Chapter I

INTRODUCTION
The occurrence of stones in the urinary tract has been documented since the beginning of history. Reports of urinary stone disease from 4800 B.C. to the present era were reviewed by Anderson (1972). Despite many modern advances in treatment and prevention of urinary calculi, it still constitutes a significant medical problem for about 4% of the general population (Schmucki and Asper, 1986). The prevalence of urinary tract stone disease is estimated to be 2-3%. The recurrence rate of calcium oxalate renal stones, without treatment, is about 10% after 1 year, 33% after 5 years and 50% after 10 years of its first occurrence/removal (Uribari, 1989).

Urolithiasis is a multifactorial disease and both intrinsic as well as extrinsic risk factors are involved in the pathogenesis of disease. These include heredity, age, sex, climate, geographical pattern, occupation etc. Abdel-Halim et al. (1989) showed that incidence of urolithiasis is more predominant in males than females. Other important risk factors include nutritional deficiencies of certain vitamins viz. vitamin A (Sharma et al., 1990), vitamin B₁ and B₆ (Sidhu, 1985; Ravichandran and Selvam, 1990), diets deficient in magnesium (Al-modhefer, 1986; Rattan, 1992) and other dietary factors like high protein and carbohydrate intake (Conyers et al., 1990; Kok et al., 1990).

Three interrelated factors which significantly promote the precipitation of crystalloids within the urinary tract are (i) the degree of supersaturation of the urine with respect to constituents of calculus (ii) development of nucleation site and (iii) the concentration of inhibitors of crystallization. Werness et al., (1981) demonstrated that crystal formation within the urinary tract is a dynamic process involving several steps, many of which occur simultaneously. These include nucleation, growth, phase transformation, aggregation, disaggregation and dissolution. Two fundamental critical factors involved in the crystal precipitation and stone formation are the solute and the solution (urine) which must be supersaturated in the precipitating crystalline phase (Smith, 1990). In addition, solute concentration, ionic strength, complexation and pH, all influence the state of saturation of urine. Urine contains certain inhibitory substances which can complex or bind with potential solutes, thereby
effectively reducing their concentration in solution e.g., citrate and phosphate bind calcium, while magnesium and sodium bind oxalate. Removing solute in the form of soluble complexes reduces the free ion activity (concentration), decreasing saturation for the specific crystal growth (Smith, 1990). An imbalance between urinary oversaturation with stone constituent ions and concentrations of protective inhibitors can result into stone formation. Therefore, studying the process of calcium oxalate stone formation and measurement of inhibitory potential of urine is an important aspect of calcium oxalate nephrolithiasis.

Hyperoxaluria plays a critical role in stone formation and changes in urinary oxalate concentration have been shown to be fifteen times more potent as changes in calcium concentration in altering urinary saturation with calcium oxalate (Finlayson, 1974). Trinchieri et al. (1991) also suggested hyperoxaluria rather than hypercalciuria as more significant for the causation of calcium oxalate stone disease, thus making oxalate metabolism a focal point of scientific investigations. Since animals have a limited ability to metabolize oxalate, the primary route of oxalate elimination is by urinary excretion. Three major sources of urinary oxalate causing hyperoxaluria are increased dietary absorption or intake, increased endogenous production or increased renal excretion. Approximately 80-100 mg of oxalate per day is ingested in the diet, majority of which is bound by calcium in the lumen and excreted in the feces, leaving 10-20% available for absorption. Hyperoxaluria and nephrolithiasis could be the complications of gastrointestinal disorders e.g., inflammatory bowel disease, coeliac disease etc. The origin of increased urinary oxalate in such cases is the diet. Oxalic acid absorption by the intestine depends upon the availability of soluble or bioavailable oxalate (Brinkley, 1990). Tiselius et al., (1981) demonstrated the hyperabsorption of oxalate in patients of enteric hyperoxaluria. Dobbins and Binder (1976) have shown oxalate absorption by the large bowel whereas Prenen et al., (1980) described the small bowel as a principal site of oxalate absorption. Hatch et al. (1984) have established that the net mucosal to serosal transfer is an energy-dependent process.
The second source of increased oxalate excretion could be the abnormal endogenous production of oxalate. Endogenously, oxalic acid is synthesized mainly from ascorbate and glyoxylate. Baker et al. (1966) have shown that oxalate is derived from carbon atoms 1 and 2 in the oxidative metabolism of ascorbic acid and both dehydro-L- ascorbic acid and 2,3-diketo-L-gluconic acid are the intermediates in this process. The other major precursor of oxalate is glyoxylate, a nucleus for a multitude of metabolic routes. Its immediate precursors are glycine, glycolic acid and alpha-keto-gamma-hydroxyglutarate. Glycine and glycolate are both precursors and products of glyoxylate. Glycolate is produced by a reduction reaction using lactate dehydrogenase as a principal enzyme. The other major enzymes involved in the synthesis of oxalate via glycolate involved in the synthesis of oxalate via glycolate-glyoxylate-oxalate pathway are glycolic acid oxidase and xanthine oxidase (Yanagawa et al., 1990).

