3. LITERATURE REVIEW

3.1 Macrotyloma uniflorum (Lam.) Verde. (Varier, 1996)

(=Dolichos biflorus Linn.)

**Family** : Fabaceae

Eng : Horse gram

Hin : Kulthi

Kan : Huruli

Mal : Mutira

San : Kulatthah

Tam : Kollu

Tel : Ulavalu

**Distribution** : Cultivated throughout India

**The Plant**: A much branched suberect or trailing annual, leaves trifoliolate, leaflets lanceolate or oblong, entire, membranous; flowers yellow, 1-3 in the axils of the leaves, one at the base and two placed laterally; fruits sword-shaped, compressed pods about 5 cm long, tipped with the persistent style, seeds 5-6 compressed, reniform, usually reddish brown.

**Parts used** : Seeds.

**Properties and uses** : The seeds are bitter, acrid, thermogenic, anthelmintic, astringent, Diaphoretic, diuretic, emmenagogue, expectorant, febrifuge, ophthalmic and tonic. They are useful in haemorrhoids, tumours, bronchitis, cardiopathy, nephrolithiasis, urolithiasis, splenomegaly, asthma,
strangury, hiccough, ophthalmopathy, verminosis and vitiated conditions of vata.

The natural product *Cucumis melo* (non-edible portion), *Eriobotrya japonica* (leaves), *Macrotyloma uniflorum* (seeds), *Pedalium murex* (fruit) and *Zea mays* (silk) were tested for their inhibitory effects on the growth of carbonate apatite (CA) crystals. The most efficient growth retarding effect was exhibited by *Macrotyloma uniflorum* (Ahmed et al., 1987).

The alterations in the content of free sugar residues were studied in the samples of the experimentally induced rat breast carcinoma in the course of sacromatoid reversal. Glycosylation alteration were determined by immunohistochemical methods detecting the binding sites for lectins from *Arachis hypogaea*, *Canavalia ensiformis*, *Dolichos biflorus*, *Triticum Vulgaris* and *Ulex europaeus*. The results indicate that the content of most studied saccharides decreases in the course of sarcomatoid development (Kolar et al., 1990).

Six lectins from *Arachis hypogaea*, *canavalia ensiformis*, *Dolichos biflorus*, *Ricinus communis tox*, *Triticum vulgare* and *Ulex europaeus* have been used for demonstration of glycosylative changes in the hyperplastic, adenomatous and carcinomatous mucosa of large bowel. The results obtained were rather heterogenous with regard to lectin binding capacity in the single cases but they proved a certain uniformity of changes inside every tested group (Kolar et al., 1989).

The activity of superoxide dismutase (E.C.1.15.1.1) investigated in germinated seedlings of horse gram (*Dolichos biflorus*), green gram (*Phaseolus radiatus*) and lentils (*Lens esculentus*). The occurrence of the
enzyme in these plant fruits was reported. A partial purification, metal specificity and the nature and number of iso-enzyme forms of the enzyme, in the mitrochondria and cytosol of horse-gram germinating seedlings showed a higher degree of activity than the two other seedlings (Vidyarthi et al., 1993).

A significant role of the seeds of *Dolichos biflorus* was observed against some test organisms using different fractions of defatted methanolic extract of the seeds of *Dolichos biflorus*. A few active principles were also isolated (Bask et al., 1992).

The seeds of nine leguminosae plants including *Dolichos biflorus* were investigated for their alcohol constituents. This led to the isolation and identification of 25 triterpene alcohols including one new compound, 24z-ethylidene -24- dihydroparkeol, and three series of skeletal double bond isomers of lanostane- type compounds, 9 beta, 19-cyclolanostanes, and delta 8- and delta 9(11) – lanostenes. The occurrence of eight 3-oxo steroids was also demonstrated, beta – Amyrin was the principal triterpene alcohol found in five of the leguminosae seeds investigated (Akihisa et al., 1994).

**In vitro** effect of *Dolichos biflorus* seeds on crystallization of calcium phosphate, showed a marked decrease in anticalcifying activity with post-harvest storage seed for 6 months. The inhibitors of crystallization present in seed extract of *Dolichos biflorus* were water soluble, heat stable, polar, non-tannin and non protein in nature. There were two or more different inhibitors of calcium phosphate precipitation since both the dialysate and the dialysed fractions contained inhibitory activity, though more in the dialysed fractions . The anticalcifying activity was lost completely with activated charcoal which was not recovered or eluted by any solvent tried (Peshin et al., 1994).
Defatted seeds of *Dolichos biflorus* was macerated with 10 percent NaCl solution and subjected to Chromatography. Various sugars viz. D-glucose, and D-galactose, L-Rhamnose, D-Arabinose and L-ascorhic acid were identified and also aminoacids viz., glycine, alanine cysteine, serine and aspartic acid were identified using modern techniques (Dubey *et al.*, 1998).

