal activities of all the extracts were also tested, using the food poisoning technique. The dichloromethane extract of the whole plant has been proved to be active against all fungi tested with a higher inhibition activity than standard nystatin.
Dolichos biflorus mixed with other herbal medicines has significant diuretic effect in male albino rats. The ethanolic extracts of seeds of *D. biflorus* and its combination along with ethanolic extract of *Cucumis melo* seeds were shown to have potent diuretic effect by modulating the urine volume, sodium, potassium, chloride and bicarbonate contents in urine (Ravishankar and Priya, 2012b).

Kawsar *et al.* (2009) have isolated two compounds namely, methyl ester of hexadecanoic- and ethyl ester of hexadecanoic acid mixture (I), and n-hexadecanoic acid (II) from the extract of aerial parts of *D. biflorus*. In addition, the fractionated crude extracts dichloromethane, ethyl acetate, 1-butanol and aqueous from aerial parts were tested for hemolytic activity. The extract of 1-butanol exhibited the most significant hemolytic activity.

SCAPE OF THE STUDY

Urolithiasis is a major healthcare problem worldwide because it appears to be increasing substantially in incidence and its incidence is very high in Asian countries. Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutics, natural products still remain as one of the best reservoirs of new structural types. Many plants have been in use since the ancient times for the treatment of various urolithiatic disorders. This presumptive evidence of efficacy renewed the interest in plant based medicines, especially in the treatment of urolithiasis. Instead of screening randomly selected synthesized chemicals against available targets, screening of traditionally claimed plants is more logical. Hence, there is a need to generate systematic scientific evidence for the activity and study the phytochemical aspects of the potential plants.
The central event in urolithiasis is hyperoxaluria and hypercalciuria with accumulation of large calcium oxalate monohydrate crystals. Ethylene glycol, is a potent lithogen, is known to cause hyperoxaluria and is normally used to induce urolithiasis in animal models. Therefore the initial phase of the present study was focused on determination of the best dose of ethylene glycol for induction of urolithiasis in rat model.

As medicinal plants, indeed, are gaining universal agreement as potential drugs, *B. ciliata* and *D. biflorus* was selected for the study that has several biological effects. The present study was designed to investigate antiurolithiatic property of *B. ciliata* and *D. biflorus in vivo* by biochemical and histopathological analysis.

Then the involvement of reactive oxygen species in pathophysiology of calcium oxalate stones was determined and the effect of medicinal plants on modulation of antioxidant markers was also evaluated.

Calcium oxalate is the primary constituent of the majority of stones formed in the urinary system of patients with urolithiasis. Therefore, *in vitro* anticrystallisation property of the selected plants for calcium oxalate was determined.

The protective effects of natural medicinal herbs are due to the presence of active components in them. Therefore, phytochemical studies of both the plants were also conducted and HPLC analysis was used to identify and quantify major active components present in the plant extracts.