The third abnormality contributing to hyperoxaluria relates to the renal handling and membrane transport of oxalate. Oxalate is freely filtered in the glomerulus and proximal tubule appears to be the only segment of the nephron, important in oxalate transport. Several transporting systems like Chloride, formate, hydroxide, sulphate, bicarbonate were found to be involved in the oxalate transport across the renal brush border membrane (Karniski and Aronson 1987; Kuo and Aronson, 1988; Yamakawa and Kawamura, 1990). Wandzilak and Williams (1990) have examined the transepithelial movement of oxalate in renal tissue and described the presence of two distinct transporters, one on the apical (luminal) and other on the basolateral (contraluminal) membrane. Oxalate can use both the exchangers thereby explaining the mechanism for both luminal reabsorption and secretion in the proximal tubule. Baggio et al., (1989) have reported a faster rate of oxalate exchange in erythrocytes of stone formers than from controls which is due to the phosphorylation of band-3 protein (Cl⁻/HCO₃⁻-anion exchanger). The observation led to the hypothesis that the abnormal transport of oxalate could be underlying causative factor in idiopathic calcium oxalate nephrolithiasis. If this membrane peculiarity is also present in gastro-intestinal and renal epithelial cells, an increase in
gastrointestinal absorption and renal excretion of oxalate may both contribute to the calcium oxalate stone formation.

Urolithiasis is often related to inadequate or inappropriate medical and surgical treatment. The development of extracorporeal stone destructive techniques, percutaneous lithotripsy and refinements in endoscopic surgery have contributed to the less invasive and noninvasive management of patients with stone disease. Inspite of the complicated surgical removal or the use of above mentioned sophisticated techniques, pharmacologic therapy of recurrent nephrolithiasis continues to be the foremost strategy in the prevention of calcium oxalate stone disease. The best approach in the initial treatment to prevent recurrent stone formation is the hydration and reduction in dietary intake of calcium and oxalate. Maximum fluid intake should occur during the critical periods when urine has a tendency to get supersaturated with calcium oxalate. This results in the reduction of saturation of calcium phosphate and oxalate. Yendt et al., (1981) and Jaeger et al., (1985) proposed that dietary calcium restriction enhances oxalate availability in the gut and probably leads to iatrogenic stone disease in some patients who have been advised to restrict dietary calcium. Therefore treatment consists of altering the diet to avoid an excess of oxalate intake. Menon and Koul (1992) have reported that stone growth or new stone formation is not seen in up to 60% of patients treated initially with fluid and dietary therapy alone.