Antihepatoxic effect of *Dolichos biflorus* seed in rats revealed the recovery of paracetamol - intoxicated rats, as shown by decrease in serum aminotransferase activities, bilirubin and blood urea levels, was significantly improved in Dolichos biflorus seeds treated rats 10g / Kg.p.o., providing indications of an antihepatotoxic effect of the plant seed (Laskar *et al.*, 1998).

Soxhlet extracts of seeds of *Dolichos biflorus* and rhizomes of *Beregenia ligulata* were tested for their *in vitro* antilithiatic / anticalcification activity by the homogenous precipitation method. The extracts were compared with aqueous extract of cystone (a marketed herbal preparation) for their activities. Extracts of *Dolichos biflorus* showed activity almost equivalent to cystone while *Bergenia ligulata* showed less activity and the combination was not as active as the individual extracts (Garimella *et al.*, 2001).

Lead induced changes in antioxidant metabolism of horsegram *Macrotyloma uniflorum* (Lam.) Verdc. and bengalgram (*Cicer arietumin L*) suggest that Pb toxicity causes oxidative stress in plants and the antioxidative enzymes SOD, CAT, POD, GR, GST could play a pivotal role against oxidative injury (Reddy *et al.*, 2005).

Proteinase inhibitor isolated from horsegram (*Dolichos biflorus* or *Macrotyloma uniflorum*) inhibited specifically the enzymes trypsin and
chymotrypsin. The inhibitor contained seven disulfide linkages and was free from thiol groups. The disulfide linkages play a predominant role in maintaining the three-dimensional structure of the inhibitor (Ramasarma et al., 1995).

The extracts of edible plant *Trianthema monogyna* and the pulse *Macrotyloma uniflorum* were found to be effective in the inhibition of calcium oxalate crystallization. Various physicochemical techniques, viz. conductometric and nephelometric titrations, UV and IR spectroscopy and potential measurements in the absence and presence of these extracts were employed. They reduced the particle size considerably as revealed by microphotographs. Considerable solubility was also observed by flame photometric studies (Ishwar Das et al., 2005).

3.2 *Plectranthus amboinicus* (Lour.) Spreng. (Warrier et al., 1997).

(=Coleus amboinicus Lour.)

(C. aromaticus Benth.)

**Family** : Lamiaceae

Eng : Country borage, Indian borage

Hin : Patta ajavayin, Patharcur

Kan : Karpurahalli

Mal : Kannikkurkka, Kannikkurkka, Panikkurkka

San : Karpuravalli, Sugandhavalakam

Tam : Karpuravalli

Tel : Sugandhavalkam

**Distribution** : Throughout India, cultivated in gardens
The plant: A large succulent aromatic perennial herb with hispidly villous or tomentose fleshy, stem; leaves simple, opposite, broadly ovate, crenate, fleshy, very aromatic; flowers pale purplish in dense whorls at distant intervals in a long slender raceme; fruits orbicular or ovoid, nutlets smooth.

Parts used: leaves

Properties and uses: The leaves are bitter, acrid, thermogenic, aromatic, anodyne, appetizing, digestive, carminative, stomachic anthelmintic, constipating deodorant, expectorant, lithontriptic diuretic and liver tonic. They are useful in cephalalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea and cholera especially in children, halitosis, convulsions, epilepsy, cough, chronic asthma, hiccough, bronchitis, renal and vesical calculi, strangury, hepatopathy and malarial fever.

A leaf extract of *Plectranthus amboinicus* revealed the presence of hexacosanol, beta-sitosterol, oleanolic acid, betulin and other triterpenoids. The monoterpene hydrocarbons form 53% of oil, obtained from stem and leaves by hydrodistillation. The major components of the remaining portion of the oil are delta-3 carene (16.3 percent), gamma-terpinene (11.9 percent), camphor (12.3 percent) carvacrol (13.4 percent), (Vera et al., 1993).

Volatile oil isolated from the leaves of *Coleus amboinicus* exhibited varying degree of antimicrobial activity against a number of pathogenic and non pathogenic fungi and bacteria (Alankara rao et al., 1991).

The volatile leaf oil isolated from *Coleus amboinicus* was subjected to (in vitro) antimicrobial screening against pathogenic and non pathogenic fungi and gram positive and gram negative bacteria in pure form at various
dilutions. The results are compared with known fungicides and bactericides (Abstr. No. BT-01) (Rao et al., 2005).

The origin, distribution, medicinal uses and phytochemical work of Coleus amboinicus are reviewed. Because of its aromatic nature of leaves it is often used as a substitute for borage or thyme (Morton 1992).

Cytological effects of leaf extract of Coleus amboinicus on allium cepa and Vicia-faba root tip cells were examined. Two parameters mitotic index and cytological observations in various phases of mitosis were investigated. In all the treatment there was a significant decrease in mitotic index (Sumathi et al., 1995).

