REVIEW OF LITERATURE
Kidney stone form when substances that normally dissolve in the urine like calcium oxalate, calcium phosphate, magnesium ammonium phosphate (struvite) and, occasionally, urate or cystine precipitate. Calculi vary in size and may be solitary or multiple. It may remain in the renal pelvis and damage or destroy renal parenchyma, or, they may enter the ureter. Large calculi in the kidneys cause pressure and necrosis. In certain locations, calculi cause obstruction, resulting in a condition called “hydronephrosis”. They may cause intractable pain and serious bleeding. Very small calculi (less than 4mm in size) may remain in the renal pelvis or pass down the ureter (Andrews et al., 1998).

Based on the location of the stones, the kidney stones are termed differently. Nephrolithiasis or renal calculi refer to the presence of stones in the kidney(s). Urolithiasis or urinary calculi refers to the presence of stones in the urinary tract. Ureterolithiasis is termed if the stones are found in the ureter. Stones that present in the urinary bladder are called as bladder stones.

**Prevalence**

Urinary calculi are prevalent in certain geographic areas (“Stone belt”) only because a hot climate promotes dehydration and concentrates calculus-forming substances or a person may acquire stones due to regional dietary habits (Andrews et al., 1998). According to Victor (1999) the incidences of stones among the stone formers are

a) Calcium oxalate-33%
b) Mixed calcium oxalate and phosphate-34%
c) Magnesium ammonium phosphate-15%
d) Uricacid-8%
e) Calcium phosphate-5% and 
f) Cystine-3%

Types of stones (Victor, 1999)

The chemical composition of stones depends on the chemical imbalance in the urine. The four most common types of stones are comprised of calcium, uric acid, struvite and cystine.

**Calcium stone:** Approximately 85% of the stones are composed predominantly of calcium compounds. Calcium stones are composed of calcium that is chemically bound to oxalate (calcium oxalate) or phosphate (calcium phosphate). Of this, calcium oxalate is more common. Calcium phosphate stones typically occur in patients with metabolic or hormonal disorders such as hyperparathyroidism and renal tubular acidosis. Increased intestinal absorption of calcium (absorptive hypercalciuria), excessive hormone levels (hyperparathyroidism), and renal calcium leak (kidney defect that causes excessive calcium to enter the urine) can also cause hypercalciuria.

**Uric acid stone:** Digestion produces uric acid. High acid level in the urine or higher excretion of acid leads to the formation of uric acid stones. Approximately 10% of patients with kidney stone disease develop this type of
stone. Genetics may play a role in the development of uric acid stones, which are more common in men.

**Struvite stones:** Also called an infection stone, develops when a urinary tract infection (e.g. cystitis) affects the chemical balance of the urine. Bacteria in the urinary tract release chemicals that neutralize acid in the urine that enables bacteria to grow more quickly and promotes struvite stone development. It is more common in women because they have urinary tract infections often. These stones usually develop as jagged structures called "stag horns" and can grow into bigger in size.

**Cystine stones:** Cystine is an amino acid in protein that does not dissolve well. Cystinuria, an inheritable, rare, and congenital disorder causes cystine stones that are difficult to treat and requires life-long therapy.

**Kidney stones and microbes**

Stones formed by infections are composed of magnesium ammonium phosphate and / or carbonate appetite (Rodman et al., 1996). Urea splitting bacteria like *Proteus* spp, *Staphylococci*, *Klebsiella* spp metabolize urea in urine resulting in the deposition of carbonate-appetite and struvite in a friable matrix, the so-called mixed phosphate stones.

Non-urea-producing bacteria such as *Escherichia coli* are the most common organisms found in association with secondarily infected stones. Holmgrem (1986) reported that of 796 consecutive patients at a stone clinic over a 10-year period, 52 (7%) had recurrent or chronic urinary tract infection (UTI) that
was considered to be of pathogenic importance in the stone formation. Urease producing microorganisms play a key role in the stone formation. Infection by *Proteus mirabilis* was reported to be associated with many types of stones (Neerhut and Griffith, 1982) whereas some of the *E. coli* isolates were associated with calcium oxalate type of stones.

**Present day medications and draw backs (Victor, 1999)**

Few medicines are currently being treated for the kidney stone patients that may decrease the chemical content in urine or in blood, thereby aid in preventing the formation of stones and they are

a. Orthophosphates – decrease urinary calcium, decrease oxalate crystal aggregation. Used to prevent calcium stones.

b. Alkaline phosphates – increase urinary inhibitors of stone formation in calcium stone formers.

c. Hydrochlorothiazide – prevents reabsorption of sodium and calcium in loop of Henle, which leads to increase in proximal tubular reabsorption of sodium, and calcium, thus decreasing total urinary calcium excretion.

d. Methylene blue – competes with calcium at matrix site to prevent calcium stone. Not proven completely.

f. Allopurinol – used in uric acid stones-alters pathway to uric acid by acting as a zanthine oxidase inhibitor. This prevents increase in the concentration of uric acid.

g. Penicillamine – utilizes to dissolve cystine stones by forming cystine penicillamine which is more soluble than cystine.

h. Hemiacidrin – efficacious in treatment of residual magnesium, ammonium phosphate stones. Used in irrigation techniques directly on to stone through bladder irrigation or nephrostomy tube irrigation.

