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2.1 SCOPE OF THE REVIEW

The world-wide incidence of urolithiasis is quite high and in spite of tremendous advancements in the field of medicine, there is no truly satisfactory drug for the treatment of kidney stones. The main reason for this is the obscurity of the mechanism underlying the stone formation. Modern medicine, with its sophisticated and expensive diagnostic equipments and reliance on costly drugs and treatment technology, plays a minor role in many third world countries, especially in rural areas. Information about herbal remedies has been passed from generation to generation and a number of indigenous drugs are being used in India and several other countries. Therefore the scope of the present review is to understand the mechanism of pathogenesis of urinary calculogenesis and to evaluate the role of traditional drugs in its prevention and treatment.

2.2 UROLITHIASIS IN MAN

Urolithiasis has been a perplexing problem since the dawn of history. Urinary stones constitute a major health problem all over the world. Early accounts of urinary tract stone disease have been made by Susruta, Hippocrates and Celsus. Whereas Hippocrates (460-370 B.C.) advised surgical operation for renal and vesical calculi, Galen favoured the use of stone solvents. Till date, theories proposed for stone formation have lacked scientific investigations and no single causative factor could be attributed, therefore, rendering it a multifactorial disease.

Urolithiasis is a significant problem recognizing that the incidence of this disease has been estimated to be between one to twelve per cent (Frangos and Rous, 1987). Finalyson (1974) studied that the United States has a relatively high incidence of urinary calculi. In India, two stone belt regions were demonstrated by Anderson (1969) and Colabawalla (1971). One belt starts from Amritsar in the north and extends to the north west, including Delhi and Agra and ends in U.P. The other belt starts on the west coast.
at Jamnagar and extends inwards towards central India to Jabalpur. The peak incidence of this disease occurs in the months of above average temperature and below average rainfall i.e. in the months of June-July (Elliot et al., 1975; Robertson et al., 1975). Wisniewski (1981) observed an increased incidence in the management class and low incidence in farmers, fishermen, miners and unemployed men.

Racial differences exist in that urolithiasis is 3-4 times more common in whites than blacks. Also the condition is approximately twice as common in males than in females (Rous, 1981; Sarmina and Resnick, 1987; Abdel-Halim et al., 1989). In affluent countries, it is a major cause of morbidity with considerable socio-economic costs for health care and productivity in the community (Goldsmith, 1986; Mather, 1986). Despite its low mortality, the disease is recurrent and affects people in their most productive years with pain that is legendary in severity (Ljunghall et al., 1981; Robertson, 1984).

Calcium oxalate combined in various proportions with calcium phosphate is a major component in more than 70% of renal stones (Rofe et al., 1981; Asper, 1984; Thind et al., 1989), thus emphasising the need for studying oxalate metabolism to understand the etiopathogenesis of this disease. Several aspects of urolithiasis and oxalate metabolism have been reviewed in the recent years (Nath et al., 1984; Conyers et al., 1990; Smith, 1990; Coe et al., 1992). Literature on urolithiasis is also being updated every four years at international meets. The seventh of this series was held in August, 1992 in Cairns, Australia.

2.3 RISK FACTORS RELATED TO UROLITHIASIS

The decisive steps in the formation of urinary calculi as well as pathogenesis of urolithiasis have not yet been elucidated, but it is well established to be a multifactorial disease afflicting mankind. Although both intrinsic (heredity, age, sex) as well as extrinsic (climate, diet, water, occupation etc.) factors have been described by Anderson (1972) to be involved in the genesis of renal calculi, Drach (1986) and Smith (1988) have suggested that formation
of a stone within the urinary tract is not a disease but instead a complication of many varied disorders, which can create from time to time within the urine, supersaturation of one or more crystalline phases that may precipitate. The risk factors involved in the formation of stones can be divided into two categories: (i) Urinary factors (low urine volume, its super saturation with lithogenic substances etc.) and (ii) dietary factors (vitamin deficiencies, excess protein and carbohydrate intake etc.).

2.3.1 Urinary Factors

For crystals to form in urine, the urine must be supersaturated in the precipitating crystalline phase (Finlayson, 1978). Urine is an extremely complex solution containing many varied forms of solute and its state of saturation is dependent on many factors like solute concentration, ionic strength, complexation, pH and volume (Smith, 1987). Some of these are discussed below:

2.3.1.1 Urine volume and pH

The importance of increasing urinary volume to prevent renal stone formation has been appreciated since the time of Hippocrates (Adams, 1939). A low urinary volume is an important criterion for stone formation; however, often the urinary volume in idiopathic calcium stone formers did not differ from normals (Robertson et al., 1968). Dehydration and inadequate fluid intake have been implicated in nephrolithiasis in individuals from temperate climates, who reside for long periods in the tropics. For example, British sailors stationed in the Middle East had a higher incidence of nephrolithiasis than their counter parts in Great Britain (Blacklock, 1969).

Finlayson (1974) demonstrated that increased urine flow causes a reduction in urine oxalate concentration and to be significantly effective, a urine output of more than 3600 ml per day would be theoretically necessary. The effect of urinary dilution on the crystallization of calcium salts has been quantitatively assessed by Pak et al., (1980). As the inhibitors of calcium...
oxalate crystal growth are an important urinary defense mechanism against stone formation, then diluting these inhibitors with a high urine volume may paradoxically increase the risk of stone formation (Pak et al., 1980). However, studies of the formation product ratio and the activity product ratio of urine diluted in vitro and in vivo failed to demonstrate an increase in the risk of stone formation with increasing urine dilution. Hosking and colleagues (1983) demonstrated that in patients under treatment, a urinary volume less than 1400 ml/day increases the risk of recurrence of stone formation. The prescription of a high fluid intake was one of the components of the conservative management programme suggested by these workers.

Lemann et al., (1991) noted that relative supersaturation of solution with respect to calcium oxalate increases, as daily calcium excretion rate increases and as daily urine volume decreases especially to a volume less than 1.0-1.5 L/d. The relative supersaturation for the respective stone type was reported to be significantly lowered with increase of urinary volume (Hesse et al., 1992). Stone growth or new stone formation is not seen in up to 60% of patients treated initially with fluid and diet therapy alone (Menon and Koul, 1992).

Extremes of pH in the urine can promote stone formation (Robertson et al., 1978). A pH below 5.5 increases the risk of uric acid crystal formation because of an excess of undissociated uric acid. When hourly excretion of urate and urinary pH were assessed in patients who formed calcium stones, the highest urate concentrations were found to coincide with the lowest pH level during the early morning hours (Tiselius and Larson, 1983). A pH above 7 favours the formation of calcium phosphate crystals because of an increased dissociation of phosphate, especially if hypercalciuria is present. The maintenance of the pH between 6 and 7 is therefore desirable for the control of uric acid and calcium oxalate stone disease. At this pH, the inhibiting activity of pyrophosphate and citrate is also enhanced (Tiselius, 1981). Diet and fluid intake are often responsible for the abnormalities in urine pH,
therefore both these should be modified in a beneficial way to correct the defect and to reduce the tendency towards stone formation.

2.3.1.2 Hyperoxaluria

The increased urinary excretion of oxalate has been noted in 15% to 50% of the patients with idiopathic calcium oxalate urolithiasis (Robertson and Peacock 1980; Smith et al., 1984; Larson and Tiselius, 1987). The amount of oxalate excreted in the urine is determined by dietary intake, intestinal absorption, renal tubular secretion and the rate of endogenous synthesis (Discussed in detail in section 2.4).

2.3.1.3 Hypercalciuria

Hypercalciuria is defined as calcium excretion exceeding 7.4 mmol/d (300 mg/24 h) among men, greater than 6.25 mmol/d (250 mg/24 h) among women or greater than 0.1 mmol/kg body weight/d (4 mg/d) regardless of age or sex (Flocks, 1939, 1940; Albright et al., 1953; Henneman et al., 1958). Hypercalciuria is a common abnormality found in 50-70% of patients with nephrolithiasis (Pak et al., 1980) though it has been suggested to play a less critical role in stone formation than hyperoxaluria, because an increase in calcium concentration in raising the urinary saturation of calcium oxalate (Nordin et al., 1973). Smith (1990) suggested that increased calcium does play a role in the pathogenesis of stones as the calcium excretion was found to be higher in stone formers than in normal individuals. However, a majority of stone formers have 24 h urinary calcium within the normal range and a big portion of the normal population have hypercalciuria without stones thereby suggesting that hypercalciuria does not in itself cause kidney stones but is an important risk factor among several that predispose to renal calculi as suggested earlier by Robertson et al., (1980).

Dietary calcium is undoubtedly by far the major ultimate source of the extra calcium appearing in the urine of hypercalciuric stone formers (Lemann et al., 1991). Absorption of dietary calcium is regulated by many factors. It
has been shown that phytate (McCance and Widdowson, 1942), animal protein (Goldfarb, 1988) dietary phosphorus (Insogha et al., 1989) sugars (Knowles et al., 1988) fiber (Kasper, 1990) and high salt intake (Goldfarb, 1990) may influence calcium absorption. Potassium administration (Sakhaee et al., 1983; Lemann et al., 1989) reduces urinary calcium excretion and brief deprivation of dietary potassium increases urinary calcium excretion (Lemann et al., 1991). Calcium absorption appears to be dependent on two processes, the vitamin-D dependent process and an independent process (Smith, 1990). 1,25-(OH)₂-D is the only currently known normal stimulus of intestinal calcium absorption and hypercalciuric stone formers have long been known to exhibit increased rates of intestinal Ca absorption (Pak et al., 1974). Moreover, many but not all such stone formers exhibit high normal or elevated serum total and free 1,25-(OH)₂-D concentrations (Kaplan et al., 1977; Bataille et al., 1987). Since 1,25-(OH)₂-D is known to inhibit transcription, synthesis and secretion of PTH (Silver et al., 1985), the consequent decrease in PTH could contribute to hypercalciuria. Intestinal calcium absorption has been observed to be increased in hypercalciuric stone formers in the presence of normal or only slightly elevated serum 1,25- (OH)₂-D concentrations (Vitamin D independent process) (Insogna et al., 1985; Lemann and Gray, 1989). Since, 1,25- (OH)₂-D is known to upregulate its own receptor (Favus et al., 1988) it could amplify subsequent biochemical events leading to increased intestinal calcium absorption.

Calcium is excreted primarily through filtration, with modulation in both the proximal tubule, where calcium is reabsorbed, and in the distal tubule, where it is also reabsorbed variably depending upon hormones such as PTH and the physical factors such as acidemia.

Approximately 10,000 mg of calcium is filtered at glomerulus per day. Augmented rates of urinary calcium excretion may result from either increased glomerular filtration rates (GFR) of calcium or reduced renal tubular reabsorption of filtered calcium. However, Lemann and his associates (1991) revealed that the hypercalciuria observed in stone formers is not due to high GFR but because of the decreased renal tubular reabsorption of calcium.
The differential handling of calcium between the proximal tubule and distal tubule of the nephron, could be attributed to the presence of vitamin D dependent 28 Kd calcium binding protein (Borke et al., 1988). This protein is inducible by 1,25-(OH)$_2$-D and is very likely related to the movement of calcium across the cell (Bronner and Stein, 1988). Urinary calcium excretion rates among normal subjects, normocalciuric stone formers and hypercalciuric stone formers have been observed to be directly correlated to the activity of erythrocyte Ca-Mg-ATPase (Bianchi et al., 1988). Since Ca-Mg-ATPase is known to be present in the distal renal tubule, alterations in its regulation may contribute to hypercalciuria.

2.3.1.4 Hyperuricosuria

Uric acid is a nitrogen waste metabolite having no other physiologic function. The upper limit of normal for 24 hours is somewhat arbitrary. Gutman and Yu (1968) suggested an upper limit of 800 mg/24 h for men and 750 mg/24 h for women. Using this upper limit Coe (1977) reported that the incidence of hyperuricosuria was significantly higher in stone formers than in normal subjects. The high frequency of calcium oxalate stone formation in patients with gout has directed attention to hyperuricosuria as a risk factor (Coe, 1978a,b).

Uric acid is eliminated through the gut and the kidneys. Under normal physiological conditions two-third to three-fourth of uric acid is eliminated by the kidney (Levinson and Sorenson, 1980). Using a selective inhibitor of tubular secretion these workers have found that only 0.7% of the filtered urate is excreted and 99.3% must be reabsorbed at a site proximal to the site of tubular secretion. Levinson and Sorenson (1980) found that 50% of the originally filtered uric acid load is subsequently secreted. The amount of uric acid finally excreted by the kidneys is only 20% of the amount secreted and approximately 80% of the secreted urate is reabsorbed.

Serum urate concentration like urine uric acid concentration, parallels the prevalence of uric acid nephrolithiasis (Yu, 1981). This could be due to
an overproduction of urate in case of gout patients, by increased dietary protein and by an increase in nucleic acid breakdown, therefore to maintain urate homeostasis, the urinary excretion must increase, as the serum urate or urate pool size increases.

