Introduction
1. GENERAL INTRODUCTION

Nephrolithiasis is a common multifactorial disorder and is a major cause of morbidity involving the urinary tract. The process of renal calculi formation is a multifaceted process that initiates with the formation of microcrystals in the urine and terminates with the formation of mature renal calculi. The attachment of crystals by the urothelium is a major event in the successful formation of the mature stone (Mandel, 1994).

1.1 NATURE OF STONES AND CRYSTALS

Ultra structural organization of renal calculi depicts a disordered mineralized tissue interlaid with protein (Coe and Parks, 1997). The renal stones generally consist of calcium salts, uric acid, cystine or struvite (the triple salts of magnesium, ammonium and phosphate).

About 80% of stones are composed of calcium salts and usually occur as calcium oxalate and less commonly as calcium phosphate (Daudon et al., 1995) and the remaining 20% of stones are composed of uric acid, struvite or carbonate, apatite, cystine and rare substances.

1.2 EPIDEMIOLOGIC ASPECTS OF URINARY CALCULI

Andersen (1973) presented an interesting, multifaceted theory of epidemiology of urinary calculi. According to him, two separate epidemiologic factors are involved in the genesis of urinary calculi. They are intrinsic and extrinsic factors.
1.2.1

Intrinsic factors are ethnic, racial, familial background that involves inherited physiological or anatomical disturbances predisposing to urinary calculi formation.

1.2.1.1 Heredity

Genetic studies performed by Resnick and co-workers (1968) and by McGeown (1960) concluded that urolithiasis is associated with a polygenic defect and partial penetrance. Several disorders that cause renal stones are hereditary. Familial renal tubular acidosis (RTA) is associated with nephrocalcinosis in almost 70% of patients (Dretler et al., 1969). Cystinuria is a prime example of familial transmission type of urinary lithiasis that is definitely hereditary. It is a homozygous recessive disease (Crawhall and Watts, 1968) that leads to the excessive excretion of cystine, ornithine, lysine and arginine (COLA).

1.2.1.2 Age and Sex

The peak incidence of urinary calculi occurs in the age range of twenty to forty (Fetter and Zimskind, 1961; Blacklock, 1969; Pak, 1987a,b). About three males are afflicted for every female. A relatively greater proportion of upper urinary tract calculus disease is caused by chronic urinary tract infections or defects, such as cystinuria or hyperparathyroidism in women than in men (Baker et al., 1993). Several investigators have commented on the apparently equal tendency toward urinary lithiasis in male and female during childhood (Prince and Scardino, 1960; Malek and
Welshman and McGeown (1975) have demonstrated increased urinary citrate excretion in the urine of females, which may aid in protecting females from calcium urolithiasis.

1.2.1.3 Anatomic abnormalities

Anatomic abnormalities such as medullary sponge kidney or uretero-pelvic junction obstruction can predispose to increased sheikness of the tubular epithelium resulting in crystal retention. (Kumar et al., 1991; Menon and Koul, 1992; Scheid et al., 1996).

1.2.2 Extrinsic Factors

Extrinsic factors include geography, climate, drinking water, dietary patterns, presence or absence of trace elements in foodstuffs and occupation.

1.2.2.1 Geography

Geography influences the incidence of urinary calculi and the types of calculi that occur within a given area. It influences lithogenesis in terms of temperature and humidity, which also seems to influence the incidence of human urinary calculi. The prevalence of urinary calculi is higher in those who live in mountainous, desert and tropical areas. Finlayson (1974) reviewed several worldwide geographic surveys and stated that the United States is relatively high in the incidence of urinary calculus disease for its population. Other high-incidence areas are the British Isles, Northern Australia, Central Europe, China, Northern India and Pakistan. Low-incidence areas include Central and South America, most of Africa and those
areas of Australia populated by aborigines. In India, northwestern part covering Rajasthan is considered to be the stone belt (Singh et al., 1978).

1.2.2.2 Climate

Elevated environmental temperature is related to a greater risk of stone disease in those populations capable of forming stones. Parry and Lister (1975) presented that increased exposure to sunlight caused increased urinary calcium excretion. This possibly indicates increased synthesis of vitamin D3. This occurrence may lead to a higher incidence of urolithiasis. Hallson and Rose (1977) have demonstrated increased crystalluria in patients who form stones during summer months.

1.2.2.3 Water Intake

The relationship between urolithiasis and water intake is governed by two factors. These are 1) the volume of water ingested and 2) the mineral or trace element content of the region supplying the water. Finlayson (1974) pointed out that the dilution effect of water diuresis probably outweighs the changes in ion activity and therefore helps to prevent stone formation. The mineral content of water may also contribute to renal stone formation. Excessive water hardness (e.g. calcium sulfate) contributes to calcium calculi (Rose and Westbury, 1975) and excessive softness (e.g. sodium carbonate) causes a greater incidence of carbonate stones (Juutti and Heinonen, 1980; Sierakowski et al., 1976). The presence or absence of certain trace elements in water has been implicated in the formation of urinary calculi. For e.g. Zinc is an inhibitor of calcium crystallization (Elliot and Eusebio, 1967).
1.2.2.3 Diet

Ingestion of excessive amounts of purines, (uric acid) (Hodgkinson, 1976), oxalates (Thomas, 1975), calcium phosphate and other elements often results in excessive excretion of these components in urine. Use of large amounts of Worcestershire sauce with its high oxalate content (Holmes, 1971), a vegetarian diet associated with childhood urolithiasis, and the habitual excessive ingestion of milk products in the form of cheese or ice cream may lead to stone formation. Not only the diet but also its source may be important. Vegetables grown in various parts of Thailand contain amounts of oxalate that differ by 50% or more (Suvachittanont et al., 1973).

1.2.2.3 Occupation

Mates (1969) reported that of all epidemiological factors studied, the occupation of the individual was of greatest importance. Lonsdale (1968 b) indicated that urinary calculi are much more likely to be found in individuals who have sedentary occupation. Blacklock (1969) reported that the incidence of urinary calculi was higher in administrative and sedentary personnel of the Royal Navy, cooks and engineering room personnel's. The lowest incidence of stone disease was found in agricultural and border populations.

In summary, this review of the epidemiology conclude that multiple factors like heredity, age, sex, geographic location, environmental temperature, water intake, diet, social status and occupation of the individual plays a part in the genesis of urinary calculi.
1.3 ETIOLOGIC FACTORS OF STONE FORMATION

1.3.1 Hypercalciuria

Hypercalciuria is defined as the excretion of greater than 4mg of calcium / kg body weight / day or greater than 7mmol / day in men and 6mmol / day in women (Parks and Coe, 1986). In 1974, Pak and his associates classified hypercalciuria into three types 1) absorptive hypercalciuria, in which the primary abnormality was an increased intestinal absorption of calcium. 2) Renal hypercalciuria, characterized by a primary renal leak of calcium and 3) resorptive hypercalciuria, characterized by increased bone demineralization.