Though these medicines are presently in practice there are many side effects. For example, thiazides, low dose of this diuretic causes potassium loss, which in turn reduces citrate levels and can increase the risk in the formation of calcium stones. It is also known that people with struvite stones, UTI, bleeding disorders, kidney damage, or peptic ulcers should not use diuretics or citrate products. Diarrhea is a possible side effect of phosphate drugs and phosphates increase the oxalate levels and decrease magnesium level. With cholestyramines, bloating and constipation are common side effects and may also contribute to calcium loss and osteoporosis. Deficiencies of vitamins A, D, E, and K can also result if it is taken for a long period.

Allopurinol can cause skin rashes, leucopenia (a reduction in the number of white blood cells), thrombocytopenia (a reduction in the number of platelets), diarrhea, headache, and fever. If organic acids are administered as
medication for struvite stones, the side effects can be severe. The organic acids reduce iron in the body, so anemia is a common problem. Other side effects include nausea and vomiting depression anxiety rash persistent headache, and rarely small blood clots in the legs (http://www.health-cares.net).

**Surgical treatments of stone disease**

The above medicines and drawbacks in the treatment of kidney stones drive the patient and the physician to undertake the inevitable option, the surgical removal of stones. Extra corporeal lithotripsy is the latest methodology currently in practice that uses shock waves to fragment the stones by laser beams and the fragmented stones are passed through patient’s excretory system (Victor, 1999).

The use of shockwave to disintegrate kidney stone had been thought of a God-send to thousands of people giving them rapid relief without surgery. But lithotripsy may also carry a significantly higher risk of diabetes (4 times) and high blood pressure (>50%) years later. It was also speculated that the shockwave can damage the pancreas and kidney organs that produce chemicals important for maintaining blood sugar and normal blood pressure (Krambeck, 2006).
Complementary and alternative medicine (CAM) in the treatment of kidney stones

The National Center for Complementary and Alternative Medicine (NCCAM) a component of the National Institute of Health, United States, classifies CAM therapies into five categories or domains. They are

- Alternative Medical System
- Mind –Body Interventions
- Biologically based Therapies
- Manipulative and Body based Methods and
- Energy Therapies

Complementary and alternative medicine is a generic term for a range of therapies and practices that include plant-based medicine. It is the most widely used form of CAM (Barnes, 2003). The general public has a more positive view of plant-based medicines compared with conventional medicine largely because they are perceived as being “natural” and “safe”. In US, 43% of the people used CAM at sometime during their life span (Eisenberg, 1998). Approximately one third of patients undergoing surgery admit in using herbal medicines for their illnesses (Kaye, 2000).

Drugs for kidney stone from Plants

There are several plants mentioned in Ayurveda, Siddha and other alternative medicines for the treatment of kidney stones. The formulations were single or a combination of more than than one plant. Most of the published results on
the efficacy of individual herbal formulations were on rats or mice or canine with chemically induced kidney stones.

According to Atmani et al., (2003), Herniaria hirsute has an impressive prophylactic effect on calcium oxalate stone in nephrolithic rats and the effect did not seem to be mediated by biochemical or diuretic changes. The popularity of Memordica charantia in various systems of traditional medicine for several ailments including kidney stones was known, but no report was available on the clinical use of M. charantia for kidney stone (Grover and Yadav, 2004). Increased urinary excretion of the crystalline constituents along with lowered magnesium excretion found in stone forming rats was partially reversed by Crataeva nurvala decoction treatment (Varalakshmi et al., 1990). The extracts of Dolichos biflorus showed activity almost equivalent to cystone, (a commercial drug), while Berginia ligulata showed less activity and the combination was not as active as the individual extract (Garimella et al., 2001).

An old Native American remedy for kidney stone, the combination of Hydrangea (Hydrangea arborescence), Gravel root (Eupatorium purpureum) and Marsh mallow root (Althea officinalis) was used in the treatment of kidney stones. Corn silk (Zea mays) and Dandelion (Taraxacum officinale), Parsley (Petroselinum sativum), Golden rod (Solidago canadensis), lemon juice (Citrus lemonum), black cherry juice and water melon juice were also attempted by several researchers (Bown, 1995).
Visnaga (*Ammi visnaga*), an effective muscle relaxant and has been used for centuries to alleviate the excruciating pain of kidney stone (Bown, 1995). Cystone, a commercial drug, contains Visnaga as one of the ingredients is marketed for the prevention of formation and elimination of kidney stones. But Borisov *et al.*, (2004) stated that, treatment by Prolit, a food additive helped to evacuate small concernsments from renal calices and ureters in eleven (27.5%) patients, while that of cystone was only 16%.

Kampou medicine, a traditional Japanese therapeutic system, originated in China was used to treat various diseases including kidney stone (calcium oxalate) for hundreds of years until it was superseded by Western medicine. Unfortunately no scientific validation was performed with this medicine (Koide *et al.*, 1995).