A low urine volume (Lonsdale and Mason, 1966) and decreased pH (Yu et al., 1962; Yu and Gutman, 1967; Simkin et al., 1973) increase the risk for uric acid stone formation. As urine pH becomes more acidic, the solubility of uric acid decreases and it has been found that idiopathic uric acid stone formers and gouty stone formers commonly have a lower fasting urinary pH probably due to a deficit in urine ammonium excretion. Another important risk factor for uric acid urolithiasis is a high protein intake. Protein is the major source of dietary purines as well as proteins contain ash acids, which lower urinary pH (Bogash and Dowben, 1954). Thus a high protein diet decreases uric acid solubility by increasing its urinary concentration and decreasing urinary pH.

Although several theories have been suggested, the specific role of uric acid in the formation of calcium stones within the urinary tract has not been established. It was suggested by Coe et al., (1975) and Meyer (1981) that uric acid or urate salts might induce the heterogeneous nucleation of calcium oxalate. A second observation offered an alternative hypothesis, uric acid or urate salts present in the colloidal form can bind to urinary glycosaminoglycans (GAGs), thereby abolishing or attenuating their inhibitory potency and indirectly increasing the risk of calcium oxalate crystallization in vivo (Robertson et al., 1976). Most abundant GAG, chondroitin sulfate has been found to bind to sodium urate (Fellstrom et al., 1986; Hess et al., 1987) and its preincubation with sodium urate reduces its inhibitory effect in calcium oxalate crystal aggregation in aqueous solutions (Ryall et al., 1986). Recently, Ryall et al., (1991) have reported that urate dissolved in urine at normal physiological pH values, directly provokes calcium oxalate crystal nucleation by the phenomenon of salting out. The possibility of urate promoting calcium oxalate stone formation is further strengthened by its ability to increase
significantly the amount of calcium oxalate precipitation from solution to cause aggregation of individual crystals into large clusters.

2.3.2 Dietary Factors

Dietary patterns have been implicated as major contributors to the high prevalence of upper urinary tract stones in affluent countries. A number of nutrients may influence the excretion of calcium, oxalate, urate and citrate predisposing to calcium stone formation (Robertson, 1987): they include increased consumption of protein refined carbohydrates, sodium, calcium, magnesium and decreased consumption of various vitamins.

2.3.2.1 Vitamins

Vitamins play an important role in the pathogenesis of urinary calculi. Diets deficient in vitamins are known to induce kidney calcification. Water-soluble vitamins are known to have a significant role in oxalate metabolism while fat-soluble vitamins control the metabolism of various lithogenic substances viz. calcium and phosphorus.

Vitamin B$_1$ or thiamine is involved in the physiological actions in the body in the form of thiamine-pyrophosphate. It is involved in the metabolism of glyoxylate as a cofactor, for the glyoxylate-2-oxoglutarate carboligase enzyme. Thiamine deficiency is known to result in increased tissue glyoxylate levels and also in its urinary excretion in man and various experimental animals (Liang, 1962; Buckie, 1963; Hauschildt et al., 1972; Sidhu, 1985). A 34% increase in urinary excretion of oxalate in thiamine deficient rats was noticed (Sidhu, 1985) and this increased excretion of oxalate was attributed to complete blockage of mitochondrial oxidation of glyoxylate accompanied by 36% increase in liver GAO activity and increase of LDH-I and LDH-II isoenzymes in kidney. Thiamine deficiency of 6-weeks duration significantly increased hepatic GAO, while activity of liver GAD and LDH were significantly decreased in this deficiency (Sidhu et al., 1987). Vitamin B$_1$ deficiency of four weeks in rats has been shown to produce significant hyperoxaluria by augmenting the
liver GAO, while LDH and GAD levels remained unaltered (Sharma et al., 1990). Thiamine pyrophosphate (TTP) effect has been used as suitable index to evaluate nutritional status of thiamine (Takeuchi et al., 1990). Metabolism, biochemical functions, deficiency signs and various other aspects of thiamine have been reviewed by Gubler (1991).

It is well known that the deficiency of vitamin B$_6$ is one of the factors involved in the pathogenesis of stone formation (Farooqui et al., 1981; Nath et al., 1984; Nath et al., 1990). Pyridoxal-5'-phosphate (PALP) the coenzymic form of vitamin B$_6$ is required for a number of transamination reactions with glyoxylate as one of the substrates. Thus it was hypothesized that deficiency of vitamin B$_6$ lowers these reactions, thereby increasing glyoxylate pool and oxalate production (Gershoff, 1964). Deficiency of vitamin B$_6$ leads to increased levels of oxalate-synthesizing enzymes in liver and kidney (Murthy et al., 1982), hyperabsorption of oxalate from the intestine (Sidhu et al., 1986a) and increased reabsorption of oxalate by renal brush border membrane vesicles (Gupta et al., 1988). Pyridoxine deficient rat urine exhibited a significant decrease in citrate excretion and mean urinary inhibitory activity towards calcium oxalate crystal growth (Sidhu et al., 1986b). Murthy et al., (1982) showed a significant positive correlation between urinary oxalate excretion and pyridoxine nutritional status. Clinical trials by the administration of low doses of pyridoxine in stone formers for long periods corrected the hyperoxaluria.

Role of vitamin B$_6$ in lipid metabolism has also been reported by Abe and Kishino (1982). Cho and Leklem (1990) demonstrated that vitamin B$_6$ is required in carnitine biosynthesis in vivo. Ravichandran and Selvam (1990) have observed an increased lipid peroxidation in the kidneys of vitamin B$_6$ deficient rats. A significant increase in calcium and oxalate levels in nuclear, mitochondrial and microsomal fractions in the kidneys of vitamin B$_6$ deficient rats has been demonstrated. All the three fractions of vitamin B$_6$ deficient liver and kidney showed increased susceptibility to lipid peroxidation in the presence of stimulators such as copper, iron, ascorbate and oxalate, thereby showing that accumulation of calcium and oxalate in kidney membrane fractions
may be the site for stone formation through peroxidative damage (Ravichandran and Selvam, 1990). Wolfson et al., (1991) have suggested that in rats with chronic renal failure, vitamin B$_6$ deficiency reduced the glomerular filtration rate and increases renal scarring.

The contribution of ascorbic acid towards endogenous oxalate was first demonstrated in guinea pigs by Burns et al., (1951) and was further confirmed by various workers in other animal species (Curtin and King, 1955; Abt et al., 1962; Takenouchi et al., 1966). Studies with $^{14}$C- ascorbic acid revealed that the main excretory products of vitamin C metabolism are dehydroascorbic acid and oxalic acid, which contribute 35-40% of total urinary oxalate excretion (Atkins et al., 1964; Baker et al., 1966; Spittle, 1970). It has been shown that intake of large amounts of ascorbate by humans results in increased urinary oxalate output (Knappwost and Ruhe, 1979; Hatch et al., 1980; Schmidt et al., 1981).

Excessive feeding of vitamin C in guinea pigs resulted in significant hyperoxaluria accompanied by hyperabsorption of intestinal oxalate (Farooqui et al., 1983). The same findings were supported by Chalmers et al., (1986) that increased urinary oxalate was primarily the result of increased intestinal absorption of oxalate. Recently Urivetzky et al., (1992) concluded from their studies, that excessive intake of ascorbic acid (amounts 0.5 g/day) will significantly increase the intrarenal urinary oxalate concentration and the risk of formation of calcium oxalate stones. Therefore patients with a history of stone disease or those who have decreased renal function must exercise caution in their daily vitamin C intake.

The deficiency of Vitamin A has also been implicated as one of the possible etiological factors of stone formation (Sadre and Ziai, 1977). Harris and Navia (1978) proposed that deficiency of vitamin A increased the sulfation of glycosaminoglycans (GAGs) which in turn can lead to excessive precipitation of calcium and stone formation. Parson et al., (1980) postulated that in hypovitaminosis A, disintegration of epithelial cell surface followed by their replacement with keratinized stratified epithelium of urinary tract
may serve as nidus for the genesis of urinary tract calculi, but the exact mechanism underlying this, still remains to be elucidated. Gershoff and McGandy (1981) reported that vitamin A deficient lactose supplemented diet leads to hypercalciuria, hyperoxaluria and urolithiasis. Trechsel et al., (1982) showed that vitamin A stimulated the activity of vitamin D in chick kidney cell cultures, thereby altering calcium metabolism, though the mechanism of this kind of regulation is not clear. Increased excretion of urinary calcium and oxalate in vitamin A deficient rats has been related to enhanced intestinal absorption of both calcium and oxalate (Kancha and Anasuya, 1989; Sharma et al., 1990). Various aspects of vitamin A related to its metabolism, biochemistry, function and deficiency etc. have recently been reviewed by Olsen (1991) and Blomhoff et al., (1991).

The role of vitamin D in urolithiasis is proposed via its action on calcium metabolism which in turn effects oxalate absorption from the intestine (Hodgkinson and Zarembski, 1968). The metabolically active form of vitamin D, 1,25-(OH)2-D3 is known to stimulate active calcium transport in the intestine, bone mineral mobilization and also increased urinary calcium excretion by decreasing tubular renal reabsorption (Deluca, 1984; Kumar, 1984; Reicher et al., 1989). The resultant hypercalciuria is one of the major risk factors in the genesis of urinary calculi. Hildman et al., (1982) have shown that calcium permeability of BBM is maintained by 1,25-(OH)2-D3 and decreased when there is interference with the endogenous production of vitamin D metabolism. Various aspects of vitamin D, related to its metabolism, biochemistry, deficiency, etc. have extensively been reviewed (collins and Norman, 1991). Giannini et al., (1993) studied the possible link between vitamin D and hyperoxaluria in patients with renal stone disease. They found a positive correlation between serum concentration of 1,25-(OH)2-D and urinary calcium and oxalate excretion; thereby showing a role of vitamin D in the pathogenesis of calcium nephrolithiasis, not only due to increased intestinal calcium absorption but also because of increased intestinal absorption of oxalate, thus leading to hyperoxaluria.
The relationship between stone formation and vitamin K is not clearly established. γ-carboxyglutamic acid (GLA), the synthesis of which is dependent on vitamin K, is present in stone matrix proteins (Lian et al., 1977). Stone formers have been shown to excrete large amounts of GLA in urine than control subjects (Fernlund, 1976; Joost et al., 1981). It is excreted in urine both as free amino acid and in undegraded GLA-containing proteins (Lian and Gunberg, 1988). GLA can chelate calcium thereby trapping calcium and forming matrix on which stone may grow. Nishio et al., (1990) have implicated GLA as a promoter of calcium oxalate crystallization. Nakagawa et al., (1981) however reported that a GLA containing polypeptide is present in normal human urine and also in kidney cell cultures has potent inhibitory activity on calcium oxalate crystallization and crystal growth. Thus it is not known whether the GLA-containing peptides act as stone nidus or as inhibitor of stone formation (Coe et al., 1991).

2.3.2.2 Minerals

The pathogenesis of renal calculi is strongly linked with disorders of mineral metabolism because urinary stones are mainly composed of minerals like calcium phosphorus and magnesium besides oxalate. The intrarenal environment of calcium, magnesium and phosphate, both in the fluid within the various parts of nephron and in the tissue of the papilla must vary considerably according to the diet and metabolic state of the individual (Nath et al., 1984). Such extreme conditions may be responsible for both stone formation and stone growth. The role of these minerals as risk factors in relation to urolithiasis is discussed below.

It has been observed by Coe (1977) that 45-65% stone formers have hypercalciuria and evidences have shown that idiopathic hypercalciuria has a major role in stone formation (Insogna et al., 1985). Several workers (Parks et al., 1981; Coe, 1983; Coe and Bushinsky, 1984) have shown that treatment of hypercalciuria reduces the stone formation.
Calcium is the most abundant cation in the body and as almost all calcium is present in the bones, the total body calcium can be assessed by the bone mass which averages about 1 kg in man. Only 1% calcium occurs in the extra cellular fluid and various other soft tissues (Lyles et al., 1981). Intracellular concentration of calcium is very low (10^{-7} -10^{-8} M) and it must be regulated in order to play a role as an intracellular signal and trigger and modulate specific functions of the cells which are maintained by binding and sequestering of calcium in the cell interior and by calcium transporting systems of plasma membrane and the intracellular membranes (Sarkadi, 1980). Serum calcium levels are precisely regulated within a narrow range (8.7-10.5 mg/100 ml). The regulation of cell calcium metabolism is under the influence of action of various regulators, modulators and controllers. The schematic representation for regulation of cytosolic-free calcium is shown in Fig. 1. The various aspects of calcium metabolism like its intestinal absorption, renal handling and its relation to stone formation have already been discussed in section 2.3.1.3 under the title hypercalciuria.

The daily intake of phosphorus in man is approximately one gram and most of the absorbed phosphorus is in inorganic form. It is widely distributed in calcified tissues like bone and teeth, as well as in various macromolecules like lipids, proteins, nucleic acids and carbohydrates (Glimcher and Krane, 1968). There are several reports that oral phosphate supplementation given on a long term basis reduces the recurrence rate of stone formation in idiopathic calcium stone disease without causing any serious side effects (Edwards et al., 1965; Smith et al., 1973; peacock et al., 1981; Churchill, 1987). Oral phosphate supplementation affects the calcium stone formation by reducing urinary supersaturation of calcium salts and secondly by increasing the excretion of crystallization inhibitors mainly pyrophosphate. A combined potassium phosphate and thiazide therapy was employed for the successful treatent of recurrent idiopathic urolithiasis (Klein and Griffith, 1982).