1.3.2 Hyperoxaluria

Hyperoxaluria is associated with calcium oxalate nephrolithiasis in three clinical scenarios. Primary hyperoxaluria is a rare genetic disorder resulting from increased hepatic production of oxalate. Enteric hyperoxaluria occurs in patients with short bowel syndrome or malabsorption. Recurrent idiopathic calcium oxalate lithiasis exhibits mild hyperoxaluria or increased transport of oxalate by red blood cells.

1.3.2.1 Primary Hyperoxaluria

Primary hyperoxaluria is classified into two types. Type I is an autosomal recessive inborn error of metabolism. It is characterized by increased urinary excretion of oxalic, glycolic and glyoxylic acids (Menon and Mahle, 1982). Primary hyperoxaluria type I is due to a defect of the enzyme alanine – glyoxalate aminotransferase (AGT) in the liver (Danpure
and Jennings, 1986). In normal human liver, AGT catalyzes the transamination or detoxification of glyoxalate to glycine, and its deficiency results in glyoxalate being oxidized to oxalate (Danpure, et al., 1994).

Primary hyperoxaluria Type II or L-glyceric aciduria is due to deficiencies of the hepatic enzymes D- glycerate dehydrogenase and glyoxalate reductase. It leads to increase in urinary oxalate and glycerate excretion (Chelbeck et al., 1994). In both forms of primary hyperoxaluria, there is increased oxalate production and urinary excretion of oxalate (Itami et al., 1990; Scheimann, 1991)

1.3.2.2 Enteric hyperoxaluria

Ingested oxalate is absorbed through the stomach (Hautmann, 1993) and the colon (Madorsky and Finlayson, 1977). Malabsorption from any cause, including small bowel resection (Smith et al., 1972), intrinsic disease, or jejunoileal bypass surgery (Cryer et al., 1975), increases the colonic permeability of oxalate as the result of exposure of the colonic epithelium to bile salts. The principal and perhaps only source for increased oxalate excretion is through the dietary oxalate malabsorption (Earnest et al., 1974), coupled to fatty acid and bile acid malabsorption. Fatty acids form calcium soaps that effectively reduce the concentration of calcium in the intestinal contents. As a result, calcium is not available for oxalate and hence oxalate absorption is high in the free ionized form. Kleinschmidt et al (1995) reported that patients with recurrent calcium oxalate stone disease have decreased in intestinal Oxalobacter formigenes and thus exhibit decreased oxalate degradation in the intestine.
1.3.3 Hyperuricosuria

24% of calcium stone formers exhibit hyperuricosuria (Coe and Parks, 1988). Uric acid promotes calcium oxalate crystallization by acting as a nidus (epitaxial nucleation). Addition of crystals of uric acid to supersaturated calcium oxalate solutions induces the deposition of well orientated crystals of calcium oxalate over the uric acid (Deganello and Chou, 1984). Fellstrom and his Colleagues (1982) have demonstrated that calcium oxalate stone formers with hyperuricosuria have higher rates of stone formation than with those without hyperuricosuria.

1.3.4 Hypocitraturia

Hypocitraturia is reported in 15-63% of patients with nephrolithiasis (Menon and Mahle, 1983a; Pak, 1987a). Hypocitraturia is defined as a urinary citrate excretion lower than 1.7 mmol/day (Mossetti et al., 2003). In the course of lithiatic disease, hypocitraturia coexists with subtle changes in the excretion of hydrogen ions in basal situations (Araujo and Rebelo, 2000). Hypocitraturia is more common in stone forming women than in men (Pak, 1990). Low urinary citrate excretion found in patients with nephrolithiasis may play an important role in the pathogenesis of the disease (Kaminska et al., 2000).

The primary mechanism of action of citrate is a complexing agent for calcium. Calcium citrate complexes are more soluble than calcium oxalate and are thus excreted harmlessly. Thus, citrate inhibits the
spontaneous nucleation and aggregation of calcium oxalate and calcium phosphate crystals (Tiselius et al., 1993b).

1.4 PHYSICO-CHEMICAL ASPECTS OF STONE FORMATION

Understanding the theories of stone formation is necessary to explain some of the basic processes involved in crystallization. Finlayson (1974) put forward some descriptions and examples of crystallization processes. It includes saturation, super saturation, solubility product, formation product or formation saturation, metastable region of super saturation, crystal nucleation, crystal growth, crystal aggregation, epitaxy and Zeta potential.

1.4.1 Saturation

If increasing amounts of substances capable of crystallizing are added to pure water at a given pH and temperature, eventually a high enough concentration is reached for crystals to form. When crystals begin to form, the solution has become saturated with the substance. The point at which saturation is reached and crystallization begins is referred to as the thermodynamic solubility product (Ksp). Pak et al. (1977a) developed a relatively simple method of estimating calcium oxalate and calcium phosphate saturation. They term it as the activity product ratio. A second technique uses a computer program (EQUIL93) to measure the state of saturation (Brown et al., 1994). This program has been used by many
prominent investigators in urolithiasis to monitor the results of therapy on urinary supersaturation. Temperature and pH are always specified for any crystallization process. Alteration in either factor may greatly change the amount of solute that may be held in solution.

1.4.2 Supersaturation

The central event in stone formation is supersaturation. If the product of calcium and oxalate concentrations in urine exceeds its thermodynamic solubility product (Ksp), calcium oxalate crystals should precipitate. Urine, however, contains inhibitors and other molecules that allow higher concentrations of calcium oxalate to be held in solution. Thus, urine is said to be metastable with respect to calcium oxalate. When the concentration is increased further, a point is reached at which it can no longer be held in solution. This concentration or kF is the formation product of calcium oxalate in urine. The concentrations of most stone components in non-stone formers urine are in the metastable range between Ksp and kF.

1.4.3 Crystal Nucleation

Nucleation of crystals occurs when active ions and molecules in a solution no longer flow randomly in a completely dissociated fashion but cluster together closely enough to form the earliest crystal structure that will not dissolve. There are two types of nucleation. They are homogenous and heterogeneous nucleation.
1.4.3.1 Homogenous nucleation

In normal urine, the concentration of calcium oxalate is four times higher than its solubility (Coe and Parks, 1988a). Once calcium oxalate concentration exceeds its $K_{sp}$, crystallization can occur. Because of inhibitors and other molecules, however, calcium oxalate precipitation in urine occurs only when its super saturation is 7 to 11 times its solubility. The process by means which nuclei form in pure solution is called homogenous nucleation. These nuclei form the earliest crystal structure that will not dissolve and have the form of a lattice that is characteristic of that crystal.

