Chapter 1

Introduction

Stone formation in the kidney is one of the oldest and most widespread diseases known to man. Urinary calculi have been found in the tombs of Egyptian mummies dating back to 4000 BC [1] and in the graves of North American Indians from 1500 - 1000 BC [2]. Unfortunately, kidney stones are one of the most common disorders of the urinary tract. Each year, almost 3 million patients visit health care providers and more than half a million go to emergency rooms for problems related to kidney stones. Urolithiasis refers to the condition of having calculi in the urinary tract (which also includes the kidneys), which may form or pass into the urinary bladder. Nephrolithiasis refers to the condition of having kidney stones. Ureterolithiasis is the condition of having a calculus in the ureter, the tube connecting the kidneys and the bladder.

Kidney stones are small, hard lumps like stones that form in the kidneys. Kidneys clean blood by removing waste products and water to produce urine. Normally, the waste products in urine are present in very small amounts so they stay dissolved in the fluid. But sometimes they can become solid and form crystals on the inner surface of kidneys. Over time, these crystals may combine to
form a small and hard stone. These stones so formed are referred to as kidney stones. Kidney stone is a common chronic disorder affecting 10 - 15% of the general population world wide. Calcium containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%). Calcium oxalate stones are found in two different varieties, calcium oxalate monohydrate (COM) or Whewellite, and calcium oxalate dihydrate (COD) or Weddellite. COM is the most stable form among all calcium stones. Its frequency of occurrence in the clinical stone is much greater than COD and it has a high affinity for tubular cells of renal tissue.

In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage [3]. Thus, the disease is as widespread as it is old, particularly in countries with dry and hot climate. "Stone belt" regions of the world are located in countries of Middle East, North Africa, the Mediterranean Regions, North Western state of India and Southern State of USA and areas around the great lakes. In India, the "stone belt" occupies parts of Maharashtra, Gujarat, Punjab, Haryana, Delhi and Rajasthan. In these regions, the disease is so prevalent that most of the members in a family suffer from kidney stones at some point in their lives. In United States, patients with kidney stones have been increasing over the past 30 years. In the late 1970s, less than 4 percent of the population had stone forming disease. By the early 1990s, the portion of the population with the disease had increased to more than 5 percent. Caucasians are more prone to develop kidney stones than African Americans.

Brimoneralization is the process by which living organisms produce minerals, often to harden or stiffen existing tissues. It is an extremely widespread phenomenon which is used by all six taxonomic kingdoms to form minerals [4].
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Five examples of biomineralization are:

1. The formation of siliceous spicules and frustules in sponges and diatoms, respectively.

2. The structure of skeletal spicules composed of amorphous calcium carbonate in some tunicates.

3. The secretion of the prism and nacre of some molluscan shells.

4. The development of skeletal spicules of sea urchin embryos.

5. The formation of bones and enamel of teeth in vertebrates.

The skeleton and teeth are normally the only mineralized tissues or organs in the human body. Such mineralization takes place under controlled mineral balance in the body. Any imbalance of minerals in the body may lead to some pathological condition. Such a condition where inappropriate biomineralization takes place in soft tissues, is called as ectopic calcifications. Ectopic calcifications are typically composed of calcium phosphate salts, including hydroxyapatite, but can also consist of calcium oxalates and octacalcium phosphate as seen in kidney stones [5]. In uremic patients, a systemic mineral imbalance is associated with widespread ectopic calcification, referred to as metastatic calcification [6]. In the absence of a systemic mineral imbalance, ectopic calcification is typically termed as dystrophic calcification, where blood levels of calcium are normal, and abnormalities or degeneration of tissues result in mineral deposition. Metastatic calcification can occur widely throughout the body but principally affects the interstitial tissues of the vasculature, kidneys, lungs, and gastric mucosa. For the later three, acid secretions or rapid changes in pH levels contribute to the formation of salts. Increased level of the salts in urine leads to the supersaturation of urine and increased salt, usually calcium, gives rise to hypercalcuria. Hypercalcuria is the most common metabolic abnormality observed in patients with
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nephrolithiasis. Hypercalcemia raises urine supersaturation with respect to the solid phases of calcium oxalate and calcium phosphate, leading to an enhanced probability for nucleation and growth of crystals into clinically significant stones [7].

