CHAPTER 1
INTRODUCTION

Urinary stone disease is an ailment that has afflicted human kind for many centuries. Nephrolithiasis is a significant clinical problem in everyday practice with a subsequent burden for the health system [Joshi et al., 2013]. Nephrolithiasis remains a chronic disease and our fundamental understanding of the pathogenesis of stones as well as their prevention and cure still remains rudimentary [Worcester et al., 2010; Aggarwal et al., 2013a]. Regardless of the fact that supersaturation of stone forming salts in urine is essential, but abundance of these salts by itself will not always result in stone formation [Evan, 2010]. The pathogenesis of calcium oxalate stone formation is a multistep process and essentially includes nucleation, crystal growth, crystal aggregation and crystal retention [Kumar et al., 2012; Tsujihata, 2008]. Various substances in the body have an effect on one or more of the above stone forming processes thereby influencing a person’s ability to promote or prevent stone formation. Histological and archaeological 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 [Lopez and Hoppe, 2010]. Many researchers are attempting to elucidate the mechanism of CaOx renal stone formation. Archaeological findings give profound evidence that humans have suffered from kidney and bladder stones for centuries [Eknoyan, 2004]. Mineralization can occur both under physiological and pathological conditions. 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
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with formation of such stones. Although the urinary calculi can be lodged in any part of urinary tract, 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. The risk of developing urolithiasis in adults appears to be higher in the western hemisphere than in the eastern hemisphere, although the highest risks have been reported in some Asian countries with lifetime recurrence rates of up to 50%. 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]. Under physiological conditions urinary supersaturation with CaOx is never high enough to result in homogenous nucleation; a promoter is likely to contribute to the precipitation of this salt [Tiselius, 1997]. Pure promoters of urolithiasis are rare, but some substances can act as promoters at particular stages of crystal formation and as inhibitors at other stages, e.g., 1amm-Horstall glycoprotein (1HP), depending on its stage of aggregation, may act as a promoter or an inhibitor of crystal formation [Hess, 1992]. Long-standing interest in the possible role of macromolecules in nephrolithiasis stems from the observation that all human kidney stones consist of a complex amalgam of mineral and organic material [Ryall, 2004]. The study of stone matrix has come a long way in recent years, but the wealth of knowledge we have gained has been offset to a large extent by conflicting findings, some of which have simply served to deepen the mystery of the role of matrix in stone formation. About 80% of stones are composed of calcium oxalate (CaOx) and 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 [Boyce, 1968]. Macromolecules are suggested to direct the course of crystallization by inducing crystal nucleation on the surface and acting as an adhesive or bridge for the binding of crystals together to form large aggregates and in providing a platform for the deposition of more solute, thereby leading to crystal growth [Stapleton et al., 1993; Aggarwal et al., 2013b]. 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]. Extracorporeal shock wave lithotripsy (ESWL) is widely used and valuable for small stones [Lingeman et al., 2003]. Modern instruments facilitate passage of endoscopes up the
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ureter into the kidney pelvis and permit local stone disruption with high-powered lasers [Bagley, 2002]. 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. Therefore, determination of the molecular mechanisms by which urinary constituents modulate calcium oxalate crystallization is crucial for understanding and controlling urolithiasis in humans. Thus, the aim of present study is to fractionate renal stone matrix proteins, to purify and characterize most potent proteins from organic matrix of calcium oxalate renal stones and study their interaction with calcium oxalate in silico with following objectives:

OBJECTIVES

1. To prepare the renal calculi extract from surgically removed stones (calcium oxalate) and study its effect on in vitro mineralization by the assay system involving the precipitation of calcium and oxalate as mineral phase.

2. To study the effect of renal calculi extract from calcium oxalate stones on oxalate injured MDCK renal epithelial cells.

3. To purify, characterize and identify protein(s) from the renal calculi extract which are capable of influencing in vitro mineralization and to study their effect on oxalate injured MDCK renal epithelial cells.

4. In silico study on interaction of calcium oxalate crystals onto the active binding sites of purified proteins.