1.4.3.2 Heterogeneous nucleation

In urine, however, crystal nuclei usually form on existing surfaces. Other particles that start nucleation "catalyze" the process. This type of secondary nucleation is most probable in urine and is referred to as heterogeneous nucleation. Epithelial cells, cell debris, urinary casts, macromolecules like THP, other crystals and red blood cells can act as heterogeneous nuclei (Brown and Purich, 1992).

Another aspect of crystallization that has received considerable attention is epitaxy (Hench, 1972). Epitaxy is the process by which crystalline material of one salt is laid down upon the surface of another. If the crystal structure has a regular and predictable pattern or organization of ions, it is called a lattice. This surface lattice may resemble very closely that of a second but different type of crystal. Depending on the closeness of resemblance, the second type of crystal may actually grow on the surface of
the first. Both calcium oxalate and uric acid have similar crystal lattices to permit this process of epitaxy.

1.4.4 Crystal growth

Once nucleation has occurred in the complex solution known as urine, certain nuclei may continue to grow, if the urine remains supersaturated. Not only will such nuclei continue to grow in the zone above the formation product, they will continue to grow even if the saturation of urine falls into the metastable zone between solubility product and formation product.

1.4.5 Crystal Aggregation

Another concept necessary to promote understanding of the genesis of urinary calculi is that of aggregation. If multiple nuclei and crystals are formed spontaneously and float freely, these nuclei become active kinetically and bounce about in the urine. If they remain small, free and independent within the solution, they will pass through the urinary tract within a given amount of time and will be voided. Under suitable conditions, however, these nuclei can aggregate into large clumps within a minute.

1.5 INHIBITORS OF CRYSTALLIZATION

Urine contains substances that alter or modify crystal formation (Drach and Boyce, 1972; Coe et al., 1980a). These substances can be divided into inhibitors, complexors and promoters. Inhibitors poison the crystal surface by binding to them (Bowyer et al., 1975). The entire crystalline
surface need not be bound by them in order to prevent growth. They become adsorbed to the crystal surface at sites of defects or dislocations where growth occurs and prevent the further growth of the crystal (Smith et al., 1973). Inhibitors may be classified into micromolecular and macromolecular.

1.5.1 Micromolecular inhibitors

Most inhibitors of crystallization that have been reported are related to inorganic elements that affect the calcium phosphate or calcium oxalate systems are magnesium, citrate and pyrophosphate are considered to be the three major micromolecular inhibitors.

1.5.1.1 Magnesium

Magnesium decreases the incidence of calcium oxalate calculus formation by forming soluble complexes with oxalate and thereby reduces the solubility of calcium oxalate (Li et al., 1985).

1.5.1.2 Citrate

The role of citrate in preventing stone formation is its ability to work as an inhibitor as well as to form soluble complexes with Ca$^{2+}$. Both citrate and magnesium act not only as inhibitors but also as complexors (Malagodi and Moyce, 1981). Oral potassium citrate treatment restores normal urinary citrate and has been suggested to bring about a preventive effect for recurrent calcium stone disease in children with hypocitraturia (Tekin et al., 2002).
1.5.1.3 Pyrophosphate

Among the inorganic inhibitors, pyrophosphate is one of the major components (Drach et al., 1983). The action of pyrophosphate as a crystal poison of calcification has been known for many years. It is estimated that less than 15% of total inhibitory activity is due to inorganic pyrophosphate and that this may not be, therefore, of any great importance in the pathophysiology of stone formation.

1.5.2 Macromolecular inhibitors

The most important of the known inhibitors of calcium salt crystallization and growth are the highly charged, macromolecular constituents of urine. Robertson et al. (1984) have shown that all these macromolecular inhibitors (Molecular weight 10,000-90,000) were significantly reduced in the urine of recurrent stone formers compared with that of control subjects. The most likely way by which the inhibitors function is by adsorption on to the crystal surface according to the principles of the Langmuir theory of adsorption equilibrium (Nakagawa et al., 1981). Most of the inhibitory activity in urine is due to macromolecules such as glycosamino glycans, inhibitory proteins like Nephrocalcin, Tamm-Horsfall Glycoprotein, Uropontin, Crystal Matrix Protein (FI prothrombin fragment), Uronic Acid rich Protein (bikunin) and renal lithostathine. Most of the molecules are anionic, with many acidic amino acid residues, with post-translational modifications such as phosphorylation and glycosylation. These macromolecules appear to exert their effects by binding to calcium
oxalate surface (Worcester, 1996) and play an important role during the aggregation phase of calcium oxalate crystallization (Ebisono et al., 1993).

1.5.2.1 Glycosaminoglycans

Glycosaminoglycans (GAGs) are found in urine and are polysulfated, polyanionic substances. Human kidneys contain primarily heparan sulfates, hyaluronic acid and dermatan sulfate. Chondroitins A and C, an over sulfated chondroitin sulfate and a nonsulfated chondroitin represent about one sixth of the kidney GAG content (Murata, 1975). GAGs promote crystal nucleation but inhibit crystal aggregation and growth (Malek and Boyce, 1977). Heparin and chondroitin A are found to be very strong inhibitors of calcium oxalate crystal growth and aggregation. Angell and Resnick (1989) have demonstrated that chondroitin sulfate is first adsorbed to COM crystals followed by heparin and then by hyaluronic acid. Recently, two-dimensional electrophoresis of GAGs extracted from stone matrix has shown that heparin, heparan sulfate and hyaluronic acid are detected in calcium oxalate stones, while, chondroitin sulfate is not detected in these stones (Nishuo et al., 1985). In animal experiments, hyaluronic acid is the major GAG in the early stone forming period (Wakatsuki et al., 1985).

1.5.2.2 Nephrocalcin

Nephrocalcin (NC), an acidic glycoprotein with a molecular weight of 14kD is present in urine and prevents kidney stone formation. Nephrocalcin is a calcium binding protein, composed of 110 amino acid residues rich in glutamic acid and aspartic acid residues that constitute
nearly 25%. Two to three γ carboxy glutamic acid residues that can bind calcium are present in NC. However, the sequence is undefined owing to its multiple glycosylations. This monomeric protein has a tendency to form dimer, trimer and tetramer and exhibit a wide range of molecular weights in SDS PAGE gels. It has been isolated from the urine of human (Nakagawa et al., 1983) and rat (Nakagawa et al., 1984).

NC inhibits various stages of calcium oxalate lithogenesis. It inhibits calcium oxalate nucleation and aggregation. The aggregation inhibition property of NC is exerted by its ability to coat the crystals and change the surface charge to more negative via its aspartic, glutamic and γ carboxy glutamic acid residues, leading to increased electrostatic repulsive forces between crystals (Hess et al., 1989). NC inhibits the growth of calcium oxalate seed crystals in metastable supersaturated solutions of CaOx in vitro. The ability of NC to inhibit crystal growth is due to its binding to the growth sites of the crystals (Worcester et al., 1988). When the crystals of CaOx are grown in the presence of NC, growth along one crystal face is preferentially altered, resulting in the distorted and abnormal crystal morphology (Deganello, 1991), suggesting the selective binding of NC to crystals. Data suggest that divalent metal ions may be involved in the calcium oxalate crystallization through interaction with NC (Chang et al., 2001).