Broadus et al., (1984) showed that oral phosphate therapy was capable of lowering plasma 1,25-(OH)_{2}-D_{3} and reduced calcium absorption and
REGULATORS
1. Membrane depolarization
2. Hormones
3. Cyclic nucleotides
4. Calmodulin

CONTROLLERS
1. Plasma membrane
2. Mitochondria
3. Endoplasmic Reticulum

CELL FUNCTION
1. Contraction
2. Secretion
3. Metabolism
4. Ca transport etc.

MODULATORS
Ionic composition
Phosphate, Na\(_0\),
Na\(_i\), pH\(_0\), pH\(_i\)

Fig. 1 Control, modulation and regulation of cytosolic-free calcium
subsequent excretion. However, the antihypercalciuric effect of phosphorus, mediated through the plasma 1,25-(OH)$_2$D$_3$ is still open to speculation (Kaplan et al., 1977). Phosphate transport in isolated BBMV from intestine exhibit biphasic transport kinetics indicating two components, a saturable Na$^+$-dependent uptake and a linear nonsaturable Na$^+$-independent passive diffusion (Paterlik, 1978; Lee et al., 1984). Phosphorus is absorbed throughout the small intestine and is stimulated by vitamin D$_3$. Hildman et al., (1982) suggested that vitamin D affects the Na$^+$-dependent active transport component. Dietary phosphate supplementation (Ullrich et al., 1977) or deprivation (Caverzasio and Bonjour, 1985; Levine et al., 1991) rapidly invokes a compensatory increase or decrease in renal phosphate reabsorption.

In the human body magnesium is the second most abundant intracellular cation (second only to potassium) and the fourth most abundant total cation in the body. It is recognized as a cofactor in over 300 enzymatic reactions involving energy metabolism, protein and nucleic acid synthesis. Most of the magnesium in human foods comes from plant and animal sources with cereals and vegetables contributing more than 67% of the daily magnesium intake. Studies have demonstrated that the human body contains approximately one mole of magnesium (Aikawa, 1981). About 10% of the total body magnesium is present in serum and interstitial body fluid. Serum concentration of magnesium in humans is about 0.85 mmol/l (Lowenstein and Stanton, 1986) and about one third of serum magnesium is bound by protein. An optimal intake of magnesium of 6 to 10 mg/kg/day has been recommended (Seelig 1981). In the normal individual consuming a balanced diet approximately 40-70%, the ingested magnesium appears to be absorbed (Schwatz et al., 1984; Graber and Shulman, 1986) and the primary site of its absorption is small intestine. However, other studies have demonstrated that major sites of magnesium absorption are colon (Chutkow, 1966; Meneely et al., 1982) and duodenum (Urban and Schedl, 1969; Aldor and Moore, 1970). Intestinal magnesium transport has been reported to occur by solvent drug (Behar, 1974) and/or a saturable process that may or may not (Roth and Werner, 1979) require metabolic energy or by diffusion (Ebel and Gunther, 1980).
filtration-reabsorption mechanism for the renal handling of magnesium has been suggested. Approximately 70-80% plasma magnesium is unfiltrable. In a normal individual, about 2g of magnesium passes from the plasma into glomerular filtrate each day (Quamme and Dirks, 1986) but only about 6% of the filtered magnesium appears in the urine, due to effective tubular reabsorption of magnesium by the kidney. Approximately 65% of the filtered magnesium is reabsorbed in the loop of Henle, while 5-10% is reabsorbed in distal tubule at nephron.

Nephrocalcinosis has been reported by many investigators studying laboratory rats fed on magnesium deficient diet (MacIntyre and Davidson, 1958; Bunce et al., 1980; Al-Modhefer et al., 1986). Magnesium deficiency has also been found to accelerate renal tubular calcium oxalate deposition in rats fed ethylene glycol (Rushton and Spector, 1982; Ebisuno et al., 1987). Tongyai et al., (1989) and Okuno et al., (1990) have shown that magnesium deficiency results in hypomagnesemia, hypophosphatemia, hypercalcemia and hyperphosphaturia. Magnesium has been known to have an inhibitory action on the calcium oxalate crystallization (Hallson et al., 1982). Tiselius et al., (1980) showed that magnesium can increase the solubility of calcium oxalate presumably by forming ion pairs or complexes with oxalate ions, which are thus unavailable for precipitation with calcium. Magnesium ion can act as a stabilizer of thermodynamically unstable calcium oxalate dihydrate and prevents its conversion to calcium oxalate monohydrate which is a major constituent of stones (Toshitsugu et al., 1987). Lindberg et al., (1990) also showed the beneficial effect of magnesium oxide and magnesium citrate against calcium oxalate crystallization among stone formers. It has been found by Koenig et al., (1991) that potassium-magnesium citrate is more useful for the hypomagnesuric or hyperoxaluric patients.

2.3.2.3 Proteins

It has been suggested by Robertson et al., (1979) that animal protein is a dietary source of oxalate precursors (amino acids) in metabolism. Dietary
proteins have a significant influence on the excretion of various constituents of urine, since the major portion of endogenous oxalate is derived from amino acids. Glycine is one of the most important urinary oxalate precursors and nearly 40% of the urinary oxalate is thought to be formed from body glycine pool (Crawhall et al., 1959).

Ribaya and Gershoff (1979) reported that feeding large amounts of hydroxyproline resulted in hyperoxaluria which is more marked than that induced by vitamin B₆ deficiency. Tawashi et al. (1980) also revealed that intra-peritoneal injections of hydroxyproline in rats led to the formation of calcium oxalate dihydrate in the kidneys. Robertson et al., (1981) reported that the excess consumption of high protein diet (i.e. animal protein) increases urinary calcium excretion. Animal proteins contain a significantly higher proportion of aromatic amino acids than the vegetable proteins. These amino acids can contribute to the urinary oxalate in substantially larger amounts when they are present in higher concentrations. Hostetter (1986) has shown that high protein intake, in the form of a meat meal, by young adults raises glomerular filtration rate. The increase in glomerular filtration rate after excess protein ingestion was shown to be mediated by vasodilator hormones such as glucagon, glomerulopressin and dopamine-2 receptor agonists (Mendez et al., 1990). Goldforb (1990) reported that the risk of stone formation is higher in northern and western regions of India as compared to southern and eastern regions, because of the higher animal protein intake by the northern and western region people. Iguchi et al., (1990) also suggested that excessive intake of meat should be avoided for the reduction in stone recurrence rate. Kok et al., (1990) and Trienchieri et al. (1991) reported hypercalciuria, hyperuricosuria, hypocitraturia and decreased ability of urines to inhibit the agglomeration of calcium oxalate crystals, with high animal proteins as compared to controls. Schwille and Herman (1992) have shown that excretion pattern of various inhibitors and promoters of crystallization are changed during the excess dietary protein intake and there is an increased risk of calcium oxalate stone formation.
2.3.2.4 Carbohydrates

Bird (1853) and Suzuki (1934) have shown that intravenous injections of glucose raised the oxalate levels in rabbit blood. Runyan and Gershoff (1965) in their studies have reported that $2^{-14}C$ glucose is a better precursor for oxalate production than $6^{-14}C$ glucose, thereby suggesting that the conversion of glucose occurs via formation of glycolate and may not be primarily via ascorbic acid. Thom et al., (1978) and Rao et al., (1982) have also related the incidence of urolithiasis in the community to the consumption of refined carbohydrates. It was explained by Rofe et al., (1980) that glucose is not a better precursor of oxalate than fructose because hepatocytes utilize fructose more efficiently than glucose. Ribaya et al., (1981) showed that lactose and galactose are also the efficient precursors of oxalate in rats. Thom et al., (1981) and Li et al., (1986) indicated that the urinary excretion of oxalate increases by the high intake of sucrose in humans which can lead to calcium oxalate renal stone formation. Conyers et al., (1990) have shown that probably the conversion of glucose to oxalate occurs through the polyol (glucose-sorbitol-fructose) pathway.

Recently, Kaul (1992) have studied the effect of sugars (galactose and fructose) either fed alone or in combination with pyridoxine deficient diet, and observed maximum hypercalciuria and hyperoxaluria in rats fed vitamin B$_6$ deficient diet supplemented with galactose.

2.3.2.5 Fats

Fats are not known to be directly converted to oxalate in the animal systems. However, excess consumption of fat in the diet may lead to hyperabsorption of dietary oxalate because of the binding of calcium to fatty acids in the intestinal lumen, leaving the free oxalic acid. Even though fatty acids do not contribute to the endogenous oxalate synthesis, phosphatidyl-ethanolamine (PE) may be of some significance since, ethanolamine is released by the action of phospholipase D on PE which is a known precursor.
of oxalate (Nath et al., 1984). Meikle et al., (1990) have shown that diet with high fat content (saturated fatty acids) may affect blood concentration of androgens and thereby the development of the disease related to sex hormones. The urinary calcium and oxalate excretion in the recurrent, hypercalciuric stone formers was significantly reduced with fish oil treatment with its high concentration of eicosapentaenoic acid (EPA) - a polyunsaturated fatty acid, thereby indicating that the incorporation of EPA in the diet could be a unique way of correcting the biochemical abnormalities of idiopathic urolithiasis (Buck et al., 1991). Nutritional deficiency of vitamin B₆ is well known to increase the synthesis of endogenous oxalate and urinary excretion of oxalate. Ravichandran and Selvam (1991) reported the increased lipid peroxidation in vitamin B₆ deficient rats predisposing a condition favourable for calcium oxalate stone formation. Schwille and Herman (1992) reported that the blood levels of cholesterol in numerous stone patients were found to be above the upper limit of normals.

2.4 OXALIC ACID IN IDIOPATHIC CALCIUM OXALATE UROLITHIASIS

Oxalic acid occurs extensively in nature, in rocks, soil, microorganisms, fungi, plants and animals, sometimes as the free acid but more commonly as the potassium or calcium salt. It was discovered by Angelus Sala in the sixteenth century and was found to be associated with urinary stones. Gaglio (1887) and Pohl (1896) postulated that it is not destroyed in the body. In the biological systems, oxalate predominantly occurs in the form of calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) the more stable forms rather than calcium oxalate trihydrate (COT) which is an unstable form. It has been reported by Wiessner et al. (1986) that conversion of COD to COM in biological systems triggers crystalluria and aggregation of this salt, as COM is more membranolytic as compared to COD.

Oxalate the end product of ascorbate and glyoxylate metabolism in mammals, is excreted unchanged in urine (Cuppage and Chonko, 1989). It
is sparingly soluble in biological fluids at physiological pH. Urine is commonly supersaturated with calcium oxalate, however, a number of substances in urine can inhibit calcium oxalate precipitation. When urine exceeds the upper limit of metastability of either calcium or oxalate, spontaneous precipitation of calcium oxalate may occur. This relative insolubility of calcium oxalate in urine bestows upon oxalic acid and its most important physiological role in man, namely, the formation of kidney stones (Smith, 1990).

It was reported by Lonsdale (1968a) and Sutor and Wooley (1974) that calcium oxalate is a common constituent of the nucleus of most calcium stones, moreover, the smallest stones, which probably correspond most likely to a nucleus are composed predominantly of calcium oxalate (Hodgkinson et al., 1969). Also, the commonest crystals observed in freshly voided urine from stone forming patients are those of calcium oxalate (Robertson et al., 1971). Elliot and Ribeiro (1973) concluded from a microscopic examination of 150 consecutively obtained ureteral calculi that whewellite (calcium oxalate monohydrate) is the most important initiating calculus crystal and weddellite (calcium oxalate dihydrate) the most significant secondary deposit. Thind et al., (1989) also classified the urinary stones according to their composition and reported calcium oxalate stones to be most frequently occurring.

Robertson et al., (1979) have provided evidence that when the urinary oxalate concentrations 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. Although precipitation of calcium oxalate depends on the urine saturation with both calcium and oxalate ions in a metastable state it has been proposed that oxalate ion concentration in urine is more significant in the formation of calcium oxalate stones and mild hyperoxaluria is a major risk factor of calculogenesis (Robertson and Peacock, 1980).

The average urinary excretion of oxalic acid for humans is about 30 mg/d with a range of 15-50 mg (Hodgkinson, 1977a). Hyperoxaluria is defined by oxalate levels exceeding this normal range, observed in at least
20% of calcium oxalate stone formers by Hodgkinson (1977b). The amount of oxalate excreted in the urine is determined by dietary intake, intestinal absorption, endogenous synthesis and renal tubular secretion (Wilson, 1990). Jaegar et al., (1985) and Goldfarb (1988) have suggested diet to be an important factor for the causation of hyperoxaluria whereas two other abnormalities playing a role in the development of hyperoxaluria have been emphasized as:

i) Abnormal membrane transport of oxalate in red blood cells (Baggio et al., 1986) and kidney (Wilson et al., 1988 and Manoharan et al., 1989).

ii) Abnormal endogenous production of oxalate (Harrison et al., 1981).