In most of the formulations mentioned in Ayurveda, Siddha and other forms of medicines from Temperate countries for the treatment of kidney stones contain fruits of *Tribulus terrestris* as one of the ingredient. It was considered as a powerful diuretic (Devarajan, 1997) and showed anti-microbial activity against urinary tract infecting bacteria (Abbasoglu and Tosun 1994). The phytochemistry of this plant was also studied in detail. Shi *et al.*, (1999) elucidated the flavonoids in fruit, root, leaf, stem, and aerial parts of *T. terrestris*. Tosun *et al.*, (1994) reported the isolation and identification of alkaloids in *T. terrestris* that grew in Turkey by TLC. The diosgenin content of roots, stems, and fruits of *T. terrestris*, and the saponin was analysed quantitatively by TLC (Wang and Lu, 1991). Sangeetha *et al.*, (1994)
described the effect of an aqueous extract of *T. terrestris* on the metabolism of oxalate in rats fed with sodium glycolate.

Bryophyllin - a, a new antibacterial substance from the leaf of *B. calycinum* (*B. pinnatum*) was studied by Mehta and Bhat, (1952) and this plant is believed to heal urinary calculi. The juice from this plant at 5% v/v was found to be bactericidal to a wide spectrum of gram positive and gram negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *S. faecalis*, *E. coli*, *Proteus spp.*, *Klebsiella spp.*, *Shigella spp.*, *Salmonella spp.*, *Serratia marcescens* and *Pseudomonas*. Anthocyanins a & b, biocytols, lactones and palmitic acid were isolated from fruits and root bark (Rastogi *et al.*, 1979 and Satyavati *et al.*, 1987). The flavonoid glucosides (Gaind and Gupta, 1971), alkanes, alkanols, triterpenes and sterols (Gaind and Gupta, 1972a), fumaric acid (Gaind and Gupta, 1972b) were also studied in *B. pinnatum*.

*Butea monosperma* (Lam.) Taub, (Flame of the forest) was one of the ingredients of indigenous drug for the treatment of ureteric calculus and gout. The diuretic action of this plant was already established, and the flowers yield butin and its 7-glucoside (isocoreopsin), 3-β-D-glucoside (monospermoside), butein (major glucoside), isomonospermoside, coreopsin, sulphurein, palasitrin etc. The chalcone and aurones present in them are responsible for the bright color of the flower (Asima and Satyesh, 1992).

Another plant that is believed to cure polyuria in human is *Euphorbia microphylla* (Asima and Satyesh, 1994). This plant has also been used in
tribal medicine for the treatment of gout, rheumatic disorders, and anti-inflammation. The chemical constituents of *Euphorbia microphylla* documented are Apigenine, Isovitexin Luteolin-3-o-glucoside, quercetin-3-o-galactoside and vitexin (Rastogi and Mehrotra, 1993).

Preliminary phytochemical tests *Bombax ceiba* L. showed the presence of glucosides and tannins in the root stem and leaf. The flowers and young unripened fruits of this plant are considered diuretic traditionally. The isolation of lupeol and β-sitosterol from the petroleum ether extract of the stem bark was elucidated. The flowers contained β-D-glucoside of β-sitosterol, free β-sitosterol, hentriacontane, hentriacontanol, traces of essential oil, kaempferol and quercetin and phenolic compounds. A highly branched polysaccharide that was isolated from flowers had continuous backbone of 4- β-linked D. galactopyranose with L-arabinopyranose (Satyavati et al., 1987).

**Validation of plant extracts in Animal models**

The efficacy of individual plant extracts on different types of stones was studied in animal models by many authors. For example, the effect of aqueous extract from *Herniaria hirsute* on adhesion of calcium oxalate monohydrate crystals (COM) to cultured renal cells of Medin Darby canine was studied by Atmani et al., 2004. They have also postulated a methodology by which *H. hirsute* extract was able to prevent and possibly eliminate pre existing kidney stones. Another theory was presented by Cao et al., 2004 on the inhibition of renal stone formation in rat kidney by the active constituents
of *Alisma orientalis*. He speculated that the extract could have down-regulate the bikunin mRNA expression, thereby decreasing the calcium oxalate formation.

Christina *et al.*, 2002, studied the decoction of *Rotula aquatica* on its antilithic effect in male Wistar rats. They opined that the ionic changes in both the urine and serum as attributed by the extract could have reduced the calcium and oxalate ion concentration in urine, confirming the stone inhibitory effect. They also presented histopathological studies of kidney tissue samples to substantiate their findings.

The effect of different extracts of *Alisma orientalis* on urinary calcium oxalate stone formation in rats was carried out by Cao *et al.*, (2003) and observed that ethyl acetate fraction of the extract of *A. orientalis* significantly inhibited urinary calcium oxalate stone formation.

Soundararajan *et al.*, (2006) administered aqueous suspension of *Aerva lanata* (2g/kg body wt/dose/day) for 28 days to CaOx urolithic rats and reduced the oxalate synthesizing enzymes and diminished the markers of crystal deposition in the kidney.

Vargas *et al.*, (1999) tested the aqueous extract of *Raphanus sativus* for its antiurolithiatic and diuretic activity, and the extract had significantly inhibited the formation of new stones in rats.

The increased excretion of calcium, oxalate, uric acid, phosphorus, and proteins in hyperoxaluric rat was reduced by the administration of Vediuppu
chunnam and *Aerva lanata* leaf extract as observed by Selvam et al., (2001). They observed that both the ingredient together increased the urinary volume and there by reducing the solubility of the product with calcium oxalate and uric acid.