There are four isoforms of NC called as NC-A, NC-B, NC-C and NC-D. Non-stone forming people excrete more NC-A and NC-B isoforms and stone formers excrete more NC-C and NC-D (Nakagawa, 1997). NC – A is a strong calcium oxalate crystal growth inhibitor while NC – C is a poor
crystal growth inhibitor. The daily excretion of NC in human urine is about 5-16 mg (Coe et al., 1991). NC excretion is markedly increased in the urine of hypercalciuric pregnant women (Davison et al., 1993). NC isolated from urine of stone formers with X-linked recessive nephrolithiasis is identified to be abnormal with respect to phosphorylation and amino acid composition (Nakagawa et al., 1993).

1.5.2.3 Uropontin and Osteopontin

Another important inhibitor of calcium oxalate crystal growth present in urine is Uropontin (Shiraga et al., 1992). This aspartic acid rich protein shares N-terminal amino acid sequences with osteopontin present in bones. Uropontin is produced by mouse kidney cortical cells in culture (Worcester et al., 1992) and is present in the distal tubules of stone forming rats (Kohri et al., 1993). Both Uropontin and Osteopontin are major components of the matrix of CaOx monohydrate stones. The molecular weight of uropontin ranges between 45 to 75 kDa, the anomalous migration being attributed to its differential phosphorylation and glycosylation (Ashkar, 2000). Rat OPN has 319 residues of which 36% are aspartic and glutamic acid residues. It also contains 30 serine, 12 phosphoserine and phosphothreonine residues, 10 sialic acid residues and no γ carboxy glutamic acid residues.

A reduction of uropontin expression in epithelial cells induces a reduction of CaOx stone formation. Uropontin is largely involved in the lithogenesis of urinary stones, but its mechanism of action has not been fully elucidated (Paulhac et al., 2002). It contains functional Arg-Gly-Asp-cell
binding sequences that may facilitate interactions between cells and mineralizable matrix. Thus, these proteins under certain conditions may promote crystal adherence to renal epithelial cells (Reinholt et al., 1990). Immunohistochemical localization studies have shown the expression to be heterogeneous in nature and present in thick and thin ascending limb of the loop of Henle and in DCT and macula densa (Kohri et al., 1993).

Uropontin isolated from human urine is a potent inhibitor of COM crystallization. The structural features responsible for its effect on crystallization were investigated by synthesing peptides and phosphopeptides with sequences corresponding to potential crystal binding domains within the protein sequence of Uropontin. The increased inhibition of crystal growth due to phosphorylation results from altered local patterns of charge density (John et al., 2001).

1.5.2.4 Inter α-trypsin inhibitor (IαI) and Bikunin

IαI, a family of glycoproteins play an important role in urolithiasis and function as Kunutz- type serine protease inhibitors (Atmani et al., 1999). IαI consists of 3 distinct protein chains with separate genetic origins. The 2 heavy chains, H1 (65-101 kDa) and H2 (70-106 kDa) are linked covalently via chondroitin sulphate moiety to a light chain known as bikunin (~ 30kDa) (Enghild et al., 1989). Combinations of these chains gives rise to two molecules i.e. inter-inhibitor (II or ITI) and pre-inter-inhibitor (PI). Marengo et al. (1998) reported that increased amounts of intact IαI were detected in the urine of stone forming individuals when compared with normal individuals. However, these data were only semi - quantitative, and it is
reasonable to postulate that raised levels in stone formers could simply be the result of an inflammatory response arising from stone formation itself. There is an increase in bikunin gene expression in LLC-PK1 and MDCK cells exposed to oxalate and CaOx crystals (Iida et al., 1997). Bikunin gene is upregulated by hyperoxaluria and calcium oxalate crystal deposition. Bikunin and heparan sulfate proteoglycan (HSPG) expression is increased in nephrolithic kidneys in medulla and papillary tips. The production of bikunin was increased in both the distal and proximal tubules of nephrolithic kidneys. These findings suggest that the increased expression of both HSPG and bikunin play an important role during CaOx stone formation. In addition, this phenomenon might be associated with the progression of urothelial damage (Eguchi et al., 2002). Bikunin incorporated into the experimentally induced CaOx crystallization is a strong inhibitor of calcium oxalate crystallization (Atmani et al., 1996). Bikunin isolated from stone formers has less inhibitory activity that relates to low sialic acid content when compared to that of the control subjects (Atmani et al., 1994).

1.5.2.5 Uronic-acid-rich protein (UAP)

UAP is a macromolecule with a molecular weight Of 35 kDa. This protein is named so, because of its rich uronic acid content. Its carbohydrate content is 8.5% and 24% of its amino acids are mainly of glutamic and aspartic acids. Human and rat UAP exhibit similar biochemical characteristics. Western blots with ITI antibody cross-reacts with UAP concluding that UAP belongs to the ITI super family of molecules (Atmani et al., 1996a). The antibody raised against bikunin cross-reacts with UAP in
western blot analysis; it has been also demonstrated that first 25 N-terminal amino acid residues of UAP and bikunin are identical.

1.5.2.6 Tamm-Horsfall Protein (THP)

THP also called as uromucoid, is a glycoprotein synthesized in the kidneys and isolated by Tamm and Horsfall in 1952 (Hoyer et al., 1979). Monomeric THP has a molecular weight of 85-100 kDa and consists of 70% protein and 30% carbohydrate. It has 616 amino acid residues and many disulfide bridges. Mannose rich oligosaccharide residues are incorporated into the protein backbone by N-linked glycosylation (Van Rooijen et al., 1999). THP, the most abundant human urinary glycoprotein (Kokot and Dulawa, 2000; Kobayashi and Fukuoka, 2001) is synthesized and secreted by the epithelial cells of thick ascending limb of the loop of Henle and distal convoluted tubules (Kumar and Muchmore, 1990).

THP is a most potent aggregation inhibitor. Hallson et al. (1997) established that normal THP inhibits urinary crystal aggregation and THP with the properties of low sialic acid contents are consistent with the promotion of crystal aggregation and hence stone formation THP depending on its molecular size and state of self-aggregation, may act either as an inhibitor or as a promoter of crystal formation (Meyer, 1981). The matrix of many stones is shown to contain THP (Grant et al., 1973). THP may be a specific ligand for cytokines in the kidney (Kumar and Muchmore, 1990).