Thus, either abnormal synthesis or hyperabsorption or a defective transport of oxalate across renal tubular membrane can lead to the serious condition referred to as hyperoxaluria. Hyperoxaluria can be congenital or acquired (Seftel and Resnick, 1990). Congenital hyperoxaluria is subclassified into primary type I, glycolic aciduria and type II, L-glyceric aciduria. Both are autosomal recessive disorders that do not have a sex predilection. In type I hyperoxaluria, there appears to be deficiency of the enzyme 2-oxo-glutarate: glyoxylate carboligase, which leads to an accumulation of glyoxylate, forming glycolate and oxalate via oxidation. The enzyme defect in type II hyperoxaluria is in D-glyceric acid dehydradenase, which leads to excessive synthesis and excretion of L-glyceric acid and oxalate (Williams and Smith, 1978). Acquired hyperoxaluria can be found in patients with pyridoxine deficiency or with enteric hyperoxaluria. Bile acid malabsorption, steatorrhea, diarrhea and increased permeability of colon by fatty acids, associated with increased oxalate intake aggravate the hyperoxaluria leading to stone formation (Smith, 1980). Enteric hyperoxaluria due to malabsorption has been well documented to cause renal calculi and chronic tubulointerstitial renal damage (Gelbart et al., 1977; Drenick et al., 1978; Smith, 1980; Wharton et al., 1990).
The conditions like loss of water, magnesium, electrolytes, bicarbonate, lead to calcium, oxalate and uric acid crystallization and are ideal for spontaneous nucleation of calcium oxalate resulting in the formation of urinary stones (Fig. 2).

2.4.1 Sources Of Oxalic Acid In The Body

Oxalic acid, the end product of metabolism and the normal constituent of human urine is derived partly from the diet and partly from metabolic processes (endogenously) in the body. Various endogenous precursors of oxalate biosynthesis are ascorbic acid, glycine, serine, glycolate, hydroxyproline and glyoxylate etc.

2.4.1.1 Dietary oxalate and glycolate and their intestinal absorption

The oxalate content of many processed and unprocessed foods and vegetables have been measured by Hodgkinson (1977a). In India, leafy vegetables are consumed in large amounts and the daily intake of oxalate varies from 78 mg to 2.0 g per day depending on the seasonal variation (Singh et al., 1972). The authors have determined the calorific value, oxalic acid and calcium content of various Indian diets and demonstrated a marked increase in oxalate intake among the rural population during the season when amaranthus, purslane and spinach are abundant and the oxalate intake exceeds the calcium intake by almost 2:1 on a molar basis. Further, Singh (1973) described that beet root, rhubarb and spinach by themselves are not good sources of minerals because in rhubarb and beet root the molar concentration of oxalate equals or exceeds the concentration of calcium while in spinach it may exceed the combined concentrations of calcium and magnesium. Spinach, rhubarb, chocolate, tea and peanuts etc., when taken in excessive amounts have been reported to cause hyperoxaluria in patients of calcium oxalate nephrolithiasis (Finch et al., 1981; Nordan-Vall, 1982). The oxalate absorption by the intestinal tract can be determined by measuring the increment in urinary oxalate, after oral ingestion (Barilla et al., 1978; Brinkley et al., 1981). In this way bioavailability
Fig. 2  Gastrointestinal disorders and pathogenesis of urolithiasis
of oxalate from various oxalate rich food items was calculated by expressing oxalate absorption as the percentage of total oxalate contained in foods. Brinkley et al., (1990) categorized the oxalate rich food as high risk, moderate risk, mild risk and negligible risk food depending upon the bioavailability of oxalate, and the amount of bioavailable oxalate that could be used to rate the stone forming potential of various oxalate rich foods. Spinach represented the high risk food item whereas instant tea, peanuts chocolate and almonds represented the moderate risk food items.

Oxalate absorption in rat and rabbit ileum mucosa has been shown to be linear (10 \mu M-2.0 mM) and remains unaffected by ouabain and 2,4-dinitrophenol, suggesting that oxalate is absorbed by a passive, nonsaturable, non-energy dependent mechanism (Binder, 1974). A small percentage (2%-5%) of the ingested oxalate is known to be absorbed (Prenen et al., 1984) and jejunum in the small intestine is the major site of oxalate absorption (Madorsky and Finlayson, 1977). Hyperabsorption of oxalate from the gut has been shown to be an important factor in etiopathogenesis of calcium oxalate calculi. In idiopathic stone formers, an increased absorption of oxalate has been reported by Marangella et al., (1982) and Lindsjo et al., (1989).

The \(^{14}\)C-oxalic acid uptake using intestinal everted rings, was measured in pyridoxine deficient and pair-fed control rats by Farooqui et al., (1984). The oxalate uptake rates were significantly increased from the intestines of B\(_6\)-deficient rats as compared to controls. Moreover, uptake in control rats followed a passive diffusion, whereas in B\(_6\)-deficient rats, uptake indicated a two component system - a saturable component at low concentrations and a linear nonsaturable passive diffusion component at higher concentrations, thereby suggesting the involvement of an oxalate transport carrier in B\(_6\) deficiency. Further kinetic studies done by Koul et al., (1991) also suggested the involvement of a membrane protein in oxalate binding and transport in rat intestinal brush border membrane in pyridoxine deficiency.

Bile acids and long chain fatty acids are known to increase oxalate absorption in the colon probably by causing a non specific increase in permeability.
(Kathpalia et al., 1984). In the gut, calcium forms soap with fatty acids, leaving less ionized calcium available to complex with oxalate, thus more oxalate remains in a soluble, easily absorbable state (Anderson and Gillberg, 1977; Hylander et al., 1978). It has been reported by Sharma and Schwille (1992) that in rat, oxalate transport along the gastrointestinal tract was markedly delayed when oral calcium was given simultaneously while diets restricted in calcium result in hyperoxaluria (Larson and Tiselius, 1987). Magnesium has also been found to decrease oxalate absorption as measured by decrease in urinary oxalate (Barilla et al., 1978; Berg et al., 1986). Studies by Knickelbein et al., (1986) and Yamakawa and Kawamura (1990) demonstrated the presence of oxalate: OH⁻ and oxalate: Cl⁻ exchange system on the rat and rabbit, intestinal brush border membrane.

Glycolate, an immediate precursor of both glyoxylate and oxalate, leads to hyperoxaluria and calcium oxalate crystal deposition (Richardson, 1965; Murthy et al., 1981). Glycolate can be oxidized directly to oxalate by glycolic acid dehydrogenase (Fry and Richardson, 1979a) or via glyoxylate catabolised by glycolic acid oxidase (Gibbs and Watts, 1973; Butz et al., 1980). Glycolate feeding in laboratory animals has been a widely used model for induction of hyperoxaluria and stone formation (Varalakshmi et al., 1990; Selvam and Varalakshmi, 1990; Selvam and Bijikurien, 1992; Sangeeta et al., 1993). Glycolate is known to be present in various food items in substantial amounts. Leafy vegetables and fruits like lemon, grapes and pear, and beverages like tea and coffee contain considerable amounts of glycolate (Harris and Richardson, 1980). Nearly 5% or more of the urinary oxalate is derived from the dietary glycolate. Glycolate absorption leads to hyperoxaluria and calcium oxalate crystallization in kidneys when fed in diet to rats (Murthy et al., 1981).

However, little is known about biochemical mechanisms of glycolate absorption. Talwar et al., (1984) showed the existence of a carrier-mediated glycolate transport system in the rat intestine. Glycolate absorption is maximum in the ileum and jejunum, followed by duodenum and is lowest in the colon. Yendt and Cohanim (1981), observed hyperoxaluria and hyperglycolicaciduria
in patients with calcium oxalate urolithiasis; but they could not find any correlation between these two abnormalities. The increased urinary excretion of glycolate had a positive correlation with dietary proteins and the urinary excretion of uric acid, suggesting that this increase was due to dietary effects. Recently, Sutton et al., (1992) have estimated the plasma and urinary oxalate and glycolate levels in normal subjects and idiopathic calcium stone formers. Both plasma and urinary glycolate levels were found to be lower in female stone formers than female non-stone formers and male stone formers. The urinary glycolate correlated positively with urinary urea, urate and sulphate and again probably it is attributable to dietary factors.

2.4.1.2 Endogenous synthesis of oxalate

The endogenous synthesis of oxalate amounts to be 80-90% in normal cases (Hodgkinson, 1977a; Arora et al., 1985). The biosynthesis of oxalate mainly takes place in the liver and the main immediate precursor in man and other animals are ascorbic acid and glyoxylate each accounting slightly less than one-half of the total endogenous production (Gambardella and Richardson, 1977; Schmidt et al., 1981). The remainder of urinary oxalate is derived from glycolate, various amino acids and other food sources. The various precursors of endogenous oxalate synthesis and their relative contribution to the oxalate pool is shown in Fig. 3. Except ascorbate, all other precursors converge to central glycolate-glyoxylate pathway (Fig. 4). The major enzymes involved in oxalate biosynthesis are glycolic acid oxidase (GAO), glycolic acid dehydrogenase (GAD) and lactate dehydrogenase (LDH) and xanthine oxidase (XOD). However, studies have demonstrated that XOD plays a minor role in oxalate production (Gibbs and Watts, 1973).

2.4.1.2.1 Glycolic acid oxidase (GAO) (E.C.1.1.3.1)

The enzyme which converts glycolate to glyoxylate and then to oxalate is glycolic acid oxidase (GAO) (Dohan, 1940). Kun et al., (1954) identified and partially purified this enzyme from rat liver and showed it to be a flavoprotein containing FMN and requiring molecular energy for its activity.
Fig. 3  Precursors of endogenous oxalate synthesis
Ushijima (1973) purified rat liver GAO and showed that this enzyme has a pH optima of 8.5 and it has high affinity for glycolate ($K_m$ $2.5 \times 10^{-4} \text{M}$) as compared to glyoxylate ($K_m$ $1.4 \times 10^{-4} \text{M}$). This enzyme is inhibited by p-chloromercuribenzoate, potassium cyanide and copper sulphate. Oxalic acid is a partial competitive inhibitor of both glycolate and glyoxylate with $K_i$ of $2.5 \ \mu\text{M}$. The major role of GAO is suggested by the correlation between the increased level of this enzyme in the liver and hyperoxaluria in the rat (Liao and Richardson, 1973). Askar and Davis (1983) purified GAO from rat liver and showed that this enzyme has a $K_m$ of $0.25 \ \mu\text{M}$ for glycolate and $2.9 \ \mu\text{M}$ for glyoxylate. The increased activities of glycolic acid oxidase in sodium glycolate fed rats (Murthy et al., 1983), Vitamin B$_6$ deficient (Murthy et al., 1982; Nath et al., 1990), Vitamin B$_1$ deficient (Sidhu et al., 1987) and in Vitamin A deficient (Sharma et al., 1990) rats, suggest an important role of this enzyme in endogenous oxalate synthesis, thereby leading to hyperoxaluria. Recently, Rattan et al., (1993) have also reported an enhanced liver GAO activity in magnesium deficient rats.

2.4.1.2.2 Glycolic acid dehydrogenase (GAD) (E.C. 1.2.1.17)

Glycolate may be directly converted to oxalate without involving free glyoxylate as an intermediate by glycolate dehydrogenase (GAD), the enzyme located in the liver of both rat and man (Fry and Richardson, 1979a). The pH optima for the enzyme is 6.1 and does not require FMN, NAD or NADP). This enzyme has been shown to have a molecular weight of 14,000 daltons and its $K_m$ for glycolate is $6.3 \times 10^{-5} \text{M}$. It is specific for glycolate, while glyoxylate is inhibitory. Experiments carried on Vitamin B$_6$ deficient rats showed that the conversion of glycolate, ethylene glycol and ethanolamine to oxalate, increased 18,10 and 14 fold respectively, while the conversion of glyoxylate and glycine only increased 1.2 and 1.4 fold respectively (Runyan and Gershoff, 1965). Liao and Richardson (1972) observed that perfused rat liver converts about 61% of glycolate to oxalate whereas only 28% of glyoxylate is oxidized to oxalate. These observations indicate that glyoxylate is not an obligatory intermediate in the oxidation of glycolate to oxalate. On the contrary,
Metabolic pathways of oxalate biosynthesis in animals

Fig. 4

2,3-DIKETO-L-GULONATE
recent studies by Yanagawa et al., 1990 demonstrated that in rat and human liver, the formation of oxalate from glycolate takes place predominantly via glyoxylate by the combined action of GAO and xanthine oxidase (XOD) or LDH. The hepatic GAD activity was significantly found to be increased in both Vitamin A and B₆ deficient rats (Sharma et al., 1990).