The administration of the water extracts of *Costus spiralis* Roscoe. to rats with experimentally induced urolithiasis reduced the growth of urinary stones supporting the plant’s antiurolithiatic activity. The mechanism underlying this effect is unknown but presumed to be unrelated to increased diuresis and excretion of urinary salt forming stones (Viet et al., 1999).

The effect of oral administration of *Crataeva nurvala* bark decoction on calcium oxalate lithiasis has been studied in rats by Varalakshmi et al., in 1990. The increased urinary excretion of the crystalline constituents along with lowered magnesium excretion found in stone forming rats was partially reversed by this treatment.

Treatment with ethanolic extract of *Ammannia baccifera* had significantly reduced calcium and magnesium levels in the prophylactic group while it had reversed the levels of these ions to normal values in the curative group as researched by Prasad et al., in 1994 in male albino rats.

Hirayama et al., (1993) studied the inhibitory effect of *Desmodium styracifolium*-triterpenoid (Ds-t) on the formation of calcium oxalate renal stones that can be induced experimentally by ethylene glycol in rats. Ds-t inhibited the formation of calcium oxalate stones in rat kidneys by increasing
the output of urine, decreasing the excretion of calcium, and increasing the urinary excretion of citrate and so the authors opined Ds-t may be useful in preventing the recurrence of urinary calcium oxalate stones.

Different diet factors (standard, high glucidic and high protein) coupled with infusion of *Hemiaria hirsute* L. and *Agropyran repens* L. was studied by Grases *et al.*, (1995) using Wistar rats. The possible effect of the infusion of *A. repens* infusion could not be assigned to any positive effects on the main urolithiasis risk factors and the tentative antilithiasic effects of the infusion of *H. hirsuta* was clearly dependent on the diet.

An ethnolic extract of the fruits of *Tribulus terrestris* showed significant dose dependent protection against urinary stones that were induced using glass bead implantation in albino rats by Anand *et al.*, (1994). Campos and Schor (1999) studied the effect of the aqueous extract of *Phyllanthus niruri* L. on a model of CaOx crystal endocytosis by Madin - Darby canine kidney cells *in vitro* and found that the extract exhibited a potent and effective non-concentration dependent inhibitory effect on the calcium oxalate crystal internalization. Fractionation of the ethanol extract led to the finding that the activity was located in the 10% aqueous methanol fraction. The extract had also provided significant protection against deposition of calculogenic material around the glass bead and also protected leucocytosis and elevation in serum urea levels.

The anticrystallization mechanism by vitamin B₆ and banana stem was studied by Li *et al.*, in 1998, and he concluded that both vitamin B₆ and
banana stem extract may be useful agents in the treatment of patients with hypercaluric urolithiasis as deduced by morphometrical studies in China -1 mice. The effect of banana stem (Musaceae) extract on urinary risk factors in an animal model of hyperoxaluria was also studied by Poonguzhali and Chegu in 1994. The extract reduced urinary oxalate, glycolic and glyoxylic acid and phosphorus excretion in the hyperoxaluric rats, but appeared to have no effect on urinary calcium excretion.

**Validation studies in Human**

A conservative therapeutic protocol in the management of calcium oxalate stone formation in patients using cranberry juice was studied by McHarg *et al.*, 2003. The urinary biochemical and physiochemical risk factors were studied and found that there was a decrease in the relative super-saturation of calcium oxalate and phosphate excretion while citrate excretion was increased.

The plant, golden rod (*Solidago virgaurea* L.) being used as anti-inflammatory, anti-microbial, diuretic, antispasmodic and analgesic also prevented the formation of kidney stones in human successfully as observed by Melzig (2004).

The inhibitory effect on the growth of stone, anti-inflammation and diuretic by the extract of *Quercus stenophylla* in the treatment of urolithiasis in humans was studied by Rodriguez and Rull (1980). The plant also had antibacterial property.
Soxhlet extracts of seeds of *Dolichos biflorus* and rhizomes of *Bergenia ligulata* were tested for their *in vitro* antilithiatic/anticalcification activity by the homogenous precipitation method in humans and compared with Cystone (a marketed preparation). *D. biflorus* extracts showed activity almost equivalent to Cystone while *B. ligulata* show less activity; the combination was not as effective as the individual extracts as envisaged by Garimella *et al.*, 2001.

Reduction of calcium in the urine was also noticed with intake of *Phyllanthus niruri* in patients with hypercalciuria (Barros *et al.*, 2003). Regular self-administration of *Phyllanthus niruri* after extracorporeal shockwave lithotripsy (ESWL) for renal stones results in an increased stone free rate that appears statistically significant for lower calciceal location as envisaged by Micali *et al.*, 2006. Barros *et al.*, 2003 also suggested that the plant may interfere with the early stone formation and represent an alternative form of treatment and/or prevention of urolithiasis by inhibiting the endocytosis of calcium oxalate crystals in MDCK cells. However, larger trials are to be performed to determine the possible clinical consequences of urinary calcium reduction with *P. niruri* or its aqueous extract (Nishiur *et al.*, 2004).