THP is a weak inhibitor of crystal growth, however it is a powerful inhibitor of crystal aggregation (Carvalho et al., 2002; Robertson and Scurr,
Negatively charged particles such as THP mainly exert their effect on crystallization through calcium ions (Fleisch, 1978). When bound to specific sites on crystal surfaces, this macromolecule induce a negative electrostatic surface charge, thus lowering the rate of crystal aggregation (Hess et al., 1991). Marengo et al (2002) studied the expression of THP in ethylene glycol (EG) treated rats by northern and western blot analysis and by immunohistochemistry. Unlike other calcium oxalate inhibitors such as Osteopontin, renal mRNA and protein expression for THP was decreased in EG treated rats, THP expression did not decrease until aggregates of crystals deposited in the kidneys, while Osteopontin expression began to increase almost immediately.

The daily urinary excretion of THP in human ranges between 20-100 mg / day with a urinary volume of 1.5 liters and 11 mg / day in rats (Gokhale et al., 1997). THP from healthy individuals have a pl of 3.5, while THP from recurrent stone formers have pl values between 4.5 and 6 and both exhibit different IEF patterns (Schneirle et al., 1996) due to the presence of asialo THP (Jefferson et al., 1996). The oligosaccharide residues are responsible for the gelling effect of THP (Wangsinspaisan et al., 2001). The exposure of renal epithelial cells to oxalate ions and COM crystals can cause free radical generation and increased lipid peroxidation. THP has a protective effect on the production of free radicals invitro. The effects of THP on the protection of oxalate induced radical injury may be partly due to its intact glycosylation and its adhesion to cell membrane. This effect of THP is lost when it was deglycosylated (Hsiew et al., 2003).
1.5.2.7 Crystal Matrix Protein (CMP)

When CaOx crystallization is induced in human urine by addition of an oxalate load, the inclusion of proteins onto the crystals is found to be a selective phenomenon and the protein that was included was termed as CMP, which is a minor urinary constituent. The molecular weight of CMP is found to be 31 kDa and it contains γ-carboxy glutamic acid. CMP is present in stones (Stapleton et al., 1996) and is precisely distributed within the nephron (Stapleton et al., 1993a) suggesting that it may play an important role as an inhibitor of calcium oxalate crystallization and thereby stone formation.

CMP is related to blood coagulation protein, Prothrombin present in human plasma (Stapleton et al., 1993b). CMP has a N-terminal homology with urinary prothrombin fragment I. Urinary prothrombin fragment I exist in two forms, one with a low pI and other with a high pI. The lower pI form, is the fully carboxylated form and it is present in both the urine of normal as well as in the crystal matrix, while the high pI form is the decarboxylated form and it is not detected in CaOx crystals (Buchholz et al., 1996).

Prothrombin gene expression has been noted in normal human as well as in rat kidneys (Grover et al., 1999). PT mRNA expression seems to be increased in stone forming rats as well as in acute tubular necrosis (Suzuki et al., 1999). This family is reported to be involved in the inflammation mediated tissue repair (Verkoelen and Schepers, 2000).
1.5.2.8 Other Inhibitors

A protein, Lithostathine, co localizes with Nephrocalcin in the kidney but appears to be immunologically different (Verdier et al., 1992). Another protein an S100 protein, called Calgranulin (Calprotectin) is also synthesized in human kidney and excreted in urine, is an inhibitor of COM crystal growth and aggregation. The ability of Calgranulin to inhibit crystal growth is possibly related to its ability to bind to the crystal surface (Sokalingum et al., 1998).

1.6 OXALATE BINDING PROTEINS

1.6.1 Intestinal oxalate binding protein

Hyper absorption of dietary oxalate has been attributed to be one of the causative factors for idiopathic calcium oxalate nephrolithiasis (Hodgkinson 1977). Studies by Pinto and Paternain showed for the first time the presence of an oxalate transport system mediated by a carrier protein with a molecular weight of 73kD (Pinto and Paternain, 1978). They speculated two different oxalate transport systems, one operating at low oxalate concentration mediated by the transport protein and the other at high oxalate concentration by passive diffusion.

Pyridoxine deficiency causes hyperoxaluria in man and animals (Gershoff, 1970). In pyridoxine deficiency, oxalate absorption takes place in biphasic manner in which a carrier - mediated saturable component facilitates oxalate uptake from lumen into enterocytes at low mucosal oxalate concentration (Farooqui et al., 1981).
Koul et al. (1991) identified an oxalate binding protein in the brush border membrane having kinetic properties of reversibility, saturability, temperature sensitivity and inhibition by substrate analogues. The protein is induced under pyridoxine deficiency with two distinct classes of receptor sites for oxalate, one with high affinity and the other with low affinity. The protein has a molecular weight of 79 kDa.

1.6.2 RBC Band 3 Protein

The anion transporter, band 3 protein is ubiquitous which is present not only in cell membranes, but also in nucleus, golgi and mitochondrial membranes (Kay et al., 1994). It is involved in respiration, acid base balance and in the major structural protein linking the plasma membrane to the cytoskeleton. The transport of chloride and bicarbonate, the physiologically important anions, is rapid in human red blood cells from adult, late fetuses (Braham and Wimberley, 1989) and in adult chicken RBC (Braham and Wleth, 1977). Band 3 protein has been cloned in many species (Lux et al., 1989) and the gene is located on chromosome 17. It is a protein with 911 amino acid proteins having a hydrophilic cytoplasmic domain, a hydrophobic transmembrane domain and an acidic C-terminal domain. The amino acids involved in anion exchange are lysine, arginine, histidine and glutamic acid (Ramjee et al., 1980). Band 3 proteins are shown to be involved in the transport of oxalate across the human RBC membrane (Baggio et al., 1986). Band 3 proteins behaved as an oxalate exchanger in a phosphorylation dependent manner (Baggio et al., 1993). Increased arachidonic acid content
of the RBC membrane of stone formers was attributed for the enhanced oxalate exchange through the phosphorylation reaction.

Band 3 like proteins is present along the gastrointestinal tract as well as the renal tubule (Alper et al., 1987). Immunological studies using highly purified antibodies raised against the intramembranous domain of band 3 have shown specific fluorescence in the alpha intercalated cells of collecting duct (Verlander et al., 1988). A link between the red blood cell abnormality and renal stone formation has been suggested (Borsatti, 1991). Patients with primary calcium oxalate nephrolithiasis have a significantly elevated red blood cell oxalate exchange (Baggio et al., 1984; Motola et al., 1992).