About 90% of stones are 4 mm or less in size and usually pass spontaneously. However, 9% of stones, larger than 6 mm require some form of intervention. There are various measures which are used to encourage the passage of stones. These include increased hydration, medication for treating infection and reducing pain, and diuretics to encourage urine flow and prevent further stone formation. Cautions are usually taken in eating certain foods with high concentrations of oxalate which may precipitate and lead to acute renal failure in patients with chronic renal disease [8]. Surgery is necessary when pain is persistent and severe in renal failure and when there is a kidney infection. It is also advisable if the stone fails to pass or move after 30 days of other treatments. Finding a significant stone before it passes into the ureter allows physicians to fragment it surgically before it causes any severe problems. In most of these cases, non-invasive extracorporeal shock wave lithotripsy (ESWL) is used. Percutaneous nephrolithotomy (PCNL) or rarely open surgery may ultimately be necessary for large or complicated stones or stones which fail other less invasive attempts at treatment. A single retrospective study in the USA, at the Mayo Clinic, has suggested that lithotripsy may increase subsequent incidence of diabetes and hypertension [9]. More common complications related to ESWL are bleeding, pain related to passage of stone fragments, failure to fragment the stone and the possible requirement for additional or alternative interventions. Phytotherapy is the alternative to avoid complications arising due to medical treatments. It is well documented in Indian Ayurvedic system and relies on the use of plants, either whole or In the form of prepared extractus and essences.
For thousands of years, plants were a primary source of therapeutic medication for cultures all over the world. The European Scientific Cooperative on Phytotherapy (ESCOP), established in 1989, defines phytomedicines as "medicinal products containing as active ingredients only in plants, parts of plants or plant materials, or combinations thereof, whether in the crude or processed state. Plant materials include juices, gums, fixed oils, essential oils, and any other directly derived crude plant product. They do not include chemically defined isolated constituents, either alone or in combination with plant materials" (European Society Cooperative on Phytotherapy, n.d.). ESCOP supports clinical studies on the safety and efficacy of phytotherapeutic agents. The primary role of phytotherapy in the problem of kidney stones is to use plants with the following purposes:

- Antispasmodic and sedative plants to reduce pain caused by renal colic.
- Diuretic plants to increase urination, and permit the removal of the stones.
- Bactericide plants to prevent infections.
- Plants that prevent the formation of kidney stones.

The marketed composite herbal formulations, Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India) and Chandraprabha bati (Baidyanath, India) have been widely used clinically to dissolve urinary calculi in the kidney and urinary bladder [10].

Nearly, 15% of the population of northern India suffers from kidney stones [3]. However, fewer occurrences of urinary calculi are found in southern India, which may be due to regular dietary intake of tamarind [3]. Imli or Tamarindus indica also called Indian Date, is a large, broad-leaved, tropical tree found in India and large parts of Asia. It is routinely used by practitioners of Ayurvedic system of medicine in the treatment of urinary stone disease. Many plants have
been reported all over the world which are able to inhibit kidney stones. Interestingly, the consumption of some of these plant products is also very high in areas where the incidence of the disease is reported to be very low. *Tamarindus indica* belongs to the Dicotyledonous family Leguminosae. Another plant, *Terminalia arjuna*, belongs to family Combretaceae. It is a large tree distributed throughout India. It is a commonly occurring medicinal plant growing as a 20-30 m high tree. In India, plant is found in plenty throughout in sub Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi, Deccan region mainly along riverside, riverlets and ponds. The bark of *Terminalia arjuna* is known for treating heart diseases, coronary artery diseases and hypercholes- terolemia [11]. Aqueous extract of *Terminalia arjuna* bark is shown to protect the liver and kidney tissues against CDCl4- induced oxidative stress probably by increasing antioxidative defense activities. Its aqueous extract prevents carbon tetrachloride induced hepatic and renal disorders [12]. Keeping in mind the complications arising due to the surgical treatment of kidney stones and medicinal value of *Tamarindus indica* and *Terminalia arjuna* in this context, the scientific basis of their antilithiatic properties have been investigated using both *in vitro* and *in vivo* methods with the following objectives:

1. To study the effect of aqueous extract of *Tamarindus indica* and *Terminalia arjuna* on the extent of precipitation of calcium and phosphate (CaP) and calcium and oxalate (CaOx) using the homogenous system of *in vitro* mineral phase formation.

2. To study the effect of aqueous extract of *Tamarindus indica* and *Terminalia arjuna* on the growth and demineralization of the preformed mineral phase using the homogenous system *in vitro*.

3. To isolate, purify and characterize the new biologically active compounds from aqueous extract of *Tamarindus indica* and *Terminalia arjuna* which
have the ability to influence mineralization and demineralization reactions.

4. To investigate the effect of *Tamarindus indica* and *Terminalia arjuna* on experimentally induced hyperoxaluria and nephrocalcinosis in rats.