**Urolithic agents in plants**

Though many plants and their extracts have been traditionally used in folk medicines there are certain plant materials that promote the stone formation. The efficacy of the fruit of *Randia echinocarpous* (Rubiaceae) has been shown as potent antiurolithic agent in folk medicine when tested against experimental rats by Solis and Gutierrez in 2002 and a significant increase in
diuretic activity and marked induction of the formation of stones was observed. Such information indicates that the continuing use of this plant in traditional folk medicine must be discouraged.

Urolithiasis was initiated in albino rats with implantation of zinc discs in urinary bladder (foreign body insertion method) and the effects of *Mimosa pudica* Linn. was studied by Joyamma et al., in 1990. *M. pudica* was not effective in either preventing stone deposition or dissolving preformed stones.

**Disadvantages or lacunae of plant drugs**

Many limitations regarding safety and efficacy of the plant-based drug preparations were overlooked as the active principles of herbal preparations were not defined, information on toxicity and adverse effects of these formulations were also lacking. Selection of plant materials based on quality, standardized methods of preparation, enforcement of regulation on appropriate labels to improve the quality and acceptability of herbal preparations as therapeutic agents were also absent in many of the plant based formulations (Kuruvilla, 2002).

Approximately 80% of India’s more than one billion population use traditional medicine with more than half a million Ayurvedic practitioners working in 2860 Ayurvedic hospitals and 22100 clinics (Gogtay et al., 2002). Its popularity in Western country has also increased (Hontz et al., 2004). But to fulfill the role in the Global Health Care system, the plants that can be used as an alternative medicine need to undergo several studies that include
standardization of drug, toxicology, efficacy through randomized trials, statistical validation and if possible drug-drug interactions.

**Heavy metals in plant-based medicines**

Herbs, minerals, and metals are used in Ayurvedic herbal medicine products (HMPs) (Gogtay et al., 2002). Since 1978 at least 55 cases of heavy metal intoxication associated with Ayurvedic HMPs in adults and children have been reported in the United States and abroad (Ernst 2002; Ibrahim 2002; Traub et al., 2002; Weid et al., 2003; Spriewald et al., 1999; McElvaine et al., 1990; Aslam et al., 1979; Ko, 1998).

Ayurvedic HMPs containing heavy metals are readily available in most of the South Asian grocery stores recommended for adults and children are relatively inexpensive. One of the five available Ayurvedic HMPs contained lead, mercury, and/or arsenic was above the regulatory standards. In England, 30% of Ayurvedic HMPs sampled contained lead, mercury and 41% contained arsenic (McElvaine et al., 1990). Traditional medicines from China (Ko, 1998), Malaysia (Ang et al., 2003) Mexico (Baer et al., 1998) Africa (Lekouch et al., 2001) and the Middle East (al Khayat et al., 1997) have also been shown to contain heavy metals. These heavy metals may result in insidious adverse effects on decreased childhood IQ (Canfield et al., 2003), increased blood pressure (Nash et al., 2003) and progression of chronic renal insufficiency (Canfield et al., 2003). Low level of lead exposure previously through to be acceptable but HMPs with relatively lower levels of lead (<100 μg/g) may be deleterious. Lead toxicity has been associated with use of
Ayurvedic HMPs (Hontz et al., 2004) causing fatal infant encephalopathy, congenital paralysis and sensor neural deafness (Tait et al., 2002), and development delay (Moore and Adler, 2000).

Ayurvedic theory attributes important therapeutic roles to metals such as mercury and lead (Gogtay et al., 2003; Acharya and Joshi, 1998). Ayurveda experts estimate that 35 to 40% of the approximately 6000 medicines in the Ayurvedic formulary intentionally contain at least 1 metal (Gogtay et al., 2003). Metal containing HMPs are purportedly “detoxified through multiple heating/cooling cycles and the addition of specific herbs (Acharya and Joshi, 1998). Whether the heavy metals in such sample were already present in raw plant materials (Dwivedi and Dey, 2002) intentionally or incidentally added in the manufacturing process is uncertain. The metal’s chemical forms which can impact bioavailability and toxicity, especially in the case of mercury is one the important limitations (Saper et al., 2004) in its use.

**Need for new herbal medicine for kidney stone**

A large number of individual herbal drugs and their combinations have been used in Siddha and Ayurveda systems of medicine from time immemorial against urinary stone disease in humans (Nambiraj et al., 2002). 62 plants, 9 animal based remedies, 1 mineral preparation and 4 medicinal substances of different uncertain origin had already been in use since the classical period for kidney stone and several are still in use today in traditional medicine (Lev and Dolev, 2002). None of these plants or the compounds or the mineral substances was tested for clinical validation against the stone formation and
dissolution. This was also true with many Siddha formulations used against this disorder for centuries. For example, Bhoger and Agastyan Pa U have described drugs formulations for kidney stone and many of these formulations contained mercury, sulphur, arsenic substances, gems, salts, shells and several other organic and inorganic ingredients (Veeraraghava Iyer, 1932; Subramanian and Madhavan, 1984).

**Standardization of plant formulation used as drug**

One of the great myths about natural medicines is that they are not scientific. For most common illness, there is greater support in the medical literature for a natural approach then there is for drugs or surgery. For the betterment of safety, efficacy and validation of natural plant products, standardization through screening of natural products, evaluation of natural products and formulation of natural products for drug development is necessary (Fong, 2002).