Volatile compounds of Plectranthus amboinicus leaves obtained by various methods were compared. The yield obtained from steam distillation was 0.55 percent, from hexane extraction 6.52 percent, and by supercritical CO2 extraction 1.40 percent. Twenty six components were identified and eleven for the first time (Pino et al., 1996).

The essential oils of Coleus aromatics (syn. Coleus amboinicus) distilled in different seasons and the oils were found to contain carvacrol, P-cymene and gamma –terpinene as major constituents. The oil produced in September was found to contain higher contents of carvacrol and beta-caryophyllene and oxygenated constituents than the oil produced in May (Mallavarapu et al., 1999).

The air dried leaves of Coleus amboinicus afforded three flavones: salvigenin cirsimaritin and chrysoeriol by silica gel chromatography.
Antimicrobial assay on salvigenin and crsimaritin showed low antimicrobial activities against the microorganisms tested (Ragasa et al., 1999).

The chemical investigations of the leaf essential oil of *Coleus amboinicus* indicated the presence of six components, accounting of 97 percent of the total oil. The major component was thymol (94.3 percent), followed by carvacrol (1.2 percent), 1,8-cineole (0.8 percent), P-cymene (0.3 percent), spathulenol (0.2 percent) terpinen-4-ol (0.2 percent) and an unidentified component (1.4 percent). The oil was insecticidal to white termites and also more active than the synthetic insecticides (Singh et al., 2002).

The aqueous extracts of 64 plants species, listed as animal- or insect-bite antidotes in old Thai drug recipes were screened for their activity against fibroblast cell lysis after Hoterometrus laoticus scorpion venom treatment. The venom was preincubated with plant extract for 30 min and further treated to confluent fibroblast cells for 30 min. More than 40% efficiency (test/control) was obtained from cell treatment with venom preincubated with 12 extracts including *Plectranthus amboinicus* (Lour.) spreng. (*Labiatae*), indicating that they had a tendency to be scorpion venom antidotes. However, only Andrographis paniculata and Barringtonia acutangula extracts provided around 50% viable cells from extract treatment without venom preincubation. These two plant extracts are expected to be scorpion venom antidotes with low cytotoxicity (Uawonggul et al., 2005).

The Neuropharmacological profile of *Plectranthus amboinicus* (Lour.) spreng. (*Indian borage*) was studied (Perez saad et al., 2003).
Antiepileptic effect of *Plectranthus amboinicus* (Lour.) spreng., was studied (Buznego and Perez-Saad 1999).

A screening for genotoxic activity was carried out in aqueous and alcoholic extracts prepared from 13 medicinal plants widely used as folk medicine in Cuba: including *Plectranthus amboinicaus* (Lour.) spreng. The result revealed, the aqueous extract of the plant *Momordica charantia* alone possesses a significant genotoxic effect (Ramos Ruiz *et al.*, 1996).

There are 49 plant species used in coastal area of Bahia state, Brazil to treat skin ulceration caused by *Leishmania* species. Among the principal plants, alfavaca-grossa (*Plectranthus amboinicus, Lamiaceae*) was used by 33% of the population for the treatment of cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis* (Franca *et al.*, 1996).

The volatile constituents of the whole herb of *Coleus amboininus* (Lour.) Spreng., (Labiatae) were analyzed and ten compounds were identified among which carvacrol (50.7 percent) Gamma-caryophyllene (13.1 percent) and patchoulane (8.7 percent) were dominant (Mangathayaru *et al.*, 2005).

The medical plants data were collected from local medical practitioners and priests in Alagarkovil MPCA of Dindigul Anna district. The *Plectranthus amboinicus* for scabies was reported as one among them (Viswanathan 1995).

The fresh juice of the leaves of *Plectranthus amboinicus* was reported to possesses antilithiotic activity against ethylene glycol induced urolithiasis in rats (Alvin Jose *et al.*, 2005).
3.3 UROLITHIASIS A REVIEW

Frequency of Urolithiasis as a urinary tract disorder is more with infections and prostatic disease and is estimated to afflict 240,000-720,000 Americans per year. Men are more frequently affected by Urolithiasis than women, with a ratio of 4:1. Initial presentation predominates in the third and fourth decades. The ratio of men to women approaches parity in the sixth and seventh decades.

The occurrence of urolithiasis is more in Northern India compared to Southern states. Men get stones much more frequently than women (3:1). The recurrence rate is 70-81% in males and 47-60% in females. The incidence of upper tract urinary calculi varies greatly with age, anatomic site, and geographic distribution.

Areas of high humidity and elevated temperatures appear to be contributing factors, and the incidence of symptomatic ureteral stones is greater during hot summer months. Increased sodium intake will increase sodium and calcium excretion and increase monosodium urate saturation (that can act as a nidus for stone growth), and increase the relative saturation of calcium phosphate, and a decrease in urinary citrate excretion. All of these factors encourage stone growth. Protein intake should be limited to 1g/kg/d. An increased protein load can also increase calcium, oxalate, and uric acid excretion and uric acid excretion and can also decrease urinary citrate excretion.

