INTRODUCTION
1.1 Introduction

Urolithiasis, the deposition of stones in urinary tract has plagued man since long and continues to pose a universal health problem even today. Histological and archeological studies have clearly revealed that ancient man suffered from urinary tract stone disease. The earliest evidence dates back to around 4,800 B.C. when a bladder stone was found among the pelvic bones of a young predynastic Egyptian. The incidence of urolithiasis appears to have been generally increasing over the last 100 years, particularly in countries which have either hot climate or which have moved from an agriculture economy to one based on industrial and technological development (Zilberman et al. 2009).

Mineralization can occur both under physiological and pathological conditions. Physiological mineralization in bone and teeth seems to be a purposeful phenomenon as it offers structural integrity and maintains homeostasis by creating mineral sinks. However, the pathological mineralization in soft tissues, e.g. tendons, cartilage, aorta and salivary, biliary or urinary system do not offer any satisfactory explanation with respect to its significance.

Urinary stones have attracted greater scientific attention not only due to their high frequency of occurrence but also due to the serious functional implications associated with
formation of such stones. Although the urinary calculi can be lodged in any part of urinary tract yet during the last two decades or so, the frequency as well as the site of occurrence of stones has shown gradual shift from the lower urinary tract (bladder and ureters) to the upper urinary tract (kidneys).

The epidemiological studies have demonstrated that there are various stone belts or pockets in many developed as well as developing countries. As far as India is concerned two distinct stone belts having very high incidence of urinary calculi have been identified in Northern and Central India (Coblabawala 1971). Interestingly, the incidence of this disease has been reported to be very low in the southern and Eastern coastal parts of India. Dietary patterns have been thought to be primarily responsible for the low incidence of urinary calculi these regions.

During the process of water conservation, kidneys supersaturate urine (Carvalho and Nakagawa 1999; Khan and Canales 2009). Supersaturation (SS) in relation to calcium oxalate and phosphate salts is the driving force for crystallization in solutions like urine, which means that it will contain crystals that are formed spontaneously. If inhibitors of crystal formation were not able to act and control their size, the final result will be nephrolithiasis (Carvalho et al. 2002; Kurutz 2003).

Human renal stones are composed of crystalline and non-crystalline phases; 80% of stones are composed of calcium oxalate (CaOx) and the supporting structure (the organic matrix) accounts for 2–5% of the total stone weight (Boyce 1968), and is distributed throughout the architecture of all stones (Boyce and Garvey 1956). Proteins constitute a major portion of the matrix and the organic matrix is considered to be important in stone
formation and growth (Warporheski et al. 1981). Macromolecules are suggested to modulate the course of crystallization by inducing crystal nucleation, growth and aggregation of crystals as well as their attachment to renal epithelial cells. CaOx crystal growth inhibitors (proteins, lipids, glycosaminoglycans, and inorganic compounds) have been proposed to play an important role in renal stone disease (Zerwekh et al. 1983).

Non-obstructing stones produce no symptoms or signs apart from hematuria. Stones less than 5 mm in diameter have a high chance of passage, those of 5–7 mm have a modest chance (50%) of passage, and those greater than 7 mm almost always require urological intervention (Andrew 2009). Ideally, stone analysis is performed by infrared spectroscopy or x-ray diffraction. Renal stone burden is best gauged using CT radiographs taken with 5-mm cuts, without infusion of contrast agents. The radiographic appearance and density of stones as measured by CT is a guide to their composition (Zarse et al. 2004). Extracorporeal shock wave lithotripsy (ESWL), in which sound waves are used to break the stone into small pieces that can more easily pass into the bladder, is widely used and valuable for small stones (Lingeman et al. 2003). Modern instruments facilitate passage of endoscopes up the ureter into the kidney pelvis and permit local stone disruption with high-powered lasers (Bagley 2002). Percutaneous stone removal via instruments introduced into the kidney through a small flank incision permits disruption and removal of even very large stones (Clayman 2005).

During the last few years more and more research has been done at the cellular and molecular levels. In spite of these advances however, the clinical treatment of urolithiasis remain far from satisfactory. Stone recurrence in human beings can be predicted and is beyond the control of urologists, mainly because the mechanism of stone formation at
molecular level is not yet fully understood. Thus, determination of the molecular mechanisms by which urinary constituents modulate calcium oxalate crystallization is crucial for understanding and controlling urolithiasis in humans. Although a few initial molecular-scale investigations of mechanisms involved in kidney stone formation by these inhibitory molecules have been recently performed (Nakagawa et al. 1985; Nakagawa et al. 1987) the majority of previous studies have been concerned with the overall kinetics of crystallization rather than molecular mechanisms which remain poorly defined. Thus, the aim of present study is to fractionate renal stone matrix proteins, to purify and characterize most potent antilithiatic protein from organic matrix of calcium oxalate renal stones and study their interaction with calcium oxalate \textit{in silico} with following objectives:

\textit{OBJECTIVES}

1) To prepare the renal calculi extract from surgically removed stones and study its effect on \textit{in vitro} mineralization by the assay system involving calcium phosphate precipitation and calcium oxalate crystal growth.

2) To isolate, purify and characterize proteins from the renal calculi extract which are capable of influencing \textit{in vitro} mineralization.

3) Assessment of molecular interactions between the identified protein and calcium oxalate \textit{in silico}. 