2.4.1.2.3 Lactate dehydrogenase (LDH) (E.C. 1.1.1.27)

Lactate dehydrogenase has been suggested as a major enzyme of oxalate biosynthesis in leucocytes and erythrocytes (Richardson and Liao, 1973) and also in the 10,000g fractions of human liver and heart tissues (Gibbs and Watts, 1973). Smith et al., (1972) proposed that in tissues other than liver, LDH accounts totally for the oxalate synthesis from glyoxylate. The isoenzymes of LDH have been shown to catalyze the oxidation of glyoxylate to oxalate with different affinities (Banner and Rosalki, 1967). LDH isoenzyme V (muscle-type) has a $K_m$ of 30 mM whereas isoenzyme I (heart-type) has a $K_m$ of 5 mM for glyoxylate for the oxidation reaction. Warren (1970) showed that oxalate is a competitive inhibitor for the oxidation of glyoxylate and non-competitive inhibitor for the reduction of glyoxylate by LDH. The pH optima for the LDH catalyzed oxidation to glycolate with NADH is 6.9. Duncan (1980) showed that LDH-NADH cannot dissociate easily at pH 7.0 but can reduce glyoxylate to glycolate. As the pH increases, this dissociation is made easier and binding of LDH with NAD⁺ becomes stronger causing oxidation of glyoxylate at pH 9.6. Askar and Davis (1983) purified LDH from rat liver by affinity chromatography and showed a $K_m$ of 5.0 $\mu$M for glyoxylate in the glyoxylate to oxalate oxidation reaction while in glyoxylate to glycolate reduction reaction, its $K_m$ is 14.0 $\mu$M. Oxalate acts a competitive inhibitor in the oxidation reaction with a $K_i$ of 0.1 mM while inhibition of the reduction reaction was a mixed type. The role of kidney LDH in endogenous oxalate production in Vitamin B₁ and B₆ deficient hyperoxaluric rats was investigated by Sidhu et al., (1985). Although, the total kidney LDH activity remained unaltered in both the cases, thiamine deficiency produced a significant increase in the relative abundance of LDH I and II isoenzymes and a compensatory
decrease in LDH V, indicating that LDH contributes significantly to production of oxalate from glyoxylate. The role of LDH I in oxalate biosynthesis has also been supported by a significant increase in this isoenzyme activity in the kidneys of rats with surgical induction of bladder stones (Thind and Nath, 1977).

2.4.2 Renal Handling Of Oxalate

Hyperoxaluria is a frequent finding in idiopathic calcium oxalate stone formation (Robertson et al., 1981; Baggio et al., 1983; Nath et al., 1984; Hesse et al., 1992). Since oxalate is an important and the most common constituent of human calculi, it is of interest to know its intrarenal distribution and transport. Kidney contains more oxalate than any other tissue on wet weight basis (Hodgkinson and Zarembski, 1968) the rat kidney papilla containing maximum amount of oxalate followed by medulla and cortex (Wright and Hodgkinson, 1972). The epithelium of the proximal tubules, mediates the translocation of solutes and fluid (Gregor et al., 1980) and lots of interest has been generated in isolating its main absorptive site i.e. brush border membrane (BBM). Direct uptake measurements for a number of solutes have now become feasible with the help of isolated BBM vesicles. Renal brush border membrane vesicles have been widely used for the uptake of various substances such as D-glucose (Ghishan and Wilson, 1985; Bertloot et al., 1991) monocarboxylic acids (Nord et al., 1982), dicarboxylic acids (Gupta, 1986), urate and p-aminohippurate (Kahn et al., 1983; Dan and Koga, 1991), nucleoside (Williams and Jarvis, 1991). Therefore, emphasis has been given to study the mechanism of oxalate transport in kidney BBM vesicles, to understand the etiopathogenesis of hyperoxaluria.

2.4.2.1 Physiology and biochemistry of kidney

The starting points from which one must consider the physiology of the kidney, are the structure of nephron and a comparison of the composition of the fluid reaching the kidney (blood plasma) and the fluid leaving it
(urine). For example, to account for the absence of protein and glucose in urine, for the very high concentration in urine of ammonia and creatinine, when other substances - such as sodium and calcium may appear in nearly the same concentration in urine and plasma. The kidneys are of considerable importance in homeostasis. These perform the function of excretion of nitrogenous wastes and preserve the constancy of the extra-cellular fluid in composition, volume and pH. The kidneys each of which weigh between 120-170 g, are the paired organs, lying behind the peritoneum on either side of the vertebral column. The hilum of the kidney is an indentation, through which pass the renal arteries and veins and nerves, when cut longitudinally, the renal substance consists of an outer cortex and an inner paler medulla made up of pyramids, the apices of which project into calyces.

The functional unit of the kidney is the nephron (Fig. 5), a tubule about 5 cm long, begins as a blind dilated end, glomerular capsule and opening into collecting tubule. There are approximately 30,000 to 34,000 nephrons in each adult rat kidney. The upper end of each nephron lies in the cortex, invaginated and expanded by a cluster of capillaries. The wall of a glomerulus capsule is made of flattened cells but change fairly abruptly to cuboidal cells at a point where the neck of the capsule becomes the first segment of the proximal tubule. The first or proximal convoluted tubule, about 14 mm long is continuous with the relatively straight descending limb of the loop of Henle which passes into the medulla. The tube then forms a U-bend and returns to the cortex as the ascending limb of the loop. The tubule continues on to form the second or distal convoluted tubule, which terminates as the junctional tubule by joining with a collecting tubule which opens into one of the calyces of the ureter. The filtering membrane of the renal corpuscle consists of three layers (1) an endothelial layer (2) a basement membrane and (3) an epithelial layer.

The principal mechanism by which the nephron clears the plasma of unwanted substances is the filtration of blood plasma through the glomerulus membrane into the tubules, where the unwanted substances fail to reabsorb
Fig. 5 Structural organization of the kidney
and pass into the urine, while the substances like water and electrolytes are reabsorbed back into the plasma of peritubular capillaries. The glomerulus membrane does not normally allow large amounts of plasma protein to pass, although small quantities of protein are present in glomerular filtrate which are reabsorbed by the tubules. The presence of protein in the urine, a common finding in renal disease, implies an increased leakage through the glomeruli rather than defective tubular reabsorption. However, proteinuria is not infrequently found where there is no renal disease.

The cells of the proximal tubules are large, columnar type with a well marked brush border. Each cell has about 150 thin microvilli (about 1μm long) per square micron, which enormously increase the surface area of the cells in contact with the fluid in the lumen of the tubule. The cells of the distal convoluted tubule also have microvilli but these are too small to produce a brush. The brush border membrane is considered to be mosaic of structural and functional proteins intercalated in lipid bilayer with some carbohydrates. In the renal brush border membranes, several enzymes show characteristically large and comparable increase in specific activities relatively to that in the cortical homogenate. These include trehalase, maltase, alkaline phosphatase, 5’-nucleotidase and amino-peptidases. In mammals, about 75% of the total solids are reabsorbed from the glomerular filtrate in the proximal tubules in such a way that the fluid remaining in the lumen of the tubule remains isotonic with arterial blood although the pH may fall. The solids are reabsorbed in unequal proportions and at different situations; thus the first part of the proximal tubule reabsorbs amino acids, glucose and almost completely the small amounts of protein that pass through the glomerulus; the reabsorption of phosphate, which is however incomplete, also occurs in this area. Sodium, chloride and bicarbonate are probably reabsorbed uniformly along the length of the proximal tubule as well as in the distal tubule while potassium is reabsorbed in the proximal and secreted into the distal tubule. The loop of Henle reabsorbs only 5% of the water, whereas the distal tubule, where the fluid is approximately isotonic removes about 20% of water.
2.4.2.2 Oxalate uptake in mammalian renal brush border membrane vesicles

The epithelium of the kidney proximal tubules, which mediates translocation of fluid and solutes, is characterized by cells with determined polarity. This asymmetry is evident ultrastructurally by differentiation of the plasma membrane into two distinct compartments. In comparison to intact cells or tissues, the vesicle system has a number of advantages for studying the transport mechanisms. The major advantages are the absence of metabolism for many compounds and the control over the composition of solution on both sides of the membrane. Because molecules of biological interest are metabolised by intact cells, transport studies in the past relied to a large extent on non metabolisable analogues. In transport with isolated membranes direct uptake measurements have become feasible for metabolizable solutes also. Studies by Weinman et al., (1978) and Deetjen et al., (1979) showed that oxalate undergoes a bidirectional transport in the rat proximal tubule which results in net secretion. The secretory flux in the proximal tubule exhibits all the characteristics of an active, saturable organic anion transport mechanism while the kinetics of the reabsorptive flux are best explained by a simple passive diffusion mechanism. Knight et al., (1981) posulated the presence of high affinity, low capacity transport system operating at low plasma oxalate concentration and a low affinity high capacity oxalate transport system operating at high plasma oxalate concentration in the proximal tubule. Immunological studies done by Drenckhahn et al., (1985) have demonstrated the occurrence of a protein in the collecting ducts that is homologous to the anion channel protein of red blood cells. Karniski and Aranson (1987) using subfractionated rabbit membrane vesicle preparations have described an apical transporter capable of exchanging oxalate for chloride and formate. Bicarbonate and sulphate had little effect on oxalate exchange, which distinguished it from the isolateral transporter. The apical exchange of oxalate was inhibited by DIDS. Kuo and Aranson (1988) as well as Ulrich and Rumrich (1988) using rabbit basolateral membrane vesicles and peritubular capillary microperfusion stop-flow technique respectively, demonstrated that oxalate could
be exchanged for bicarbonate and sulfate. This oxalate/sulphate or bicarbonate exchange system was found to be located on the contraluminal (basolateral) membrane of the proximal tubular cells. On the contrary, studies by Yamakawa and Kawamura (1990) found the presence of an oxalate transport system on the brush border membrane of proximal tubular cells and demonstrated that oxalate transport consists of not only passive diffusion but also an oxalate: OH exchange pathway. Wandzilak and Williams (1990) have also examined the transcellular movement of oxalate in renal tissue and described the presence of two distinct transporters, one on the apical (luminal) and one on the basolateral (contraluminal) membrane and oxalate can use both.

In renal tubular cells from stone forming animals, oxalate uptake was markedly altered. Gupta et al., (1988) observed the elevated oxalate reabsorption by proximal tubular cells in acute subclinical and chronic pyridoxine deficient rats. Renal clearance of oxalate in hyperoxaluric rats has been shown to be higher than that of inulin, thereby indicating that oxalate is secreted by the proximal tubules (Kanazawa, 1990). Sigmon et al., (1991) examined oxalate uptake in suspensions of renal cortical and papillary cells derived from control and stone forming animals. In control animals, both renal cortical and papillary cells exhibit a time dependent accumulation of oxalate that is inhibited by DIDS and sensitive to extracellular pH, with acidic pH stimulating and alkaline pH inhibiting net oxalate accumulation. In renal tubular cells from stone forming animals, oxalate handling was markedly altered, showing a reduced uptake in cortical cells and an enhanced uptake in papillary cells. Baggio et al., (1986) predicted that oxalate flux would show similar alterations in the renal papilla from stone forming patients as was observed earlier (Baggio et al., 1984) in red blood cells from these patients. Thus Sigmon et al., (1991) suggested that, possibly these alterations in renal cell oxalate transport may contribute to CaOx stone formation.

Ebisuno et al., (1992) and Koul et al., (1992) characterized the oxalate transport in LLC-PK\textsubscript{1} cells, an epithelial cell line with characteristics of proximal tubular cells. These cells showed a time and concentration dependent
accumulation of radio-labeled oxalate, which was almost completely abolished by DIDS, the uptake was sensitive to external pH. These findings are thus consistent with the operation of at least two polarized transport systems for oxalate in LLC-PK1 cells, with oxalate: Cl⁻ exchange at the luminal membrane surface and SO₄²⁻ (oxalate): HCO₃⁻ exchange facing the growth substrate. Wandzilak et al., (1992) also reported that the oxalate transport in the same cell line occurs in both apical to basolateral and basolateral to apical directions.

2.5 PROCESS OF CALCIUM OXALATE CRYSTALLIZATION

In the formation of calculi within the urinary tract, two fundamental factors are critical when crystals precipitate and stones form (Smith, 1990). They are the solute and the solution itself, which must be supersaturated in the precipitating crystalline phase. Therefore, the simplest explanation of stone disease is that it is due to oversaturation of urine with respect to stone forming solutes. The urinary tract is a flow system, where in order to regulate body fluids, urine is produced by filtration and subsequent reabsorption processes. Urine is an extremely complex solution containing varied forms of solutes and its state of saturation depends on the concentration of these solutes, ionic strength, complexation and pH etc. All these factors determine the tendency of the urine to form crystals.

Different levels of saturation of urine are undersaturated, supersaturated or oversaturated (Fig. 6). At saturated concentration of a solution, both solid and liquid phases of stone salt are in equilibrium with each other; whereas spontaneous precipitation occurs at the oversaturation concentration. The concentration between saturation concentration and oversaturation concentration is known as the metastable region.