Excess intake of oxalate and purines can increase the incidence of stones in predisposed individuals. Although a reduction in dietary calcium results in reduced urinary calcium, the concurrent increase in urinary oxalate
may promote stone formation. The renal papilla is considered to be the site where stone formation occurs (Lawrence M. Tierney, 2002).

The renal colic associated with the passage of urinary stones is often agonizing, and, partly because of this, around 50 percent of patients forming stones are admitted to hospital. Fortunately, most stones are passed spontaneously in the urine but in 30 percent the stones have to be removed surgically to prevent secondary damage to the kidney from complete or partial obstruction or from intractable infection. The majority of stones requiring surgery are located in the ureter or kidney.

### 3.3.1 Etiology of Urolithiasis

Urinary calculi consist of aggregates of crystals containing small amounts proteins and glycoprotein. Different types occur with different frequencies in different parts of the world. The Dietary and environmental factors may also make a significant contribution (Davidsons, 2003).

Crystallization requires supersaturation of urine and reduced urinary content of inhibitors.

**A. Supersaturation can result from**

1. Too little output (a concentrated urine)
2. An absolute increase in the amount of a stone constituent excreted over a period of time such as calcium oxalate or uric acid.
3. An alteration in urine pH. Low urinary pH (<5.5) increases urinary saturation of uric acid, whereas high urinary pH raises that of calcium phosphate and magnesium ammonium phosphate.
Reduction in the concentration of inhibitors of crystallization in the urine may be of great importance in stone pathogenesis. Stone formers reportedly excrete less of this inhibitory material than non-stone formers. Magnesium is an effective competitor with calcium for oxalate ions, forming a relatively soluble complex. Those forming stones excreted more phosphorous and less magnesium than unaffected ones (King, 1967).

B. Urinary tract infection

Infective stones are composed of magnesium-ammonium phosphate, together with variable amount of calcium. They are believed to form as a result of infection of the urinary tract with organisms such as Proteus mirabilis that hydrolyse urea, with formation of the strong base ammonium hydroxide.

The availability of ammonium ions and the alkalinity of the urine favours stone formation. An increased amount of mucoprotein resulting from infection also creates an organic matrix on which stone formation can occur.

C. Diet

Diet containing low amounts of inferior quality proteins and high intake of animal proteins might augment the risk of stone formation.

D. Tumour

Pressure caused by tumours in the kidney may cause ischaemia and necrosis or predispose to infection. Necrotic debris and tumour fragments provide foci for deposition of solutes in the urine.
3.3.2 Pathologic Changes of Urolithiasis

Local changes created by stone include histopathologic evidence of inflammation and anatomic evidence of distortion. Locally there may be desquamation of epithelium, ulceration of the tissue contiguous to the calculi, and fibrosis. When a large stone occupies a thickened pelvis, interstitial fibrosis and leukocytic and round-cell infiltration are evident microscopically. Additional changes are influenced by the extent of obstruction of the outflow of urine from the renal pelvis. Hydronephrosis is evidenced by blunting of the calyces and later by various degrees of dilation of the individual calyces.

Atrophy and destruction of renal parenchyma follow, and as this process progresses, the dilated calyces may stretch almost the renal capsule.

Infection is sometimes superimposed, and various lesions such as calculous pyelonephritis, calculous pyohydronephrosis, and perinephritis may develop. With the introduction of infection, multiple stones may form renal function rapidly impaired, and the renal parenchyma is destroyed.

3.3.3 Types

- Calculous Pyelonephritis

Calculous pyelonephritis may become the most prominent lesion. The gross appearance of the kidney is influenced by the extent and virulence of the infection. With severe infection the renal pelvis is thickened, and military abscesses may develop in the swollen vascularized cortex of the kidney.
Calculous pyelonephritis may develop when infection is superimposed on a kidney that is the site of calculous hydronephrosis. Three types of this pyohydrencephrosis are recognized: atrophic, giant, and intermediate.

The atrophic kidney is very small and densely adherent to the adjacent perinephric fat. Most of the renal parenchyma is destroyed, leaving a shell of tissue attached to the pelvis.

The giant hydronephrotic kidney consists of a large multilocular sac that may fill the entire flank. The surface is irregular, owing to the variable size of the tremendously dilated calyces. Multiple stones or a solitary calculus may be present in the pelvis.

The intermediate pyohydrencephrotic kidney varies in appearance according to the degree of disease. The kidney is not as large as that of the giant type. The surface is nodular, and the elevated areas are fluctuant because of the dilated calyces and the thinning of the cortex.

- **Nephrocalcinosis**

  Nephrocalcinosis is the term applied to small diffuse calcifications distributed throughout the renal parenchyma. These calcifications usually occur in the renal papillae.

