SUMMARY

The role of dicarboxylic acid on oxalate metabolism in experimental hyperoxaluric and Calcium oxalate stone forming rats and isolation and characterisation of oxalate degrading bacteria has been studied under four major sections in the thesis and the results obtained are summarised as follows:

1. **In vitro** calcium oxalate crystal growth studies.
2. Short term studies in hyperoxaluric rats.
3. Long term studies in CPD fed calculogenic rats.
4. Isolation and characterisation of oxalate degrading bacteria.

Section I **In vitro** calcium oxalate crystal growth studies

The method of Bauman and Walker had been used for measuring the inhibitory effect of mono and dicarboxylic acids on the growth and aggregation of calcium oxalate crystals. L(+) tartaric acid, maleic acid, malic acid, succinic acid, pyruvic acid and malonic acid are able to protect a given mass of calcium oxalate from growth. The percentage of crystal growth inhibition increases with increasing concentration of the above acids. L(+) tartaric acid brought about significant reduction in the calcium oxalate crystal growth (20.92%) followed by maleic acid (39.98%), malic acid (41.94%), succinic acid (48.18%), pyruvic acid (59.47%) and malonic acid (69.97%). The results of the above study show the inhibitory action of mono and dicarboxylic acid.

Section II short term studies in hyperoxaluric rats

The role of mono and dicarboxylic acid administration on oxalate metabolism in glycollalte induced hyperoxaluric rats were investigated.
Liver GAO activity in the glycollalte induced hyperoxaluric rat was significantly increased. GAO, the major liver enzyme involved in the endogenous synthesis oxalate, which led to increased renal and urinary excretion pattern of oxalate along with the associated calcium. Mono and dycarboxylic acid treatment lowering the level of oxalate synthesising enzyme GAO in the liver, along with kidney tissue and urinary excretion stone forming constituents. LDH activity was found to increase slightly in the liver and kidneys of hyperoxaluric rats and decrease with mono and dicarboxylic acid treatment. The lack of effect of LDH is of advantage, since dicarboxylic acid can be used without affecting the normal metabolic reaction of LDH.

The possibility of regulating oxalate metabolism in hyperoxaluric condition by way of inhibiting liver GAO and super saturation of calcium and oxalate were controlled to greater extent with mono and dicarboxylic acid which indicate its beneficial action in this respect.

Section III Longterm studies in CPD fed calculogenic rats

Experimental calcium oxalate lithiasis was induced in male rats by feeding calculate producing diet for 30 days. The effect of L(+) tartaric acid, maleic acid and malic acid were investigated in kidney and liver tissue and in urine.

Liver is the major tissue to synthesis oxalate endogenously. The key enzyme in oxalate synthesis, GAO activity was elevated in stone formers. Dicarboxylic acid administration decreased GAO activity significantly compared to CPD fed rats but the activity was still higher than the control activity in calculogenic rats. The influence of dicarboxylic acid play and important role on the liver GAO activity which in turn affect the oxalate content in the renal tissue and urine. Liver and kidney LDH activity was increased in CPD fed rats compared to control rats. The enhanced LDH activity was restored to nearly normal in both liver and kidney tissue of dicarboxylic treated rats.

The renal deposition of stone forming constituents elevated in CPD fed rats. Calcium deposition was reduced considerably with dicarboxylic acid treatment when
compared to stone formers. Similarly oxalate content was reduced in calculogenic rats administered with dicarboxylic acid, but not equal to that of control rats. Urinary supersaturation with respect stone forming constituents is generally considered to be one of the causative factors in calculogenesis. CPD rats exhibited increased urinary exhibition of calcium, oxalate and phosphorous and lowered the magnesium level. Dicarboxylic treatment lowered the calcium and oxalate levels considerably increased the magnesium concentration but the values did not equal to that of control. The above investigation indicate that dicarboxylic acids, along with other inhibitors of calcium oxalate crystallization in urine, may have additive effect and thereby hinders stone formation.

Light microscopic examination of Hematoxilin-Eosin (H.E) stained liver and kidney section showed various morphological deformalities. CPD feeding increased endogenous oxalate production which inturn produced focal necrosis in liver tissues. At the same time cystic dialation and extensive necrosis observed in renal tissues. These structural changes at cellular level caused by CPD feeding was reversed considerably by dicarboxylic acid treatment.

Electron microscopic (TEM) examination in stoneforming rats showed the ultrastructural changes occurring in liver and kidney tissues of CPD fed calculogenic rats. We observed nuclear damage and enlargement and cytoplasmic vacuolization in liver tissue of CPD fed rats and calcium oxalate crystal aggregation in the renal intestinal cells and marked dialation in the distal tubules in kidney tissues which was quite evident from the photograph. Dicarboxylic acid treatment helped to regain the structural alteration caused by CPD feeding, to a certain extent.

Section IV Isolation and Characterisation of oxalate degrading bacteria

The formation of calcium oxalate in the urine is dependent on the saturation level of both calcium and oxalate. The management of these ions in individuals susceptible to calcium oxalate stone appears important. Oxalic acid, a highly toxic end product of metabolism, which is catabolised by limited number of bacterial species to yield formate and CO₂. In order to lower the plasma and urinary oxalate concentration in recurrent
calcium oxalate stone formers we have initiated a study of isolation and characterisation of oxalate degraders from vegetable waste (Colocasia stem) and water and soil sources. These organism requires oxalate as a sole source of carbon for growth and utilised oxalate at a rate of 86%, 79% and 73% respectively within 32 h of growth. The strain isolated from colocasia stem, *Xanthobacter* sp. capable of degrading oxalate at a higher rate which harboured a plasmid DNA of about 4kb, as potential strain for further studies.

In an attempt to improve the oxalate degrading ability of the intestinal bacteria, *Serratia* sp., isolated from rat intestine we introduced the plasmid DNA from *Xanthobacter* sp. by transformation. The resulting transformant carry a plasmid DNA of same molecular size as that of *Xanthobacter* sp. and capable of maximum oxalate degradation compared with the parental strain.

These results indicate the scope for utilising these bacterial strains as powerful weapons to eradicate kidney stone disease.