Crystal formation within the urinary tract is a dynamic process, which involves several steps like nucleation, phase transformation, aggregation, disaggregation and dissolution. Werness et al., (1981) demonstrated the simultaneous occurrence of many of these steps in the urines of active stone formers. Crystal nucleation is the establishment of smallest unit lattice of a
Fig. 6 Different levels of saturation for a soluble salt in urine
crystal species and can be either homogeneous (i.e. pure nucleus) or heterogeneous, a foreign body or other crystal form which can lower the formation product, with resultant crystallization. Most urines from stone formers as well as from normal subjects are metastably supersaturated with calcium oxalate (Robertson, 1976). In the supersaturated urine a crystal will thus form either by a process of homogeneous or heterogeneous nucleation and this crystal will subsequently grow or aggregate to give rise to stone formation (Finlayson, 1977). It was reported by Garside (1982) that nucleation in biologic systems usually occurs as heterogeneous nucleation because the amount of supersaturation required for heterogeneous nucleation is much less than that required for homogeneous nucleation. Several other workers (Lonsdale, 1968b; Coe et al., 1975; Meyer et al., 1975,1976; Meyer, 1981) have studied the epitaxial relationships in urolithiasis and described that crystals of one type may be nucleating another if the urine is appropriately supersaturated. In the absence of dust particle, seed crystals or other foreign surfaces, homogeneous or spontaneous nucleation occurs at elevated levels of lattice ion activities. However, in vivo, stone minerals are more likely to precipitate at pre-existing solid/solution interfaces thus giving rise to heteronucleation (Nancollsas et al., 1991). Khan (1991) has also suggested that in low to medium grade chronic hyperoxaluria, nucleation of calcium oxalate appears to be heterogeneous, because in these situations, both urinary oxalate and calcium oxalate supersaturation are not high enough for the homogeneous nucleation. Normal urinary oxalate is less than 1 mmol/l. In experimentally induced high-grade chronic hyperoxaluria, the homogeneous nucleation could be possible only if urinary oxalate reaches more than 20 mmol/l (Khan, 1991).

In stone formers, calcium oxalate dihydrate crystals get converted into calcium oxalate monohydrate, a more stable form and more prone to precipitation in urine as compared to calcium oxalate dihydrate (Ligabue et al., 1979). This precipitation then leads to crystal binding, resulting in formation of larger clusters. This aggregation could be the mechanism which distinguishes simple crystalluria, which occurs in most normal people, from stone formers (Robertson and Peacock, 1972). Thus size of the particles formed is also a
crucial element in stone formation and this is mainly governed by the kinetic processes of crystal growth and crystal aggregation. It is well known, for example, that small particles can be formed and excreted in the urine without causing any problem and healthy subjects can exhibit significant degrees of hyperoxaluria (Robertson, 1977; Werness et al., 1981). A single crystal can never reach a size large enough to remain in the narrow parts of the urinary tract by the slow process of crystal growth alone (Finlayson, 1977; Burns et al., 1984), in contrast large particles can be formed within a short period by the fast process of crystal aggregation, and the average renal stone clearly has an agglomerate structure (Ismail and Towashi 1980; Rodgers, 1983; Iwata et al., 1985). Kok et al., (1986) found a clear evidence for a disturbance in the process of crystal agglomeration in a group of stone formers with very high stone production rate. Also the impaired inhibitory activity of the urines of stone formers on this physicochemical parameter i.e. on the process of crystal agglomeration was clearly related to stone frequency. The lower the degree of control of crystal agglomeration the higher the stone frequency (Kok et al., 1990).

Another essential step in stone development, is the retention of incipient stones in the urinary tract. Urine moves from the glomerulus through the nephron into the collecting system in 2 minutes or less, a period that does not allow the usual urinary crystals to grow to such a size that they could be retained. Several theories have been suggested to explain crystal retention. Randall (1937, 1940) noted submucosal calcified plaques in renal pelvis, usually on the papillary tip, that had a crystalline composition (now termed as Randall plaques). These were postulated to be the cause of heterogeneous nucleation with crystal retention and growth. In 1953, Carr suggested that renal calculi were initiated following obstruction of the lymphatic system. Oliver et al., (1966) noted the intracellular calcification within the kidneys of magnesium-deficient rats. Studies by Vermulen et al., (1967) using oxamide for experimental induction of crystalluria, clearly demonstrated crystal retention by rat papilla with subsequent tubular necrosis. Finlayson (1974, 1978) suggested the fixed particle theory, which stated that crystals were attached
in some way along the collecting system to allow the crystals to grow to such a size that they would be retained as stones. Hautman et al., (1980) and Graves (1982) also suggested that renal anatomy can provide a favourable milieu to form crystals. (both intra and extracellular). Khan et al., (1982) while using sodium oxalate to induce crystalluria, found evidence for crystal attachment to papillary epithelium, epithelial basal lumina and areas of epithelial necrosis. Riese et al., (1988) also showed the adherence of calcium oxalate crystals to cultured papillary epithelial cells. Khan (1991) has suggested that crystals may nucleate in one part of the nephron and be retained in another part. Further the primary site for calcium oxalate crystal retention in kidneys, is the renal papilla, where retention is accomplished by the involvement of tubular epithelium and its basement membrane. Subcutaneous injections of gentamicin sulfate in moderately hyperoxaluric rats. (Khan, 1991; Kumar et al., 1991) resulted in membrane shedding from the proximal tubules, thereby explaining crystal formation and their retention. Mandel and Riese (1991) also proposed that in urolithiasis, it is likely that some form of renal tubular injury or damage to epithelial cell membranes in the collecting ducts such as loss of intercellular tight junctions exposes basolateral or basement membrane molecules, and that this event is a necessary requirement for successful crystal retention and calculi development. Further, these processes of nucleation, growth and aggregation of crystals are controlled by various promoters and inhibitors of crystallization.

2.5.1 Promoters Of Crystallization

All urinary stones contain an organic matrix, regardless of the chemical nature of their mineral content. This organic material inside the stones must have acted as a heterogenous nucleator of crystals and promoted calculogenesis. A great emphasis was laid on the role of organic compounds in urine (Boyce and Garvey, 1956). A number of calcium binding macromolecules have been isolated from stone matrices and shown to influence calcium oxalate crystallization. These macromolecules are increased in amount (Boyce, et al., 1954) and are also qualitatively different in urine samples from stone formers than those
from normal people (King and Boyce, 1963). The exact chemical nature and origin of these macromolecules is still unclear.

The stone matrix could be considered as the non-dialyzable remainder of the stone after its crystal component has been dissolved with a mild solvent and accounts for 2-3% of the stone content (Rao et al., 1978; Khan and Hackett, 1984). Malek and Boyce (1973) characterized matrix as a mixture of serum proteins and mucoproteins of urine. The only difference in the composition of the matrix protein and urinary uromucoid, is the absence of sialic acid residue in the former, which has been postulated to be due to cleavage of the acid residues by the renal sialidase. Although, Whatanabe (1972) suggested that matrix only functions to bridge crystals together, Finlayson (1974) reported that simple coprecipitation cannot explain all the interactions observed between stone crystals and the matrix and suggested that polymerization of mucoids occurs to form the stone matrix.

Immunological studies of stone matrix have shown the presence of albumin, alpha-1 and alpha-2 globulins and occasionally \( \gamma \)-globulins (Resnick and Boyce, 1979). It has been shown that 85% of the active calcium oxalate stone formers had low molecular weight urinary proteins (35 kD) present in their urine, whereas none of control patients or inactive stone formers had these proteins (Resnick and Boyce, 1979). Paternain et al., (1980) have reported the isolation and characterization of a mucoprotein possessing mineral nucleating activity. This protein has a molecular weight of 51 kD, isoelectric point of 2.8, its hexuronic and sialic acid content is quite small, while glycine and glutamic acid contents are higher.

In the urine, it has been suggested that matrix exists in soluble form of relatively small size. It condenses or polymerizes by some mechanism to form the large insoluble form of matrix in the setting of stone formation. This condensation may be stimulated by the presence of crystals or may precede the crystal formation (Smith, 1982). The role of urinary uromucoids (Tamm Horsfall Mucoprotein) as promoters of calcium oxalate has been emphasized by Rose and Sulaiman (1982, 1984a,b) and Singh et al., (1989).
Ultrafiltration of urine leads to a large reduction in calcium oxalate crystal formation, which is largely restored by the addition of human urinary Tamm-Horsfall protein (uromucoid). Nancollas et al., (1989) suggested that various protein fractions or components in urine, like human serum albumin, polyglutamic acid and polyaspartic acid are capable of nucleating calcium oxalate monohydrate at particular concentrations. Electron microscopical studies clearly indicate that matrix influences the orientation of subsequently formed crystals i.e. there is a definite promoting effect on nucleation of whatsoever type of stone formed (Nancollas et al., 1989).

The presence of γ-carboxyglutamic acid (GLA) in kidney stone matrix was reported by Lian et al., (1977) and Joost et al., (1981). The excretion of urinary GLA is 2-3 times higher in calcium stone formers than in normal subjects. GLA is an amino acid with a high affinity for calcium. It is found in urine both as free amino acid and incorporated into proteins such as osteocalcin (Lian and Gundberg, 1988). Nishio et al., (1990) reported that GLA can cause significant changes in the crystallization kinetics, but the effect was dependent on calcium concentration. While at 4 mM calcium concentration GLA decreased the growth rate, at 12 mM calcium concentration, the reverse occurred. At all concentrations of calcium tested, GLA caused a significant increased crystal mass to be produced, thus GLA is a modifier of calcium oxalate crystallization and could act as a promoter of stone formation in vivo, particularly at moderately elevated levels of calcium excretion. Dussal et al., (1992) have recently detected a protein, which they call lithostathine, in all types of stones viz. calcium oxalate, uric acid, struvite stones. This protein is immunologically related to that of pancreatic lithostathine. A major matrix component of calcium oxalate monohydrate stones, called uropontin, as aspartic acid rich protein (150 μg/100 mg of stone) has been reported by Hoyer (1992) which has also been detected in human urine by monoclonal antibody immunoadfinity chromatography. Thus presence of organic matrix, an integral part of all urinary stones, has long been thought to be essential for stone formation (Khan and Hackett, 1992).
2.5.2 Inhibitors Of Crystallization

Several inhibitors of calcium oxalate crystal growth and aggregation have been so far identified in human urine but it has not been possible to assess the accurate percentage contribution of each of these to the total inhibitory activity. It has been demonstrated that the effect of various inhibitors are additive and thus each contributes partly to the solubilization of calcium oxalate (Drach et al., 1982; Azoury et al., 1984). The inhibition of crystal growth and aggregation can be affected by diminution of supersaturation with stone forming substances such as calcium, oxalate and uric acid. Some other substances (e.g. magnesium, citrate, etc.) can modify the ability of inorganic microcrystals to grow and form stones (Goldwasser et al., 1986; Watts, 1989; Grases et al., 1989). Many investigators found an imbalance between promoters and inhibitors of stone formation in adults with urinary stones (Robertson et al., 1981; Francois et al., 1986; Laminski et al., 1990). The calcium stone formers may have deficiency of not one but several of the inhibitors that are present in the urine. The nature of some of these inhibitors is discussed below.

2.5.2.1 Trace elements

The inhibitory effect of trace elements like copper, zinc, tin, lead, aluminium on crystal growth of calcium oxalate was studied by Meyer and Angion (1977). When magnesium is excreted into urine in significant amounts it tends to increase the solubility of calcium phosphate and calcium oxalate (Hallson et al., 1982). Effect of high magnesium concentration is due to a complex formation between magnesium and oxalate. Nephrocalcinosis has also been reported by Al-Modhefer et al. (1986) in magnesium deficient rats. Boskey and Posner (1982) postulated that magnesium acts by inhibiting the formation of calcium-acidic phospholipid phosphate complex by competing with \( \text{Ca}^{2+} \) for sites on the phospholipid moiety. Once this complex was formed, magnesium ions were found to have no effect on calcium-oxalate crystallization or precipitation (Nunziata et al., 1984). Zinc has been often implicated in
the inhibition of urinary stone formation (Elliot and Eusebio, 1967; Elliot and Ribeiro, 1973). Thomas Jr. (1982) and Meyer and Thomas (1982) have studied the inhibitory effect of trace metal: citric acid complexes on calcium oxalate and calcium phosphate crystal growth. Anasuya and Narasinga Rao (1983) reported that silicon and fluoride accelerated the calcium uptake while magnesium inhibited this process. Fluoride is known to stimulate the crystallization below the ion product of $1.5 \times 10^{-6} \text{M}^2$ but inhibited it above $2.5 \times 10^{-6} \text{M}^2$ (Taves and Newman, 1964). Lin et al., (1981) demonstrated that at low fluoride concentration, fluoroapatite appears to be formed at the surface hydroxyapatite through absorption and at high fluoride concentration, $\text{CaF}_2$ was formed on hydroxyapatite by surface precipitation. Li et al., (1992) indicated that $\text{NaF}$ can inhibit renal stone formation induced by ethylene glycol, by decreasing oxalate synthesis and urinary oxalate excretion thereby suggesting a possible clinical therapeutic value of $\text{NaF}$ in the prevention of oxalate kidney stones. The lower content of serum nickel, manganese and cadmium in the active stone formers as compared to healthy individuals, could be of significance in the pathological mechanism of stone formation, not from mineralogical or crystallographic view points but for the smooth flow of enzymatic reactions in the biological systems (Hofbauer et al., 1991).