- **Xanthogranulomatous Pyelonephritis**

  A kidney damaged by calculi may be the site of replacement lipomatosi. Destruction of renal parenchyma appears to be a prerequisite for replacement lipomatosi; the fatty masses replace the destroyed tissue.
• **Squamous Cell Carcinoma**

A striking relationship between renal calculi and squamous cell carcinoma of the renal pelvis has been observed.

Gilbert and Mc Milan presented a collective review of 55 cases of squamous cell carcinoma of the renal pelvis. Higgins reviewed 59 cases and added 5 others, 3 of which were complicated by renal calculi. In a later collective review, Gahagnan and Reed reported that calculi occurred in 48 of 106 cases of squamous cell carcinoma of the renal pelvis.

• **Uric Acid Lithiasis**

Most animals filter uric acid through the glomerulus and rapidly reabsorb it through renal tubular cells. It recirculates through blood to the liver, there the enzyme uricase transforms it to allantoin. Allantoin then returns to the circulation and is easily excreted by the kidneys.

Man not only produces excessive, relatively water-insoluble uric acid, he also excretes urine that is predominantly acid because of the acid end products of metabolism. When uric acid enters human urine it exists in two forms. One is free uric acid. The other is the urate salt, which forms a complex mostly with sodium (George and Drach, 1986).

**Classification of Uric Acid Lithiasis**

The first category is termed idiopathic uric acid lithiasis. These individuals do not have hyperuricemia, and the amount of urinary excretion of uric acid per day is within normal ranges. The major Physiologic abnormality in these patients is a consistently low urine pH.
Their second category includes uric acid nephrolithiasis associated with hyperuricemia. Such as the Lesch-Nyhan syndrome. This latter disease is of interest because such patients have a deficiency in an enzyme, hypoxanthine-guanine phosphoribosyltransferase.

A third category consists of patients who develop uric acid lithiasis because of excessive loss of water to the environment. This may be due to excessive perspiration or to gastrointestinal losses such as that associated with ileitis, colitis, and similar conditions.

The final category includes patients who develop uric acid lithiasis because of ingestion of uricosuric drugs or overindulgence in foods high in purine and proteins (George and Drach, 1986).

### 3.3.4 Symptoms and Signs of Urolithiasis

Obstructing urinary stones usually present with colic pain usually occurs suddenly and may awaken patients from sleep. It is localized to the flank, is usually severe and may be associated with nausea and vomiting. Patients are constantly moving—–in sharp contrast to those with an acute abdomen. The pain may occur episodically and may radiate anteriorly over the abdomen. As the stone progresses down the ureter the pain may be referred in the ipsilateral testis or labium. If the stone becomes lodged at the ureterovesical junction, patients will complain of marked urinary frequency. Stone size does not correlate with the severity of the symptoms.

### 3.3.5 Clinical Presentation

A Clinical Presentation with renal colic and urinary tract obstruction is most commonly found with calcium stones. Haematuria may be macroscopic
or microscopic. The disease may, however, be asymptomatic; even a staghorn calculus may be a chance finding. Any type of stone may impair urinary drainage and present as a urinary tract infection; conversely, certain urinary tract infections predispose to stone formation.

3.3.6 Diagnosis of Urolithiasis

The diagnosis of urinary stones is seldom difficult. As 90% of calculi are radiopaque, most stones are visible on plain abdominal X-ray. An IVP is, however, necessary to reveal the presence of translucent stones and to show dilatation of the ureter above an obstruction. Abdominal ultrasound will also demonstrate calculi and any dilation of the urinary tract secondary to obstruction.

Although not clinically related to establishing a definite diagnosis, renal function must be determined and the urine cultured for infection. Serum urate concentration should be measured to exclude hyperuricaemia and increased serum calcium concentrations may indicate a diagnosis of hyperparathyroidism the latter may be confirmed by measuring serum parathyroid hormone concentration. When appropriate, 24-hour urinary concentrations of calcium, urate and oxalate should be assayed and a sample of urine assayed for cystine concentration.

3.3.7 Investigation of Urolithiasis

All patients with renal stone should have a carefully taken history, abdominal roentgenographic examination, urine analysis and a routine blood screen.
The objective of in-depth evaluation, applicable particularly to those with recurrent calculi, is to discern the specific metabolic cause for the nephrolithiasis. It includes a measure of parathyroid function and 24 hr urinary calcium, oxalate, uric acid, citrate, total volume, sodium and pH. Plain abdominal X-ray and excretion urography are the mainstay of diagnosis. Renal tomography is sometimes necessary. Ureteric acid stones can be missed by ultrasound.