Poly herbal medicine is making dramatic comeback and increasing number of patients are visiting alternative medicine clinics due to the alarming side effects of synthetic medicines (Singh, 2005). But plant drugs that have the potential to be candidates for approval for marketing as preparation drugs in Western countries need to undergo several safety and efficacy testing. World Health Organization (in 1993 and 2000) and USFDA (in 2004) released guidelines on assessing and evaluating the quality of plants that were promised as drugs. Scientific information on the safety, efficacy, and quality control/quality assurance of widely used 28 medicinal plants were provided by
WHO that might help regulatory authorities, practitioners of orthodox and of traditional medicine, pharmacists, other health professionals, manufacturers of herbal products, and research scientists. The first part of monograph of each plant consisted of pharmacopoeia summaries for quality assurance: botanical features, distribution, identity tests, purity requirements, chemical assays, and active or major chemical constituents. The second part summarized the clinical applications, pharmacology, contraindications, warnings, precautions, potential adverse reactions, and posology.

USFDA has released guidance for industrial botanical products that need to be marketed as drug in USA. The basic quality control tests required for the drug and they stressed the three phases of clinical studies of the drug. They have treated both the botanical products and the biomedical drugs in the same category. Though these tests in practice cannot be carried out for each of the formulation depicted in ancient literature or practice (as in folk medicine), many of the concerns in making quality product from plant-based medicines can be achieved if there is emphasis on the following:

- Proper methodology of identification of the plants used in traditional therapy in various preparations
- Availability of the raw material in a sustainable manner through appropriate conservation strategies
- Defining the quality parameters for maintaining the purity of the plant material. This may include basic finger print phytochemicals profile of
the plant, testing of heavy metal and pesticide contents, microbial limits, impurities, ash content etc.

- Establishing extraction and processing technologies under controlled conditions to avoid degradation of active principle contained in them and for manufacturing of drugs under GMP.

- Carryout biological assays and clinical trials to establish therapeutic efficacy for the identified indication through GCP.

- Minimal toxicology studies to ensure safety

- Standardization of molecules isolated from plant material or their chemically converted products for drug development modalities

- Molecular targets and natural products

Inspired to develop a product on the above lines, a five-plant extract was formulated (DCBT5678) by Dalmia Centre for Research and Development (DCRD), Coimbatore, India that had shown promising results in few volunteers with kidney stones. A limited toxicology study was also carried out in mice and the LD$_{50}$ values were >5000mg/kg body weight for both males and females (A scanned copy of the LD$_{50}$ analysis by Shriram Insitute of Industrial Research, New Delhi is also attached herewith).
TOXICOLOGY STUDY REPORT

JOB ORDER NO. : 910-160-0843-00843

SPONSOR : DALMIA CENTER FOR RESEARCH AND DEVELOPMENT
9/38 - C, SIRUVANI MAIN ROAD,
KALAMPALAYAM,
COIMBATORE – 641010

SUBJECT : ACUTE ORAL LD$_{50}$ RAT

PRODUCT : HERBAL POWDER DCBT 5678

MATERIAL DESCRIPTION : GREEN POWDER

BATCH NO. : 13101999 / 001 Dt. : 13.10.99

RESULTS : ACUTE ORAL LD$_{50}$ RAT
Male : > 5000 mg/kg B.wt.
Female : > 5000 mg/kg B.wt.

Total No. of Pages : 11

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
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Relevance of biomarkers and chromatography

For the development of the quality and standardization parameters of herbs/herbal drug, development of methods that are reliable and reproducible is necessary. Screening of phytochemicals from the extracts of plants using chromatographic techniques is an important step in determining the quality of a raw material or its extract in a drug formulation. The chromatographic techniques include separation, identification and quantification of active ingredients or biologically active markers or plain compounds alone or a profile of all the above. Several instruments are employed in the separation of the phytochemicals and they include High Performance Liquid Chromatography, Gas Liquid Chromatography, High Performance Thin Layer chromatography (Ong, 2004).

Presently standardization is regulatory and mandatory for herbal products and they are being made based on their active constituents or biologically active markers. The potential therapeutic effects of the phytochemicals are being ascertained with use of respective biomarkers and finger printing behavior of a plant/polyherbal based formulations from its/their extracts (Singh, 2005 and Hamischfeger, 2005).

Marker compounds are characteristic phytochemicals found in a plant. They are often chosen to represent the standard for a standardized plant/extract; serve a useful function in terms of quality for the purposes of identification and ensuring appropriate drying, handling, and extraction of the herbal starting
material, and to achieve a consistent level of a marker compound (or compounds).

Chromatographic techniques for the chemical standardization of marker compounds or active ingredients in botanicals and herbal preparations are well established. Phytochemicals are important for a given therapeutic effect of an herbal/herbal extract. Marker compounds are not necessarily active compounds as in Gingo biloba and St.John’s Wort (Butterweck et al., 2000 & 2003). If a marker compound is chosen that has no known useful pharmacological activity, it should not be optimized in the extract at the expense of other phytochemicals (Bone, 2004).

Puri et al., 2006, employed HPLC attached with photodiode array detection in the isolation of naphthodianthrones from St.John’s Wort. They have also purified this marker compound to 98% using preparative HPLC.

Fritillaria isosteroidal alkaloids in herbal and biological samples by HPLC evaporative light scattering detection method was found to be the most simple, selective and sensitive assay in the development of quality control method for the analysis of the principles of this herb (Lin et al., 2001).