The drugs most commonly used in patients who form calcium oxalate renal stones are thiazide diuretics. These are particularly effective in patients with hypercalciuria as these directly stimulate calcium reabsorption in the distal tubule, while promoting sodium excretion. Thiazide induced hypokalemia may cause hypocitraturia with a resultant decrease in urinary inhibitors (Menon and Koul, 1992). Besides this, various other side effects of thiazides like weakness, fatigue and impaired carbohydrate tolerance contraindicate the use of thiazides in management of renal stone disease. Long term treatment with low doses of pyridoxine was effective in lowering hyperoxaluria in renal stone patients (Murthy et al., 1982; Vathasala et al., 1989), though studies of Caudarella
et al., (1989) failed to support it. Evaluation of prophylactic effect of alkaline citrate on formation of stones has been made by several workers (Butz and Dulce, 1981; Nicar et al., 1984; Pak et al., 1985; Tiselius, 1985). Pak (1987) has demonstrated that potassium citrate given in a dose to provide 20-30 mEq of potassium and base, twice daily could prevent stone formation in many patients by increasing citrate excretion and urinary pH. Increased citrate complexes calcium and the raised pH promotes the complexation of both calcium and oxalate, thereby decreasing saturation of calcium oxalate. The results of potassium citrate treatment have been encouraging, although no long-term trials with adequate controls have been done.

The effectiveness of orthophosphate therapy for the prevention of stone formation and renal failure in patients of idiopathic calcium oxalate urolithiasis has been suggested by Thomas (1978) and Smith (1980, 1989). Orthophosphate reduces the state of saturation and increases the inhibition of crystal formation but the cathartic action of orthophosphate in some patients was severe enough to interfere with the therapy. Also orthophosphate is contraindicated when infection or obstruction is involved in the stone formation, because in these situations it may actually promote stone growth as the mechanism of stone formation is different. Magnesium supplementation, as magnesium oxide, magnesium hydroxide or magnesium citrate has been reported by several workers to be beneficial in the prevention of recurrent formation of calcium oxalate renal stones (Tiselius et al., 1980; Gulati et al., 1988; Jarrar et al., 1989). The therapeutic use of magnesium in the renal stone disease is based on the observations that magnesium binds oxalate in the gastrointestinal tract, increases the solubility of calcium oxalate in urine and also acts as an inhibitor of calcium oxalate crystallization (Barilla et al., 1978; Berg et al., 1986). However, Lindberg et al., (1990) reported that although magnesium salts have been proven beneficial in calcium oxalate nephrolithiasis, these have a limited citraturic action and do not prevent hypokalemia in patients with enteric hyperoxaluria or those treated with thiazide diuretics.
Therefore, in view of the limitations of modern medicine and economic constraints for a developing country like India, indigenous drugs can form an indispensable part of health care, in the management of idiopathic urolithiasis. Ayurveda, the oldest existing medical system and several other traditional medical systems are recognized by World Health Organization (WHO) (Zaman, 1974) and are widely practiced. Therapeutic actions in these practices include the use of diet, exercise, herbs etc. and these are maximally effective, only if appropriate dietary measures are instituted to support the restoration of physiological balance. Ayurvedic physicians prescribe a number of herbal food supplements. Although, recent studies indicate their potential applications, further investigations are clearly needed. Chopra (1958) and Nadkarni (1976) have described several indigenous drugs for the prevention and treatment of various urinary disorders and many of them are being used in our country. Therefore, to elucidate the role and therapeutic efficacy of some of these herbal drugs of indigenous origin, in urinary stone disease in terms of oxalate metabolism the present work was undertaken with the following aims and objectives:

1. Induction of acute hyperoxaluria in rats by feeding sodium glycolate.
2. To investigate the therapeutic efficacy of ayurvedic extracts of plants available in this region.
3. The drug found most suitable in controlling hyperoxaluria will be used to study its effect on
   (a) Oxalate biosynthesizing enzymes viz. GAO, GAD and LDH in the rat liver and kidney.
   (b) Urinary inhibitory activity towards COM crystal growth in an in vitro assay system.
   (c) Oxalate uptake by renal brush border membrane vesicles isolated from rat kidney.
   (d) Lipid composition of the renal brush border membrane.