3.3.8 Classification of Urinary Stones

Whenever a stone is passed, its chemical composition should be determined. There are four principal types of calculus,

- Calcium oxalate
- Triple phosphate (struvite)
- Uric acid
- Cystine

When these stone-forming salts become supersaturated in the urine there is a risk of crystal formation and calculus growth. It is believed that normal urine contains various inhibitors of crystallization known to influence the aggregation of calcium oxalate, calcium phosphate and perhaps, uric acid. These inhibitors are absent or reduced in urine from patients with renal calculi. In addition, a ‘glue’ to bind the growing crystals (and stone) to a tubular cell may also be necessary for promoting calculus growth. These risk factors in the urine are in turn modified by a number of additional influences including the age, disease state and dietary intake of the patient.
Calcium oxalate stones account for 70-75% of renal calculi and occur either in a pure form or mixed with calcium phosphate or uric acid. They are radiopaque and particularly insoluble in urine: once formed they are especially difficult to dissolve. Urine from normal individuals is supersaturated with calcium oxalate so that precipitation with stone formation is common. These stones occur most frequently in middle-aged men, tend to recur most commonly after 2-3 years and are associated with the higher social classes presumably because of an increased consumption of animal protein and dairy products. The majority of cases are idiopathic, but 10% or so may be caused by primary hyperparathyroidism.

Hypercalciuria remains a well-recognized risk factor but mild hyperoxaluria, which is often present, is less well appreciated. Severe hyperoxaluria, however, remains discussed subsequently, also increases the risk of stone formation for the minority of patients with this condition. Many patients with calcium oxalate stones, however, excrete excess uric acid in their urine—presumably because of their increased dietary intake of purine. A decrease in urinary volume, reduced urinary citrate and magnesium concentrations, and a decreased excretion of crystallization all predispose to calcium oxalate stone formation.

3.3.9 Metabolism of Oxalic Acid

The present day information in oxalic acid metabolism has been obtained mainly with studies on rats and man. Since, the 19th century oxalic acid has been known to be a normal urinary constituent. The preliminary metabolic studies conducted by Ganglio (1987) indicated that oxalic acid is an end product of metabolism of ethylene glycol in the body. Further studies
revealed that most of the urinary oxalates are endogenously formed, with about 10-15% originating from dietary oxalate.

\[ \text{α-anhydrous oxalic acid} \]

\[ \text{β-anhydrous oxalic acid} \]

\[ \text{Oxalic acid dihydrate} \]

The important endogenous precursors of urinary oxalate in man and other animals are ascorbic acid, glycine, glycolate etc., (Ganglio 1987).
In alpha Oxalic acid the hydrogen bonds and carbonyl groups form an extended chain system which links the molecule to form a puckered layer structure throughout the crystal where as the beta form possesses cyclic type of carbonyl and hydrogen bond system arranged in chains (Hendricks, 1935).

Oxalic acid forms neutral and acid salts with monovalent metals and ammonia. The principle precipitating salt is known as calcium oxalate (Lecompte et al., 1945).

3.3.10 Stone Formation

There are three requirements for clinical stone disease to occur. First, a nidus must form. Second, this nidus must be retained within the urinary tract. Third, the nidus formed must grow to sufficient size to obstruct the ureter. Three theories have been proposed to explain nidus formation.

- **Matrix Theory**

  All stones consist of 2-3% of organic material. Matrix theory proposes that this organic material is the initiating mechanism of calculus formation. Uromucoid or Tamm-Horsfall, urinary mucoprotein, is the most prominent single mucoprotein in human urine and has been believed to be involved in stone formation.

- **Inhibitor deficiency theory**

  Urine is a complex fluid that contains a number of inhibitors of crystallization. Some of these are citrate, sulphate, phosphate, magnesium, glycosaminoglycans, ribonucleotides and nephrocalcin. These inhibitors have
been shown to inhibit nucleation, crystal growth and crystal aggregation. Deficiency of these inhibitors enhances stone formation.

- **Precipitation – Crystallization theory**

  This theory relies on the recognized regions of saturation that exist in an aqueous solution containing minerals. These regions are defined by the solubility product and the formation of the product. The solubility product is the level of saturation where the liquid phase is in equilibrium with solid phase. The formation product is the level of saturation were spontaneous nucleation occurs. Above the formation products, the solution is oversaturated and spontaneous nucleation occurs. The precipitation – crystallization theory proposed that the periods of oversaturation occur that result in precipitation of a crystalline nidus that initiates stone disease.

**MECHANISM OF STONE FORMATION**

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SUPER SATURATED SOLUTION
  ↓
NUCLEATION
  ↓
SMALL CRYSTAL
   ↓
CRYSTAL GROWTH  CRYSTAL AGGREGATION
   ↓
LARGE CRYSTAL    CRYSTAL AGGREGATION
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3.3.11 Treatment for Urolithiasis

- **Idiopathic calcium oxalate stones**

  In general terms, treatment is aimed at decreasing the supersaturation of the urine and also perhaps at increasing the protective inhibitory activity against crystal formation and growth. It is easier to prevent the formation of new crystals and stones than to dissolve existing calculi.