Swertiamarin, a secoiridoid glycoside and one of the active compound from the whole plant of Enicostemma littorale was separated, purified, and quantified by HPTLC (Vishwakarma et al., 2004).

Eugenol and gallic acid from the flower buds of Syzygium aromaticum (L.) Merr&Perry have been shown to give interesting biological activites and
hence serve as biomarkers. These biomarkers were separated and quantified using HPTLC methods (Pathak et al., 2004). Pathania et al., (1996) developed precise HPTLC method for the quantification of curcuminoids, the bioactive compounds of *Curcuma longa*. The three compounds of curcuminoids viz. curcumin, demethoxy curcumin and bisdemethoxy curcumin were also separated by Gupta et al., (1999) using HPTLC.

A simple HPTLC method was developed for the simultaneous determination of the pharmaceutically important quinazoline alkaloid, vasicine and vasicinone in *Adhatoda vasica* by Das et al., (2005).

The extraction of saponins of few plants and their separation using HPTLC and HPLC were standardized and quantified using respective standards by Oleszek et al., (2004) and Kostova and Dinchev (2006).

Using HPTLC and HPLC, four marker constituents from *Cissus quadrangularis* Linn. were quantitatively determined, collected from five different geographical zones of India (Metha et al., 2001).

In quality control and stability testing of herbs/herbal medicinal products, finger print chromatograms are used as powerful tools to evaluate and compare the composition of compounds as bioactive compounds (Koll et al., 2003). Few examples were; aciteoside in the leaves of *Plantago palmate*, (Biringanine et al., 2006), *Ligusticum chuornxiong* (Van et al., 2005), Saffron (Li et al., 1999), *Tripterygium wilfordii* (Li and Wang, 2005), *Salvia miltiorrhiza* (Hu et al., 2006) and *Glycyrrhiza* spp (Cuia et al., 2005).
Bergamottin, a furanocoumarin of grape juice, capsaicin from chill peppers, glabridin, an isoflavan from licorice root, isothiocyanates found in all cruciferous vegetables, oleuropein rich in olive oil, diallysulfone in garlic resveratrol in red wine were used as biomarkers and were isolated and identified by HPLC and HPTLC (Zhou et al., 2004).

**Investigation of stones in Human**

The following are some of the laboratory guidelines in investigating the stone formation in patients. In urine analysis, hematuria patients have proteins in their urine, infection by urease splitting organism can lead to increased pH (>7.6) and a pH of lower than 5.5, favor uric and cystine stone formation. The cystine crystals are hexagonal in shape and the uric acid stone are amber brown glass silver type as observed under microscope. Nitroprusside test of urine can be used to detect cystinuria (cystine stones).

The following are the quantitative 24 hour urine analysis and the supersaturation of these compounds can occur above these levels.

1. Calcium – normal 300 mg
2. Uric acid – normal 600 mg
3. Cystine – normal 50 to 180 mg

Imaging studies on the kidney stones can give information on the size, position and some times the nature of the stones also. Plain abdominal film on the kidney, ureters and bladder (KUB) can be used to detect radiopaque stones. Intravenous pyelogram (IVP) shows the degree of obstruction and
general architecture of urinary tract. Retrograde ureterogram and pyelogram can be used when there is non visualization or poor visualization of collecting system after IVP. Other recent developments in instrument technology have resulted in the increasing roles of stone diagnosis as in the case of ultrasonography, computerized axial tomography (CAT), radionuclide renal scanning, and percutaneous nephrography.

In blood chemistry investigations of the patients, serum calcium, uric acid, and phosphorus are important to assess the nature of the stones formed in kidneys. Determination of serum protein is also important as half of calcium in body is unionized and thus bound to protein. Low protein levels would mean more unbound calcium. Serum chloride may help in diagnosis of hypercalcemia and elevation above 102mEq/l in hyperparathyroidism has been reported as a reliable method to support suspected hypercalcemia. Radio immuno assay of parathyroid hormone elevation is also used to diagnose hypercalcemia secondary to hyperparathyroidism. The following are the normal range of concentrations of some of the blood ingredients.
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood urea</strong></td>
<td>7 to 18mg/dl</td>
</tr>
<tr>
<td><strong>Serum creatinine</strong></td>
<td>0.7 to 1.5mg/dl</td>
</tr>
<tr>
<td><strong>Serum calcium</strong></td>
<td>0.4 to 8.4mg/dl</td>
</tr>
<tr>
<td><strong>Serum phosphate</strong></td>
<td>2.4 to 4.8mg/dl</td>
</tr>
<tr>
<td><strong>Serum uric acid</strong></td>
<td>3.6 to 8.2mg/dl</td>
</tr>
<tr>
<td><strong>Urine calcium</strong></td>
<td>50 to 150mg/day</td>
</tr>
<tr>
<td><strong>Urine phosphate</strong></td>
<td>0.4 to 1.3mg/day</td>
</tr>
<tr>
<td><strong>Urine Uric acid</strong></td>
<td>800 to 1000mg/day</td>
</tr>
</tbody>
</table>