2.5.2.2 Pyrophosphate and polyphosphate ions

Pyrophosphate (PPi) is a urinary constituent identified as a potent inhibitor of calcium oxalate crystal growth and aggregation (Nancollas and Gardner, 1974; Meyer and Smith, 1975). Its concentration in urine varies between 1 to $7 \times 10^{-5} \text{M}$ which is high enough to inhibit calcium oxalate crystallization. It delays various processes involved in the formation of the solid phase viz. heterogenous nucleation, crystal growth and crystal aggregation by getting tightly absorbed on the crystal surface (Fleisch, 1978). Data from several laboratories indicate that pyrophosphate is decreased in stone formers’ urine (Russel and Hodgkinson, 1966; Valyasevi and Van Reen, 1968). The inhibitory nature of PPi has also been confirmed by Hosking et al., (1983); Meyer (1984) and Sharma et al., (1992). However, study by Ramavataram et
al., (1989) has demonstrated no significant differences in the urinary pyrophosphate levels in non-stone formers and stone formers in both fasting and 24h urine samples, thereby suggesting that the amount of pyrophosphate present in normal urine is insufficient to be a major determinant of its mineralizing potential and that the reduced content of pyrophosphate found in the urine of some patients with calculi is unlikely to have been an important factor in stone development.

Liu et al., (1982) studied retardation of calcium oxalate crystallization by high molecular weight polyphosphate ions, which get strongly absorbed on the crystal surface and the inhibition increases with chain length. However, it was observed that polyphosphate ions are much more effective in inducing the precipitation of calcium oxalate dihydrate. Martin et al., (1984) showed that most of the inhibitors of calcium oxalate crystallization induce the precipitation of calcium oxalate dihydrate and strongly inhibit its phase transformation to the thermodynamically more stable calcium oxalate monohydrate.

2.5.2.3 Citrate

During the past decade, citrate has received increasing interest as an important pathogenic factor in stone formation and as an exciting modality in the prevention of new stone formation (Pak, 1987). It is now generally recognized that hypocitraturia is a frequent biochemical disturbance among patients with nephrolithiasis (Tiselius, 1981; Schwille et al., 1982; Menon and Mahle, 1983; Hosking et al., 1984; Sharma et al., 1990; Wangoo et al., 1991). Citrate retards the crystallization of stone forming calcium salts by two broad means. Firstly, it complexes calcium and reduces ionic calcium concentration in urine (Pak et al., 1982). Secondly, citrate directly inhibits crystallization of calcium oxalate and calcium phosphate. Citrate has been shown to inhibit spontaneous precipitation of calcium oxalate and to retard agglomeration of preformed calcium oxalate crystals (Kok et al., 1986; Nicar et al., 1987). Kok et al., (1990) studied the effect of urines of stone formers and non-stone formers,
on calcium oxalate monohydrate crystallization kinetics (solubility, growth, agglomeration) and found that the defective inhibition of the process of crystal agglomeration is a major physico-chemical mechanism of calcium oxalate renal stone formation, which appears to be modulated by urinary citrate concentrations. Hobarth and Hofbauer (1991) also reported that recurrent stone formers show a significantly higher calcium/citrate ratio compared with controls, which would indicate an increased risk for stone formation. Lindberg et al., (1990) has shown the beneficial effect of dietary magnesium citrate complex against calcium oxalate monohydrate crystallization, while other workers (Hauser et al., 1990) used potassium citrate to prevent the stone formation. Arroyo et al., (1992) also proposed the oral administration of citrates in mixed stone patients as treatment and prophylactic measure, because citrate inhibits spontaneous nucleation of calcium salts and crystal growth, and it also increases the urinary pH with a consequent increase in uric acid solubility.

2.5.2.4 Glycosaminoglycans (GAGs)

Glycosaminoglycans, the degradation products of high molecular weight proteoglycans, have been shown to be potent inhibitors of calcium oxalate crystal growth and aggregation in vitro. Urine contains about 3-5 mg/day of acid mucopolysaccharides of which major components are chondroitin sulphate A and C (Foye, 1982).

GAGs inhibit the crystal growth and aggregation by blocking the growth sites on the crystals, thereby preventing or delaying further development of large crystals. Bichler et al., (1983) have reported a significant decrease in the amount of GAGs excretion in patients with staghorn and struvite calculi, while patients with calcium oxalate stones showed no change in GAGs excretion. Hautmann et al., (1984) found a steep increase of GAGs from the cortex of the papillary tips in the renal tissue obtained from healthy and stone forming kidneys. These GAGs protect the papillary tips from calcification because even in healthy subjects, there is supersaturation of calcium oxalate in the interstitium of the papilla. Wakatsuki et al., (1985) demonstrated...
that hyaluronic acid is the major GAG in the early stone forming period, as well as in tissues, in stones and in the urine of stone forming rabbits. Nishio et al., (1985) and Roberts and Resnick (1986) have also suggested heparin sulphate and hyaluronic acid to be the promoters of urinary stone formation. But further confirmation is needed in this respect.

Martelli et al., (1985) and Caudrella et al., (1988) have described significant difference in the GAG excretion in urolithiasis patients and healthy subjects, but these differences are present only in men (Caudarella et al., 1988). An age dependence of GAG excretion has also been demonstrated with excretion of GAGs significantly reduced with increasing age (Hesse et al., 1986). Sidhu et al., (1989) have reported that in patients with nephrolithiasis not only is the 24h urinary excretion of GAGs significantly low but the 3-hourly concentration of GAGs is also significantly decreased as compared to healthy subjects. GAGs play a major role in cellular abnormalities associated with idiopathic calcium oxalate stone formation. Baggio et al., (1990) demonstrated that in erythrocytes, GAGs proved to be active in normalizing oxalate self exchange and affected the membrane protein phosphorylation. Recently, Hesse et al., (1991) found that GAGs reduced the risk of calcium oxalate stone formation and the inhibition of calcium oxalate crystallization is attributed to direct binding of calcium to GAGs.

2.5.2.5 Amino acids and proteins

The presence of amino acids in the urine increases the solubility of calcium substances in urolithiasis. Shaker et al., (1983) have estimated urinary amino acid levels in stone formers and normal controls by paper chromatography and revealed that there was a significant decrease in amino acid excretion in all the stone formers and the individual amino acid pattern varied according to the types of stone formed. Azoury et al., (1984) have stressed the importance of glutamic acid in stone formation and have observed a lower excretion of this amino acid in stone formers. Hussain et al., (1989) have found that both the total amount and concentration of 24-h urinary
cystine were higher in vesical stone formers. Kohri et al., (1989) added L-glutamic acid and L-aspartic acid to synthetic urine and found that glutamate inhibited both the nucleation and crystallization of calcium oxalate. Aspartate also inhibited these processes but smaller than glutamate, however, concentrations of these amino acids in human urine are not larger than those of other amino acids.

Nakagawa et al., (1978) have isolated four acidic polypeptides from human urine which inhibit the growth of calcium oxalate cystals. Another acidic amino acid rich glycoprotein was isolated from human kidney tissue culture medium (Nakagawa et al., 1981). This protein called nephrocalcin contained two residues of γ-carboxyglutamic acid (GLA) whereas, nephrocalcin from stone former urine contains no detectable GLA (Nakagawa et al., 1987; Hesse et al., 1989). Recently, Coe et al., (1991) demonstrated that kidney derived inhibitors of crystal growth and aggregation include nephrocalcin (NC), an acidic glycoprotein produced in proximal tubules and thick ascending limb of Henle’s loop and the Tamm Horsfall glycoprotein (THP), produced only in the thick ascending limb. Nephrocalcin inhibits growth and aggregation of calcium oxalate monohydrate whereas THP inhibits only COM aggregation. Taken together these two proteins offer considerable protection to nephrons. Patients who form calcium oxalate monohydrate stones produce abnormal NC molecule that lack γ-carboxyglutamic acid and fail to inhibit COM crystallizations normally (Coe et al., 1991).

2.5.2.6 Ribonucleic acid and like substances

Ribonucleic acid and four synthetic homopolyribo nucleotide s were found to inhibit the calcium oxalate monohydrate crystallization, whereas DNA was shown to have an inhibitory effect much less than that of RNA (Ito and Coe, 1977). Schrier et al., (1981) have reported that 20-40% of the inhibitory material in normal urine is RNA or RNA-like material (fragments of RNA). The effect of RNA and other potent inhibitors on the conversion of COM to COD was studied by Martin et al., (1985).
They showed that RNA, at a concentration of 20 mg/l accounted for 16-20% conversion of COM and COD, only preceeded by pyrophosphates and citrates which accounted for 45% conversion. Mandel et al., (1987) studied the interaction of calcium oxalate monohydrate (COM) and ribonucleic acid (RNA) by adsorbing RNA on the COM surface. The result showed that at low RNA concentration, polymeric bridges occured resulting in slight aggregation of crystals but at higher concentrations, the denser polymer layers at the surface prevented aggregation by steric repulsion. The role of RNA as an inhibitor of calcium oxalate crystallization has been reported by many investigators (Brown et al., 1989 and Kleboth and Joost, 1989). Wangoo (1990) reported that the amount of RNA isolated from the urine of stone formers, was significantly low as compared to non-stone formers.

2.6 MANAGEMENT OF HYPEROXALURIA AND PREVENTION OF STONE FORMATION

Urinary stones existed more than 7,000 years ago perhaps with the dawn of civilization. With the turn of 20th century, the historical trend from endemic bladder stones has shifted to upper urinary tract stones, especially in industrialized countries. Thind et al., (1989) studied the chronological variation in chemical composition of urinary calculi between 1965-68 and 1982-86 in north-western India. They have found almost complete disappearance of magnesium-ammonium containing stones and a significant increase in the number of vesicle and renal calculi containing urate. Similarly, oxalate containing stones also showed a significant increase from 1965-68 to 1982-86. Recently, Coe et al., (1992) have also reported that about three-fourth of all kidney stones are composed of calcium oxalate. Stone analysis is important from epidemiological and therapeutic view points. Knowledge of the percentage composition of a urinary calculi contributes to the ability to predict the most possible cause of that calculus. With the knowledge of physico-chemical disturbance, it has become possible to formulate selective treatment protocol to reverse the derangements, thus preventing stone formation, as well as medically treating stone disease in certain cases (Pak, 1982). Jindal and
Vaidyanathan (1985) have described renal tubular acidosis, hyperparathyroidism, hyperoxaluria, hypercalciuria, hyperuricosuria and hypocitraturia, etc. as the causative factors for the development of different types of stones. Various risk factors for stone disease have already been described in section 2.3.

The majority of patients with calcium oxalate renal calculi, excrete increased amounts of calcium and/or oxalate in the urine. The more common of these two metabolic abnormalities is hypercalciuria which is seen in approximately 50% patients with stones. More research conducted on the elucidation and treatment of hypercalciuria than on any other metabolic derangements in stone disease, does not acknowledge the primacy of hypercalciuria over hyperoxaluria, but is probably the result of a general interest in calcium metabolism and the availability of reliable methods for measuring urinary calcium (Menon and Koul, 1992). Indeed evidences have suggested that hyperoxaluria correlates better with the severity of stone disease than hypercalciuria. Excellent correlation exists between the levels of urinary oxalate and extent of calcium oxalate crystallization as well as the likelihood of stone. The classical examples of the diseased conditions in which urinary oxalate excretion leads to stone formation are the Type-I primary hyperoxaluria, Type-II primary hyperoxaluria and a new syndrome called mild metabolic hyperoxaluria, in which degree of hyperoxaluria is much less than in primary hyperoxaluria type-I, but the stone recurrence is high, until urinary oxalate is lowered (Menon and Koul, 1992).

Preventing stones from recurring, reduces the need for urologic intervention. However, prevention requires a diagnosis of the cause of the stone, with the use of urine and blood chemistry measurements and stone analysis, but the general mainstay of any treatment program to prevent stone disease is hydration. Crystallization and stone growth probably do not occur throughout the entire day but rather during certain critical periods, when urine is supersaturated with calcium oxalate. Maximum take of fluid should occur during these periods especially after dinner. Fluid intake should be adjusted to maintain a daily urine output of 2-3 litres. This results in the
reduction of the saturation of calcium phosphate or calcium oxalate (Foster et al., 1990; Iguchi et al., 1990).

The most popular form of dietary therapy has traditionally been to reduce calcium intake by restricting milk and milk products. However, a low calcium diet in the presence of normal intake of oxalate may lead to mild hyperoxaluria (Menon and Koul, 1992). High protein and high sodium diets increase urinary calcium and uric acid excretion and decrease citrate excretion resulting in an increase in calcium oxalate crystallization and crystal aggregation (Laminski et al., 1991). Treatments for enteric hyperoxaluria include reducing dietary oxalate and fat (Anderson and Jagenburg, 1974) high fluid intake, oral citrate supplements (Sakhaee et al., 1991) and oral calcium supplements, which precipitate oxalate in the intestinal lumen (Smith, 1992).

Drugs most commonly used in patients of calcium oxalate stones are thiazide diuretics. Thiazides lower urinary calcium by direct action on the renal tubules. Thiazides represent an ideal treatment in renal hypercalciuria, since they correct the renal leak of calcium, restore normal parathyroid function, serum 1,25-(OH)\(_2\)D\(_3\) and calcium absorption. Menon and Koul (1992) have reported hypocitraturia in patients under thiazide therapy. Long term therapy with thiazides is also associated with mild hyperlipidemia, which may increase the risks for cardiovascular morbidity and mortality.