- **Dietary management**

  The combination of low calcium, low oxalate diet with a high fluid intake (>3 litres/day) decreases the recurrence rate of calcium stone formation. A low calcium diet alone and treatment with sodium cellulose phosphate may decrease urinary calcium excretion but encourages the absorption of additional quantities of oxalate from the gastrointestinal tract and thus predisposes to hyperoxaluria. Moreover, sodium cellulose phosphate is contraindicated for triple-phosphate stones and may cause diarrhoea it is a less effective treatment than long-term thiazide therapy.

- **Thiazide diuretics**

  Long-term administration of thiazide diuretics diminishes urinary calcium loss and does not increase urinary oxalate concentration. This form of treatment is effective in 90% of cases.

- **Allopurinol**

  As some patients with calcium oxalate stones also excrete excessive quantities of uric acid in their urine, treatment with allopurinol may prove successful in preventing the formation of further stones. The mechanism of action is unclear: uric acid may provide a nucleus for calcium oxalate stone
growth or may bind certain inhibitors of crystallization and thus favour calcium stone formation. Either or both these factors will be corrected by allopurinol treatment.

- **Orthophosphate supplements**

  Neutral or slightly alkaline orthophosphate supplements may decrease urinary calcium losses and increase urinary inhibitory activity by increasing urinary pyrophosphate concentrations. Long-term treatment with 1.5g/day of elemental phosphorus has proved effective in preventing further stone formation and has not, despite concern, caused either ectopic calcification or hyperparathyroidism. The powder is, however, unpalatable and patient compliance may prove a problem.

- **Magnesium supplements**

  Magnesium supplementation may also prove successful in preventing further stone formation.

- **Hyperparathyroidism**

  Parathyroidectomy with removal of the adenoma is the treatment of choice for primary hyperparathyroidism and, when successful, the urinary abnormalities resolve. When parathyroidectomy is contraindicated, the patient should maintain a high fluid intake.

- **Hyperoxaluria**

  Patients with the primary hereditary disorder often present in renal failure and it is seldom possible to restore renal function. If renal function is well preserved, a high fluid intake (>3litres/day) should by recommended and may be sufficient to control hyperoxaluria. When associated with small
bowel resection (enteric hyperoxaluria), dietary oxalate restriction together with a high fluid and calcium intake may be beneficial.

- **Renal tubular acidosis**

  Patients with this disorder may be helped by oral acid supplements. It is characterized by a hypokalaemic, hyperchloraemic acidosis with urine which is usually less than maximally acic (pH>5.4), although this is not always the case. There is a qualitative abnormality of renal tubular acidification classified according to the site of the tubular defect either the distal or the proximal nephron (Grame and Catto David, 1988).

### 3.3.12. Role of Oxalate In Urolithiasis

Oxalate measurements indicate higher values of clearance implying a new tubular excretion of oxalate by active tubular transport (Hodgkinson, 1972). Tubular excretion of oxalate is by active process and oxalate undergoes a bi-directional transport in the renal proximal tubule which results in a net secretion (Knight et al., 1981).

Currently, emphasis has been centered towards the role of crystals of the salt inducing the crystallization of another salt such as epitactic induction between crystals have similarities in lattice dimensions is a well known phenomenon in crystallography. Relevant lattice similarities are present between uric acid, calcium oxalate and calcium phosphate crystals and epitactic induction does occur among them (Lonsdale, 1968b). The precipitation of calcium oxalate can be induced from metastable solution by hydroxy apatite (Meyer et al., 1975, Pak et al., 1976) brushite and urate (Coe et al., 1975).
When the urinary oxalate concentration reach the upper limit of the normal range while having normal calcium excretion, the upper limit of the solubility or formation product of calcium oxalate is exceeded in the patients leading to increased crystalluria and stone formation.

So, in the present study, hyperoxaluria was induced by ethylene glycol. Citric acid in urine plays a role in the prevention of renal stones. Citrate binds calcium ions forming a soluble complex (Chulkaratana et al., 1971). In addition to Chelating effect of citrate on calcium in solution, it also appears to act in some additional way to inhibit crystal growth (Smith et al., 1973) and it is shown that citrate appears to affect the crystal formation to a greater extent that could be expected from chelation alone (Newman and Newman, 1958).

Recent studies by Tiselius (1981) demonstrated that citrate seems to have a significant inhibitory effect on calcium oxalate crystal growth even at very low concentration. High level of urinary magnesium retards renal stone formation and low level of urinary magnesium encourages stone formation (Pyrah, 1979). High concentration of magnesium affecting the solubility of calcium oxalate have been reported (Chulkaratana et al., 1971). Magnesium forms a soluble complex with oxalate thereby decreasing the availability of free oxalate to complex with calcium ion for crystal growth (Meyer and Smith 1975a).