Diagnostic imaging is essential to confirm or exclude the presence of urinary calculi. Several imaging modalities are available, and each has advantages and limitations (Andrew and Chandru, 2001) as below:
<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra sonography</td>
<td>Accessible good for diagnosing stones. Requires no ionizing radiation</td>
<td>Poor visualization of ureteral stones</td>
</tr>
<tr>
<td>Plain radiography</td>
<td>Accessible and inexpensive</td>
<td>Poor visualization of stones in middle section of ureter, phleboliths,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>radiolucent calculi, extra urinary calcifications and non genitourinary</td>
</tr>
<tr>
<td>Intravenous Pyelography</td>
<td>Accessible, Provides information on anatomy and functioning of both kidneys</td>
<td>Variable-quality imaging requires bowel preparation and use of contrast</td>
</tr>
<tr>
<td></td>
<td></td>
<td>media. Poor visualization of non-genitourinary condition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delayed images required in high grade obstruction.</td>
</tr>
<tr>
<td>Noncontract helical computed</td>
<td>Most sensitive and specific radio logic test (i.e. facilitates fast,</td>
<td>Less accessible and relatively expensive. No direct measure of renal</td>
</tr>
<tr>
<td>tomography</td>
<td>definitive diagnosis) Indirect signs of the degree of obstruction of</td>
<td>function</td>
</tr>
<tr>
<td></td>
<td>obstruction. Provides information on nongenitourinary conditions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some of the present treatment modalities for renal and urtereral calculi, their advantages and limitations is given below (Andrew and Chandru, 2001)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Indications</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracorporeal shockwave lithotripsy</td>
<td>Radiolucent calculi Renal stones 7.2cm. Ureteral stones 7.1cm</td>
<td>Minimally invasive out patient procedure</td>
<td>Requires spontaneous passage of fragments. Less effective in patients with orib obesity or hard stones.</td>
<td>Ureteral obstruction by stone fragments. Perinephric nematoma</td>
</tr>
<tr>
<td>Ureteroscopy</td>
<td>Ureteral stones</td>
<td>Definitive out patient procedure</td>
<td>Invasive commonly requires post operative ureteral stent</td>
<td>Ureteral stricture or injury</td>
</tr>
<tr>
<td>Renal stones 7.2 cm</td>
<td></td>
<td>Definitive out patient procedure</td>
<td>May be difficult to clear fragments. Commonly requires post operative ureteral stent</td>
<td>Ureteral stricture or injury</td>
</tr>
<tr>
<td>Ureteronephrolithotomy</td>
<td>Renal stones &gt;2 cm proximal ureteral stones &gt;1cm</td>
<td>Definitive</td>
<td>Invasive</td>
<td>Bleeding injury to collecting system injury to adjacent structures.</td>
</tr>
</tbody>
</table>
Kidney stones are detected when the patient is in severe pain. The following are some of the symptoms commonly associated with the stones depending on its locations (Andrew and Chandru, 2001).

<table>
<thead>
<tr>
<th>Stone location</th>
<th>Common symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Vague flank pain, hematuria</td>
</tr>
<tr>
<td>Proximal ureter</td>
<td>Renal colic flank pain, upper abdominal pain.</td>
</tr>
<tr>
<td>Middle section of Ureter</td>
<td>Renal colic, anterior abdominal pain, flank pain.</td>
</tr>
<tr>
<td>Distal ureter</td>
<td>Renal colic, dysuria, increased urinary frequency, anterior abdominal pain, flank pain.</td>
</tr>
</tbody>
</table>

Based on the location of the stones and their size, Morse and Resnick, (1991) calculated the probability of passage of stones. But Cook et al., (1992) opined that approximately 50 percent of asymptomatic renal calculi become symptomatic within five years.
<table>
<thead>
<tr>
<th>Stone location and size</th>
<th>Probability of passage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal ureter</strong></td>
<td></td>
</tr>
<tr>
<td>&gt;5mm</td>
<td>0</td>
</tr>
<tr>
<td>5mm</td>
<td>57</td>
</tr>
<tr>
<td>&lt;5mm</td>
<td>53</td>
</tr>
<tr>
<td><strong>Middle section of ureter</strong></td>
<td></td>
</tr>
<tr>
<td>&gt;5mm</td>
<td>0</td>
</tr>
<tr>
<td>5mm</td>
<td>20</td>
</tr>
<tr>
<td>&lt;5mm</td>
<td>38</td>
</tr>
<tr>
<td><strong>Distal ureter</strong></td>
<td></td>
</tr>
<tr>
<td>&gt;5mm</td>
<td>25</td>
</tr>
<tr>
<td>5mm</td>
<td>45</td>
</tr>
<tr>
<td>&lt;5mm</td>
<td>74</td>
</tr>
</tbody>
</table>

The following flow diagram indicate the diagnostic methodology adopted by the physicians in the treatment of kidney stones (Reich and Hanno, 1997)
Suspected renal colic

Patient with abdominal pain

History and physical examination

Renal colic suspected

Diagnostic imaging

Patient is pregnant, or Patients chole cystitis or Gynecologic process is suspected

Ultrasound examination

Stone detected

Patient has history of radiopaque calculi

Plain film radiography

Stone not detected

Intravenous pyelography if CT is not available.

All other patients

Intravenous pyelography if CT is not available.

Non contrast helical CT

Stone detected

Stone not detected

Clinical suspicion of urolithiasis