Long term treatment with low doses of pyridoxine was effective in lowering hyperoxaluria in renal stone patients (Murthy et al., 1982; Vathsala et al., 1989), though studies of Caudarella et al., (1989) failed to support it. Pyridoxine supplementation (2-200 mg daily) lowered the oxalate production in some patients (Yendt and Cohamin, 1985).

Orthophosphate (neutral salt of sodium and potassium) is recommended for the treatment for hyperphosphatemic absorptive hypercalciuria as it inhibits 1,25-(OH)\(_2\)D\(_3\) synthesis, reduces urinary saturation of calcium oxalate and inhibits spontaneous nucleation of brushite and calcium oxalate (Jindal and Vaidyanathan, 1985). Orthophosphate is ideally suited for patients with
hyperoxaluria and has been used effectively in preventing stone formation, nephrocalcinosis and renal failure in patients with primary hyperoxaluria and idiopathic calcium oxalate urolithiasis (Thomas, 1978; Smith, 1980, 1989).

Evaluation of the prophylactic effect of alkaline citrate on formation of stones has been made by several workers (Butz and Dulce, 1981; Nicaragua et al., 1984; Pak et al., 1985; Tiselius, 1985). Citrate is pathogenetically important in stone formation, because it retards the crystallization of stone forming calcium salts and because its level in urine is low in many patients with nephrolithiasis. Citrate has a modest inhibitory effect against crystal growth of calcium oxalate when corrected for the reduction in activity product occurring from ion pair formation (Meyer and Smith, 1975). Citrate is believed to be a more potent inhibitor of the crystal growth of calcium phosphate. Pak and Peterson (1986) have found citrate to prevent heterogenous nucleation of calcium oxalate by monosodium urate. New drugs are under development as improvements or refinements of currently available potassium citrate. These are potassium citrate 10 mEq tablet preparation, effervescent calcium citrate and potassium magensium citrate (Pak, 1991). Sakhaee et al., (1991) studied the contrasting effects of various potassium salts on renal citrate excretion and showed that urinary citrate excretion is significantly increased by giving patients, the potassium citrate and this citraturic action of potassium citrate is largely accountable for, by provision of an alkali load. Potassium itself had no effect in the absence of potassium deficiency.

Magnesium is known to bind oxalate in the gastrointestinal tract, increases the solubility of calcium oxalate and inhibits the precipitation of calcium oxalate and calcium phosphate (Barilla et al., 1978; Bisaz et al., 1978; Berg et al., 1986). Magnesium supplementation has been reported to be beneficial in the prevention of recurrent formation of calcium oxalate renal stones (Gulati et al., 1988; Jarrar et al., 1989). Various magnesium salts like magnesim oxide, magnesium hydroxide and magnesium citrate have been proven beneficial in increasing urinary magnesium excretion but Ogawa et al. (1990) and Lindberg et al., (1990) have demonstrated that supplementation
of magnesium citrate to either hyperoxaluric rats or humans produces much better effects in not only increasing the urinary excretion of magnesium but also of citrate, an important inhibitor of calcium oxalate crystallization.

In the absence of adequate medical treatment, surgical intervention has been a significant treatment of urinary calculi since ages. Stones lodged in the proximal ureter and not progressing downwards are best pushed upwards into renal pelvis and disrupted by extracorporeal shock wave lithotripsy (ESWL) (Coe et al., 1992).

Despite all these various modes of treatment (surgical/non-surgical), no inevitable remedy has been evolved so far. Use of indigenous drugs in India and other countries has been popularizing day by day. Medical management of urolithiasis was practised by naturopaths. Ayurvedic literature has a mention of herbal drugs for breaking up calculus.

2.6.1 Traditional Systems Of Medicine And Indigenous Plants Used In Urinary Disorders

In many places of the world special ways of medical treatment are found. If one looks back into history, one can find that in all continents and in every country some kind of traditional medicine exists or has existed. The importance of the traditional systems of medicine and of certain traditional medical practices has now been recognized all over the world. While there exists a number of traditional systems of medicine and practices all over the world, some of them have been identified by Satyavati (1982) as of global importance. These are Ayurveda, Sidha, Unani, Homeopathy and Chinese. The art of practising Chinese herbal medicine stretches back over more than 5,000 years. Chinese physicians and philosophers developed a special system of physiology describing vital organs as storage houses and vital connections as meridians which became the basis of acupuncture (Porkert, 1973).

In India, Ayurveda, Sidha and Unani systems of medicine provide health care for a large part of the population. Among the various systems
of traditional medicine, Ayurveda stands out distinctly, as not only a system of great antiquity but an organized system with distinct aims and objectives (Satyavati, 1982). In fact it is the very foundation stone of the ancient medical science. The word Ayurveda is composed of two parts Ayu (life) and Veda (knowledge). The origin of this science of life have been placed by the scholars of Ayurveda at somewhere around 6,000 years B.C. (Vogel, 1991).

It is important to realize, however that Ayurveda is not just curative in its approach, but reveals a deeper insight into the various problems of human life. Modern health care poses a conundrum. Researchers are pushing the frontiers of medicine ever outward, surgeons are transplanting organs and geneticists are mapping the human genome with increasing precision, yet scientific medicine is being met by waves of discontent. The need is a search for new approaches to health and health care by people and one of these new approaches is several thousand years old, the Ayurvedic medicine. It attempts to improve health by harmonizing mind and body. To do this, it employs a seemingly eclectic combination of herbal remedies, massage therapy and yoga. Drugs used in Ayurveda are derived from a wide range of materials such as plants and minerals. The rich herbal and mineral materia medica of Ayurveda and other traditional systems of medicine is quite well known. Several hundreds of drugs of herbal origin are listed in the Ayurvedic classics. India is a veritable emporium of medicinal plants, nearly three fourth of the drugs mentioned in the British and other pharmacopoeias grow here in a state of nature. The urgent need for the cultivation of medicinal plants and for the development of sources by many natural products of vegetable origin within the British Common Wealth of Nations was voiced in 1941 (Chopra et al., 1958).

An important aspect of value of research in indigenous drugs is the reinvestigation of well known drugs. Although some of these drugs have been known for many years, the last word has not yet been said about them. In Eastern mediterranean countries and in Arabia, the local physicians often prescribe a decoction of the dried seeds of a local plant, Ammi visnaga as a diuretic and as an antispasmodic in renal colic. A number of indigenous
and herbal drugs are being used in India for several urinary disorders and calculus dissolution etc. Nadkarni (1976) in his book Indian materia medica, vol. II has mentioned about 80-100 drugs of plant origin, which have diuretic action e.g., Achyranthus aspera, Boerhavia diffusa, Crataeva nurvala, Ocimum basilicum, Pedalium murex, Tribulus languinosus and Tribulus terrestris etc and around 20 drugs for the prevention and cure of different types of calculi e.g. Citrus limonum, P. murex., C. nurvala and T.terrestris etc. Canabis sativa, Santalum album, Hibiscus rosa sinensis, Eucalayptus sps., T.terrestris etc for cystitis. Allium sativam (garlic), sodium potassium and silicon salts for anuria.

Decoctions prepared from the whole plant of Achyranthus aspera (F.Amaranthaceae) found all over India, is a good diuretic found efficacious in renal dropsies. Its fruits are known to contain a large percentage of alkaline ash containing potash. Boerhaavia diffusa has been in use in the indigenous medicine from time immemorial. Chopra and his coworkers studied the diuretic effects in the cat and the dog. The plant is known to contain unusually large quantities of potassium nitrate (1/2-2g) and other potassium salts. It produced a marked and persistent diuresis and in some cases the ascites entirely disappeared. C.nurvala or C. religiosa (F. capparidaceae) is usually cultivated in the vicinity of temples in central India, Bengal and Assam. Its bark contains saponins and root and bark are laxative and lithotriptic (Nadkarni, 1954; Kumar et al., 1980). Bark is specially useful in urinary complaints such as bladder and kidney stones. Deshpande et al.,(1982) in their study highlighted the specific role of C.nurvala in different urinary disorders (such as urolithiasis, urinary tract infection and atony of urinary bladder etc.). He showed that decoction of plant given for a month (50 ml twice a day) in urolithiatic cases, reduced the urinary calcium to a great extent, while the excretion of sodium and magnesium increased significantly. It imporves the tone of the smooth muscle and helps in the downward migration of stationary uretric calculi which encourages the spontaneous passage of calculi. By virtue of its diuretic,anti- inflammatory and tonic action, the drug helps in overcoming the stagnant infection of urinary tract. Varalakshmi et al., (1990) showed that the elevation of the oxalate-synthesizing liver enzyme glycolic acid oxidase
produced by feeding glycolic acid was remarkably reduced with the decoction of C. nurvala, also the increased deposition of stone forming constituents in the kidney of calculogenic rats was lowered. The increased urinary excretion of the crystalline constituents along with lowered magnesium excretion found in stone forming rats was partially reversed by decoction treatment.

*Tribulus terrestris* (F. Zygophyllaceae) is an annual or perennial plant trailing in sandy soils throughout India. The entire plant and especially the fruit and the root are used in the Hindu medicine. The fruits are regarded as cooling, diuretic, tonic and aphrodisiac and are used in painful micturition calculus affections, urinary disorders and impotence. It has been combined with hyoscyamus and opium in inflammatory conditions of the urinary passages. It is used in Europe as an aperient and diuretic. The fruits are known to contain alkaloid in trace amounts, fixed oils, essential oil, resin and fair amount of nitrates comprising the ingredients in medicines for urinary disorders and impotence. The chemical and pharmacological as well as diuretic properties of *T. terrestris* were reassessed by Bose et al., (1963). The chemical analysis revealed the presence of a fixed oil, sterol bodies, tannins, resin, alkaloid and potassium. The diuretic action may be ascribed to the alkaloid fraction and potassium content. Singh and Sisodia (1971) reported that the ether extract of fruits of *T. terrestris* produced diuresis and increased the creatinine clearance, which suggests increase in the glomerular filtration rate. However, the ether extract did not significantly increase the chloride clearance which excludes inhibition of tubular chloride reabsorption. Santha Kumari and Iyer (1967) also confirmed the diuretic effect of aqueous extract of *T. terrestris* which could be attributed to the presence of potassium salts in high concentration. Bhutani (1969) demonstrated that the alcoholic extraction of leaves and fruits of *T. terrestris* and crystallization of the extract deposited potassium nitrate and a mixture of saponins. The ether and acetyl acetate fractions were found to be identical and gave positive color reactions for flavanoids. The different fractions furnished compounds A,B,C,D. The ethanol soluble portion afforded compound E. Compound C was a new acylated flavonoid glycoside and called tribuloside. Saleh et al., (1982) detected 25 flavonoid glycosides in *T. pentandrus*. 
and *T. terrestris*. The glycosides belong to the common flavonols, kaempferol, quercetin and isorhamnetin with 3-gentiobiosides as the major glycosides. Besides these, Ahsan *et al.*, (1989) supported the use of *Trigonella foenum-graecum* in Saudi folk medicine. Daily oral treatment for 4 weeks significantly decreased the quantity of experimentally deposited calcium oxalate in the kidneys of male rats. Sidhu *et al.*, (1985) conducted the studies to investigate the effect of coconut water and demonstrated that coconut water contains dialysable biomolecules, which not only can inhibit the initial mineralization phase formation and its subsequent growth, but also stimulate the demineralization of preformed mineral phase formed experimentally, thereby suggesting that the lower incidence of kidney stones of coastal population could be due to high and regular consumption of coconut.

Joseph *et al.*, (1989) demonstrated the complete disappearance of both whewellite and weddellite crystals within 24 h of consumption of Tamarind, which contains a high content of tartaric acid, which forms metal complex ions with Ca in urine, thereby reducing the amount of calcium available for calcium oxalate precipitation. These authors also contradicted the popular belief about tomatoes consumption by analyzing the net calculogenic potential of tomatoes and demonstrating that the high citrate content of toamtoes could have an inhibitory effect on calculogenesis. Dolichos biflorus (*Kulath ki dal*) is also an important folk medicine of India used for urinary disorders (Pendse *et al.*, 1985; Banerjee and Roy, 1989).

AlaOpas *et al.*, (1987), Tizzani *et al.*, (1989) and Ebisuno *et al.*, (1991) showed the positive long term treatment effects of wheat and rice bran for recurrence rates of stone formation and for various urinary parameters. Recently, Kailash and Varalakshmi (1992) studied the effect of stem extract of banana on the liver enzymes glycolic acid oxidase and lactate dehydrogenase, also the calcium, phosphorus, oxalate and glycolic acid content in the liver tissues of sodium glycolate induced hyperoxaluric rats. The extract had a very good effect on lowering of GAO enzyme activity and urinary/tissue -
specify inorganic constituents of the hyperoxaluric rats. It also reduced the precursor of the oxalate formation, the liver glycolic acid content of hyperoxaluric rats.