CONCLUSION
Kidney supersaturates urine with slightly soluble salts like calcium oxalates and calcium phosphates during the process of water conservation (Kok 2002). Except for special cases, the glomerular filtrate is not supersaturated. Supersaturation for calcium phosphate (CaP) is first achieved in the loop of Henle (Kok 1996), while that for calcium oxalate (CaOx) occurs in the distal tubules. These sites move along the nephron because of changes in the concentrations of calcium, phosphate or oxalate in the nephronic fluid, or variation in the nephron function (Kok 1996; Kok and Schell-Feith 1999). High concentrations present in the deep papillary nephron segments may permeate to the basement membranes and renal interstitium notwithstanding the low trans-membrane permeability of the thin loop membranes. When supersaturation is high enough and lasts long enough or promoters are present, crystals nucleate in the urine and are either excreted as crystalluria (Robertson and Peacock 1972) or deposit in renal tissue, appearing as so-called Randall's plaques (Randall 1940). Both types of nuclei may increase in size by growth and/or aggregation (Coe and Parks 1988). In most people, crystals formed in the urine are discharged without any discomfort (Robertson and Peacock 1972; Hallson and Rose 1976; Finlayson 1978; Finlayson 1977; Finlayson et al. 1990). In hyperoxaluric animal models and hyperoxaluric patients crystal deposits remain in the urinary space, causing tubular epithelial damage and formation of large aggregates (Khan 1995; Kok and Khan 1994). In addition, in hypercalciuric stone formers subepithelial deposits are found in the loop of Henle (Evan et al. 2003). Randall's plaques remain just that in most people who never form stones (Low and Stoller 1999); but in some, they may grow through the interstitium towards the papillary surface and become a nidus for stone formation (Evan et al. 2003). The fact that the widespread occurrence of crystalluria and Randall's plaques leads to stone formation in
much fewer people and then usually only once or twice a lifetime suggests there are 
mechanisms that ensure crystals pass harmlessly and plaques stay as plaques. These 
mechanisms act at all levels of crystallization and stone formation: supersaturation, 
nucleation, crystal growth, aggregation, crystal structure and habit, crystal surface properties 
and crystal interactions with epithelial cells.

Many urinary compounds have a protective role, usually involving an affinity for the 
crystals or its constituents. The common occurrence of biomolecules in renal stones has 
prompted the present study on identification and characterization of a calcium oxalate 
crystal growth protein inhibitor from human renal stone matrix and in silico interaction of 
purified protein with calcium oxalate.

1) Three different extraction methods viz. EGTA, SDS and acetic acid were used for the 
extration of renal stone matrix. Maximum amount of proteins from human renal stone 
matrix was obtained from SDS extraction method followed by EGTA and acetic acid 
extraction methods respectively. Though, the amount of protein extracted by EGTA 
extraction method was lesser compared to SDS extraction method but its activity on CaOx 
crystal growth was quite prominent in contrast to almost no activity observed in the case of 
SDS extract. EGTA extract exhibited highest inhibitory activity (98%) towards CaOx crystal 
growth followed by acetic acid (6.47%) and SDS extract (2.64%). Therefore, EGTA 
extraction method was selected for the renal stone extraction to carry out the further studies. 
2) An anionic (MW ~ 42 kDa) CaOx crystal growth protein inhibitor was purified from 
human renal stone matrix by bioactivity guided purification using anion exchange 
chromatography and molecular sieve chromatography and SDS-PAGE analysis. Finally,
homogeneity of purified protein was confirmed by HPLC. Further, the protein was in gel tryptic digested and characterized by MALDI-TOF-MS.

3) Protein was identified as human phosphate cytidylyltransferase 1 (CCT), choline, beta when m/z data obtained after MALDI–TOF-MS of digested protein was searched in MASCOT search engine. Phosphate cytidylyltransferase 1, choline, beta is a novel CaOx crystal growth inhibitor and holds a direct relevance as it is involved in the formation of phosphatidylcholine which is a constituent of calcium oxalate stones.

4) *In silico* studies revealed that interaction between calcium oxalate and acidic amino acids present in the binding sites of wild type human phosphate cytidylyltransferase 1 (CCT) showed a good docking score, an indicator of excellent interaction with calcium oxalate. However, upon substitution of these acidic amino acids with alanine, glycine, lysine, arginine and histidine, a poor docking score was observed suggesting no binding at all with calcium oxalate.

5) Lipids isolated from calcium oxalate renal stones had an ability to inhibit growth of CaOx crystals.

6) High and low molecular weight biomolecules extracted from human renal matrix of calcium oxalate (CaOx) stones have a significant influence on calcium and phosphate (CaP) crystallization.