1.6.3 Renal oxalate binding proteins

1.6.3.1 Mitochondrial oxalate binding proteins

Oxalate binding protein has been identified in tissues like human kidney, rat kidney and liver (Laxmanan et al., 1986). Other rat tissues like heart, lung, skeletal muscle, spleen, stomach, small or large intestinal homogenate has no appreciable oxalate binding activity. About one third of total oxalate binding is found to be localized in the inner mitochondrial membrane. The binding of oxalate is specific and the other substrate analogues compete with it less efficiently. The binding of oxalate is found to be rapid, reversible, dependent on oxalate concentration, and is temperature sensitive. Scatchard Plot analysis has shown the maximum binding capacity (Bmax) with 49pmol/mg protein with dissociation constant (Kd) of 43nM. Calcium has no effect on oxalate binding (Laxmanan et al., 1986) suggesting
that the binding is oxalate specific. The purified proteins from human as well as rat mitochondria have molecular weights of 62 and 58kD respectively. Both proteins have higher percentage of both basic amino acids and acidic amino acids. Antibody raised to the rat protein inhibits oxalate binding and also cross-reacts with the human protein. Proteoliposomes prepared with the proteins show accumulation of oxalate confirming transport function of the protein (Selvam and Devaraj, 1997).

1.6.3.2 Nuclear oxalate binding proteins

About two third of the total cellular oxalate binding activity is distributed in the kidney or liver nucleus (Menon et al., 1984). Selvam and Kannabiran, 1996 have shown that most of the oxalate binding activity was associated with histone – H1 fraction, that centered on H1B fraction. Two distinct binding sites are present for histones, one with high affinity and the other with low affinity. Transport inhibitors like DIDS and phenyl succinate inhibit the oxalate binding activity. Similarly, liver H1B is found to have maximal oxalate binding activity with similar characteristics of renal protein (Selvam and Prasannalakshmi, 1996). Different tissues histone H1 fractions also exhibit oxalate binding showing its ubiquitous nature.

About 40% of the total nuclear oxalate binding is localized in nuclear membrane. Two oxalate-binding proteins have been identified on the nuclear membrane, one having a molecular weight of 68kD (Selvam et al., 1996) and the other in the nuclear pore complex having a molecular weight of 205 kD (Vijaya and Selvam, 1999). Both oxalate-binding proteins exhibit maximal binding at pH 7.4 for both rat and human.
1.6.4 Calcium Oxalate Binding Proteins

Renal calcium binding proteins have been identified in several biological systems (Hermsdorf and Bronner, 1975). Resncik et al. (1980) reported excess excretion of calcium binding proteins in kidney stone formers. Calcium binding proteins as well as molecules like sialic acid, γ-carboxy glutamic acid and phosphatidic acid allow high local concentrations of calcium inside the cell in hyperoxaluric condition (Rengarajan and Selvam, 1987; Rengarajan and Selvam, 1989; Angayarkanni and Selvam, 1998).

Using CaOx, the existence of calcium oxalate binding protein has been demonstrated in several tissues of rat (Adhirai and Selvam, 1998) of which kidneys showed maximal binding activity among the various tissues studied. Renal medulla exhibit higher calcium oxalate binding activity than that of papilla or cortex. Sub cellular studies revealed the enrichment of this protein in nucleus. The molecular weight of the protein is 45kD and it exhibits kinetic properties of concentration and time dependency, optimum temperature, and substrate saturability with single affinity site with a Kd of 41nM and Bmax of 6.5nmol/mg protein. This binding is inhibited by DIDS while EGTA and ruthenium red did not affect the binding suggesting that the binding sites of the protein was oxalate rather than calcium specific site. The richness of Lysine - E - amino groups are suggested to be responsible for its oxalate binding activity.
1.6.5 Calcium Oxalate Monohydrate (COM) Adsorbing Proteins

All urinary stones contain an organic matrix, which comprises approximately 2-5 percent of total stone weight. Organic matrix has been considered essential for the genesis and mineralization leading to the growth of urinary calculi in the process of stone formation (Morse and Resnick, 1988). As the matrix proteins are thought to be present due to the inclusion by tissue trauma or by co precipitation along with crystals, the actual proteins involved in the active crystallization process are still unidentified. So, studies have been attempted to isolate the proteins, which are adsorbed with calcium oxalate monohydrate crystals in urine. Several proteins have been identified by this method. These proteins resemble to those identified in stone matrix. (Atmani et al., 1996; Doyle et al., 1991; Hess et al., 1989; Hoyer, 1994). All these proteins, such as Nephrocalcin, Tamm Horsfall Glycoprotein, Crystal Matrix Protein or prothrombin fragment F1 and bikunin have characteristic properties of calcium binding and inhibition of crystal growth. None of these proteins had been tested for oxalate binding activity. Several calcium oxalate monohydrate crystal adsorbed proteins and stone matrix proteins with oxalate binding activity have been identified in our laboratory.

1.6.5.1 Human kidney

The proteins are adsorbed on calcium oxalate crystals by allowing them to interact with triton-extracted human kidney homogenate. The proteins are then subjected to DEAE-Cellulose column chromatography and three protein peak fractions are eluted (designated as fraction I-III).
according to their order of elution with increasing concentration of sodium chloride (Selvam and Kalaiselvi, 2000). Among the three eluted protein peaks, the protein eluted with 0.3M NaCl on DEAE cellulose column (Fraction III) has maximum oxalate binding activity of 270pmol/mg protein at pH 4.5. The protein had no oxalate binding at pH 7.4. The purified protein has a molecular weight of 23kD. Amino acid analysis showed that 18% of the total molar proportion is constituted by basic amino acids (lysine and arginine) and acidic amino acids constitute by only 11%. Modification of lysine group abolishes oxalate-binding activity. In contrast to fraction III, fraction I has oxalate binding activity at pH 7.4. The purified protein has a molecular weight of 45kD with an activity of 280pmol oxalate/ mg protein. The protein has the kinetic properties of saturability, with a Kd of 1.96nmol and Bmax of 200pmol/mg protein (Selvam and Kalaiselvi, 1999).

1.6.5.2 Rat kidney

Among the different tissues, the COM adsorption of proteins derived from kidney was found to be maximum. DEAE-Cellulose chromatographic fractionation had yielded three proteins with oxalate binding activities, fraction I-III according to their order of elution. The molecular weight of these fractions was determined to be 74,20 and 23kD respectively. Sub cellular distribution studies of these proteins had revealed that 20 kD was largely distributed in microsomes, 74kD in mitochondria and 23kD in nucleus (Selvam and Kalaiselvi, 2001). 74kD protein had exhibited maximum oxalate binding activity at pH 7.4 while 23kD protein at pH 4.5 with no activity at pH 7.4.
1.6.5.3 Human kidney stone matrix

When EDTA-extractable proteins from human kidney stone matrix were subjected to DEAE - cellulose chromatography three protein peaks were identified with oxalate binding activity in the order of elution, Fraction 1-3 Fraction 1 on further passage through Sephadex G-200 column, separated into 48kD and 29kD proteins. 48kD protein had maximal oxalate binding activity when compared to the other. Both proteins exhibited the binding characteristics similar to renal oxalate binding proteins (Govindaraj and Selvam, 2002)

1.7 THEORIES OF STONE FORMATION

1.7.1 Matrix nucleation theory

According to this theory, biological macromolecules such as mucoproteins, urinary proteins and glycosaminoglycans form the initial nuclei for crystallization and serve as a bridge or platform that binds pre-formed crystals to grow and aggregate paving the way for mature stone formation. The veracity of matrix theory is substantiated by the ubiquitous nature of organic matrix in urinary stones (Boyce and Garvas, 1956), and the observations of Khan and Hacket (1993) who have shown the presence of cellular degradation products in stones and suggesting that this may be involved in heterogeneous nucleation.