In the present study crystalluria was induced in male albino rats by giving ethylene glycol orally. There are reports which show that within short period crystalluria can be induced in rats by giving ethylene glycol. Ethylene glycol (EG) or 1,2-ethanediol is a dihydric alcohol. It is used as an anti-freeze in cooling systems of automobiles, air crafts and space modules and has wide industrial applications. Due to its toxic nature may accidental deaths have
occurred from its consumption as a cheap substitute for alcohol (Pousand and Custer, 1946; Hageman and Chiffella, 1948; Haggerty, 1959; Friedman et al., 1962 and Collins et al., 1970).

Ethylene glycol is metabolised through glycoaldehyde, glycolic acid and glyoxalic acid. Liver is the major site of EG metabolism and that all enzyme systems necessary for its oxidation are present in the liver (Richardson, 1973). Key enzymes catalyze the oxidation of glycolate to glyoxylate and then to oxalate namely xanthine oxidase, lactate dehydrogenase and glycolate oxidase. It was shown that xanthine oxidase is of no great significance in the synthesis of oxalate (Hodgkinson, 1977).

3.4 EARLIER INVESTIGATION OF VARIOUS PLANTS ON UROLITHIASIS

Urolithiasis, the process of formation of stones in the kidney and the urinary tract is one of the third most common afflictions found in humans. Several remedies have been employed to treat this clinical disorder. (manifestation). The importance of traditional systems of medicine has now gained recognition all over the world, many indigenous drugs form an indispensable part of health care. Efficacy of several traditional system of medicine has been duly acclaimed. Very commonly many plant juices are available for the treatment of urolithiasis. The plants are easily available, cheap and allow very easy mode of administration. A review of plants investigated against urolithiosis is presented briefly as hereunder.
Plants investigated against urolithiosis

<table>
<thead>
<tr>
<th>SL No</th>
<th>Plant name Family</th>
<th>Parts used</th>
<th>Extract / Active constituent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Plectranthus amboinicus</em> Lour Lamiaceae.</td>
<td>Fresh Leaves</td>
<td>Fresh juice-concentrated to a dry mass</td>
<td>Alvin jose <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>3</td>
<td>Dolichos biflorus Linn Papilionaceae</td>
<td>Seeds</td>
<td>Pentacyclic triterpenes lupeol and structural analogue betulin</td>
<td>Vidya <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>4</td>
<td><em>Bergenia ligulata</em> Rutaceae</td>
<td>Rizhiom</td>
<td>Soxhlet extraction</td>
<td>Carimella <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>5</td>
<td><em>Aerva lanata</em> Amaranthaceae Vedi &amp; Vediuppu chunnam (siddha drug)</td>
<td>Leaf</td>
<td>Leaf extract</td>
<td>Selvam <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>6</td>
<td><em>Herniaria hirsute</em> Caryophyllaceae</td>
<td>Whole plant</td>
<td>Plant extract</td>
<td>Atmani <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>7</td>
<td><em>Costus spiralis Roscoe Zingiberaceae</em></td>
<td>Whole plant</td>
<td>Water Extract</td>
<td>Araujo Viel <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>8</td>
<td><em>Hibiscus sabdariffa</em> Linn Malvaceae</td>
<td>Whole plant</td>
<td>Juice</td>
<td>Kirdpon <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>9</td>
<td><em>Ammannia baccifera</em> Linn Lythraceae (Cultivar)</td>
<td>Whole plant</td>
<td>Ethanolic extract</td>
<td>Prasad <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>10</td>
<td><em>Musa paradisiaca</em> Linn (Cultivar) Scitaminaceae</td>
<td>Stem</td>
<td>Stem juice</td>
<td>Prasad <em>et al.</em> (1993)</td>
</tr>
<tr>
<td>11</td>
<td><em>Trigonella foenum-graecum</em> Papilionaceae</td>
<td><em>Trigonella foenum-graecum</em> seed &amp; <em>Ammi majus</em> fruit</td>
<td>As such</td>
<td>Ahsan <em>et al.</em> (1989)</td>
</tr>
<tr>
<td>12</td>
<td><em>Tamarindus Indicus</em> Caesalpiniaeae</td>
<td>Paricarp</td>
<td>As such</td>
<td>Singh <em>et al.</em> (1987)</td>
</tr>
<tr>
<td>15</td>
<td><em>Crataeva nurvala</em> Buch - Ham Capparaceae</td>
<td>Stem bark</td>
<td>Lupeol</td>
<td>Annie shirwaikar <em>et al.</em> (2004).</td>
</tr>
<tr>
<td>17</td>
<td><em>Tribulus terrestris</em> Zygophyllaceae</td>
<td>Fruits</td>
<td>Ethanolic extract</td>
<td>Anand <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>19</td>
<td><em>Mimosa pudica</em> Linn Mimosaceae</td>
<td>Whole plant</td>
<td>Extract</td>
<td>Asima Chatterjee (2000)</td>
</tr>
</tbody>
</table>