1.7.2 Precipitation crystallization theory

A stone forms when urine is supersaturated with respect to its constituent crystals. This mechanism is important for at least certain types of
stones such as cystine, uric acid and xanthine. There are three different phases in crystal development: nucleation, growth and aggregation. According to this theory, the spontaneous nucleation from the solution does not occur unless the formation product is exceeded (Elliot, 1973). Supersaturation of urinary colloids results in precipitation as a crystal initiation particle, which when trapped, acts as a nidus, leading to subsequent crystal growth (Finch et al., 1981).

1.7.3 Inhibitor absence theory

This theory elaborates that deficiency of inhibitors of crystal nucleation; growth or aggregation can cause stone formation. This theory supports that urine naturally contains both high and low molecular weight inhibitors (Ryall, 1993). Many inhibitors of crystallization have been identified. Low molecular weight inhibitors like phosphate (Fleisch and Bisaz, 1964), magnesium and citrate (Robertson et al., 1984) and high molecular weight inhibitors are small peptides (Howard et al., 1967), acid mucopolysaccharides (Robertson et al., 1976), GAG's (Ryall et al., 1981) and THP (Grover et al., 1990) have been identified in urine. These inhibitors bind to the crystal surface and poison their growth rate or bind to stone forming mineral constituents and thereby reduce the degree of supersaturation.

1.7.4 Fixed particle retention theory

In the free particle mechanism, particle size has been the decisive factor, while in the fixed particle mechanism; adhesion plays a major dominant role. Finlayson and Reid (1978) have calculated the chances of forming a stone through a fixed particle mechanism in the renal tubules,
pelvis and the bladder. The calculations were based on average normal values for oxalate excretion, kidney structure and nephron dimensions and they have found that particle size could never become large enough to be retained in the nephron. They concluded that particle retention could occur through retention caused by adherence to cells or disturbed urinary flow conditions must play an important role in stone formation.

1.7.5 Injury induced crystal retention theory

This theory was proposed by Selvam (2002). Many studies have shown close relationship between tissue injury and stone disease. It is suggested that there is a self-perpetuating cycle, where, injured epithelium might either nucleate crystals or preferentially attach preformed crystals. The crystals would then further damage the cells to which they are attached, generating more injured tissue that could complete the cycle by inducing more crystal retention either by nucleation of new crystals or by attachment of crystals formed higher in the nephron. Damage to urothelial cells produced by instilling 0.1 N HCl or 5% Triton X-100 into the rat urinary bladder has produced a marked increase in binding of calcium oxalate (Khan et al., 1984) with high correlation between the extent of damage and the oxalate deposits.

Hyperoxaluria itself is found to be damaging to the endothelium (Weissner et al., 1986). Mandel and Riese (1991) have proposed that tubular injury or damage to epithelial cell membrane is a necessary requirement for crystal retention. Crystal retention leads to successful stone formation.
**FREE RADICALS AND STONE FORMATION**

Lipid peroxidation is a degenerative pathway of membrane components mediated through free radicals produced in the cells. The evidence of the involvement of oxalate in free radical-mediated LPO reaction for the membrane injury is further strengthened by the subsequent observations made in several other laboratories (Scheid *et al.*, 1996; Thamilselvan *et al.*, 1997). Free radicals have been proposed as one of the predisposing factors of oxalate and calcium oxalate binding and subsequent stone formation (Selvam, 2002). All aerobic cells generate enzymatically or nonenzymatically oxygen-derived free radicals, superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO), hydroxy radicals (OH) and peroxyl radical (ROO) (Halliwell and Gutteridge, 1989) as part of the many normal biological processes. Under pathological conditions, the rate of formation of partially reduced oxygen species is increased and/or the antioxidant defenses of the cells are weakened, eventually leading to oxidative cell injury (Halliwell and Gutteridge, 1989; Hauptmann and Cadenas, 1997). The mechanism of cell injury by partially reduced and thereby activated oxygen species is explained through the action of hydrogen peroxide (H$_2$O$_2$).

Ascorbic acid, a precursor of oxalate biosynthesis, has been shown to promote LPO *in vitro* in tissue nonenzymatically. Ernster (1967) was the first to show enhanced ascorbic acid-linked LPO in the presence of iron complexes as well as oxalate, showing the pro-oxidant nature of oxalate. The increased LPO formation by oxalate is probably associated with the
generation of oxygen free radicals. The experimental evidence for the accumulation of hydroxyl radical in both kidney and liver was prevented in urolithic rats when induced by feeding either a sodium glycolate-supplemented diet (Selvam and Bijikurien, 1991; Selvam and Bijikurien, 1992) or a B6-deficient (Ravichandran and Selvam, 1990a; Selvam and Ravichandran, 1991).

The excessive formation of hydroxyl radical observed in urolithic rat kidney was explained on the basis of inhibition of catalase activity by oxalate in vitro (Selvam and Bijikurien, 1987). The inhibition of catalase activity was found to be oxalate concentration-dependent (17% of inhibition at 0.5 mM and 30% inhibition at 1 mM), and the same effect had been suggested for the observed decreased catalase activity in urolithic kidney (Bijikurien and Selvam, 1989). In the urolithic kidney, both iron and copper were accumulated, and this process could facilitate the formation of hydroxyl radical (Ravichandran and Selvam, 1991; Ravichandran and Selvam, 1990b).

The basal levels of LPO products, thiobarbituric reactive substances (TBARS), hydroperoxides, diene conjugates, and lipofuscin were elevated. Like nicotinamide adenine dinucleotide phosphate irrespective of the nature of urolithic agents used to induce either hyperoxaluria (Selvam and Bijikurien, 1987; Selvam and Bijikurien, 1992; Selvam and Bijikurien, 1991; Bijikurien and Selvam, 1989; Thamilselvan et al., 1997). When the animals were treated with a glutathione-depleting agent such as buthionine sulfoximine or nephrotoxin, and cyclosporine along with a calculi-producing agent The
susceptibility of renal tissue for LPO in the presence of promoters oxalate was enhanced approximately 25 fold (Ravichandran and Selvam, 1990c).

The RBC of stone patients were found to be osmotically fragile, and the RBC membranes not only showed elevated basal levels of LPO products but also released an excess of it in presence of promoters, suggesting that the RBC of stone formers are prone to LPO reaction because of the loss of some protecting molecules (Anuradha and Selvam, 1988). The plasma of stone patients and urolithic rat showed significant accumulation of lipofuscin pigment, a conjugate product of LPO reaction, suggesting that a free radical-mediated LPO mechanism is highly operative in the stone formers (Anbazhagan et al., 1999; Anuradha and Selvam, 1988; Ravichandran and Selvam, 1990d).

A subtle change in the composition of fatty acids could damage the membrane, and this is often sufficient to increase greatly the susceptibility of the membrane to oxidative damage. In the chemically damaged urothelium, a direct relationship between the extent of membrane damage and the oxalate salt deposits was observed (Khan et al., 1984).

1.9 ANTIOXIDANT THERAPY

If LPO is the major cause of tissue injury, then further experiments should show that prevention of peroxidation by antioxidants prevent cell damage. (Slater, 1984). Supplementation of antioxidants either – SH generating amino acid methionine (Selvam and Bijikurien, 1991) or – SH reagents such as GSH monoester (Muthukumar and Selvam, 1997) and lipoic
acid (Sumathi et al., 1993), or cysteine (Saravanan et al., 1995) hydroxy radical scavengers such as mannitol (Thamilselvan and Selvam, 1997) or vitamin E (Kotush et al., 1996) and triterpenes (Malini et al., 2000) abolished the accumulation of LPO products in tissues under urolithic conditions. This normalization process has been suggested due to arresting of free radical-mediated reactions during antioxidant therapy in urolithiasis. This is facilitated because of restoration of the levels of pro-oxidants such as iron, copper and oxalate and the antioxidant enzymes, SOD, catalase, GPx, free radical scavengers, GSH, ascorbic acid, vitamin E and protein thiol groups (Selvam and Ravichandran, 1993). At the same time, there was no retention of calcium oxalate crystals in the tissue, even though the excretion of oxalate was not reduced in experimental urolithiasis (Muthukumar and Selvam, 1997; Selvam and Bijikurien, 1991; Thamilselvan and Selvam, 1997). This was due to the protection of vitamin E against oxalate-induced cell injury. Vitamin E is known to protect against chemically induced cell injury by maintaining cellular protein thiols as a cytoprotective mechanism (Pascoe et al., 1987).

Nephrolithiasis, which was readily produced in the control animals, was prevented in the experimental animals by pretreatment with fish oil, and urine calcium excretion was significantly reduced. The urinary calcium and oxalate excretion in the recurrent hypercalciuric stone formers was significantly reduced with fish oil treatment over an 8-week period (Coln et al., 1991). All these observations implicated the radical-mediated membrane changes, predisposing a favourable environment for subsequent crystal deposition and then retention. Antioxidants intervene in this process,
protect the membrane from injury, and prevent adherence or retention of the crystals. GAGs have an antiadherent property and prevent the development of solid concretions on urothelium. Free radical scavengers such as phytic acid prevent the deposition of crystals (Grasses et al., 1998). Pretreatment of rats with vitamin A has an inhibitory effect on lithogenesis through its action on tubular cellular repair (Sakly et al., 1994).

1.10 VITAMIN E

Vitamin E is the term used for eight naturally occurring fat soluble nutrients called tocopherols. It is comprised of a family of hydrocarbon compounds characterized by a chromanol ring with a phytol side chain referred to as tocopherols and tocotrienols. The isomers of biological importance are tocopherols, of which alpha-tocopherol is the most vitamin. Because of lipophilic property, it is widely distributed throughout the body. Vitamin E is involved in a variety of physiological and biochemical functions. The molecular mechanism of these functions is mediated by either the antioxidant action of the vitamin or by its action as a membrane stabilizer. Alpha-tocopherol is an efficient scavenger of lipid peroxyl radicals and, hence, it is able to break peroxyl chain propagation reactions (Wang and Quinn, 1999).

Alpha-tocopherol (Vitamin E) scavenges reactive oxygen species, and in the process, it is converted to alpha-tocopherylquinone. Alpha-tocopherol binds to alpha-tocopherol transfer protein (alpha TTP) in the liver cytosol, whereas alpha-tocopherolquinone binds to glutathione-S-transferase (Arita et al., 1998). Vitamin E is concluded as a universal
participant of antioxidant defense reactions in biological membranes, since it acts at all stages preventing membrane oxidation damage (Evshtneeva et al., 1998). In addition to its antioxidant effect, vitamin E also intervenes in the regulation of several enzymes and probably has impact on gene expression (Feki et al., 2001).

Experimental evidence available shows that vitamin E is capable of dose-dependently regulating mitochondrial generation of super oxide and hydrogen peroxide, by preventing electron leakage, by mediating the super oxide generation systems directly and/or by scavenging super oxide generated (Chow, 2001). Restoration of antioxidants by dietary supplementation of methionine (Selvam and Bijikurien, 1991) is found to prevent calcium oxalate crystal deposition in chronic hyperoxaluria. Although vitamin E has less effect in controlling events like nucleation and growth, it was efficient in the repair mechanism and supported the hypothesis that oxalate crystals may be destructive to renal epithelium because they are large and irregular (Sakly et al., 1994). Antioxidant administration may prevent calcium oxalate nucleation and retention in the renal tubules by preventing oxalate mediated peroxidative injury (Thamilselvan et al., 2003).

1.11 SCOPE OF THE PRESENT INVESTIGATION

Free radicals have been implicated to be one of the causes for the pathological biomineralization process within the urinary tract namely urolithiasis. This has been formed the basis of the newly proposed injury induced crystal retention theory by Selvam (2002). The veracity of the theory
comes from the experimental evidences that supplementation of antioxidants like methionine (Selvam and Bijikurein, 1991), lipoic acid (Sumathi et al., 1993) and GME (Muthukumar and Selvam, 1997) to hyperoxaluria challenged rats effectively reduces crystalluria and prevents crystal retention. Similarly, injury to the urothelium by Triton X - 100 or 0.1 N HCl or gentamycin increases crystal deposition (Khan et al., 1984).

Preliminary studies by Anbazhagan et al. (1999) and Sumuthra (2003) in our laboratory have shown that vitamin E supplementation to hyperoxaluric patients brings down the urinary risk factors like oxalate, calcium, phosphorus etc. The effectiveness of vitamin E therapy should be judged not only by the risk factors but also by the status of urinary lithogenic proteins as these proteins direct the act of stone formation by mimicking the process of biomineralization. Hence, the present study is aimed 1) to find out whether vitamin E is capable of recovering the structural and functional alterations of calcium oxalate monohydrate binding proteins induced by hyperoxaluria in humans 2) in order to assess the status of calcium oxalate binding proteins in kidneys the studies were carried out in rat model also.