Chapter 6

SUMMARY & CONCLUSIONS
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Urolithiasis, classified as one of the most painful disorders afflicting mankind since ancient times, affects 2-20% population worldwide and is the third most common affliction of the urinary tract. “Stone belt regions” of the world encompass countries of Middle East, North Africa, Mediterranean Regions, North Western states of India and south states of USA. Prevalence of urinary stones differs in different parts of the world depending on various dietary and non-dietary factors with a prevalence rate of 15% in India. One of the most important phenomena that characterize urolithiasis is its high reoccurrence rate.

The exact etiological cascade of events which leads to urolithiasis is unknown. Hypotheses ranging from oxalate induced renal injury to insufficient urinary inhibitors of calculogenesis, to nidus formation with epitaxy have been proposed. According to clinical and epidemiological studies calcium oxalate followed by calcium phosphate are the most frequently encountered crystalline components found in analysed stones. Most stones do not contain one single crystal phase but rather a mixture of several different crystal phases. The concentration of ions in the tubular fluid, the level and activity of crystallization modulators and retention of crystals in the kidney are considered major determinants in the development of a stone.

The use of herbal medicines is gaining popularity due to toxicity and side effects of allopathic medicines. Medicines manufactured from plant parts are in great demand for primary health care because of their higher safety margin and lesser costs throughout the globe. Different remedies have been employed during ages to treat urinary stones. In the traditional system of medicines, most of the remedies were taken from plants and they proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs.

The present study aimed at investigating the antiurolithiatic potency of B. ligulata rhizome. Further, the efficacy of ethanolic extract of *B. ligulata* (BLE) was tested and
used for the isolation and characterization of new biologically active compound. Both the \textit{in vitro} and \textit{in vivo} methods were employed to validate the activity. The schematic outline of the work is summarized in figure.

![Figure 6.1 Graphical outline of thesis work](image)

The key summary points of the present thesis are listed below:

1. \textit{B.ligulata} ethanolic extract (BLE) exhibited a concentration dependent inhibition of CaOx and CaP crystal growth. The phytochemical analysis of the extract was carried out by using standard laboratory tests. The analysis revealed the presence of tannins, saponins and flavonoids as the main constituents of the extract.
2. Being most potent, BLE was subjected to activity guided fractionation using silica gel column chromatography. After two rounds of purification, a white crystalline material (SFR1) was isolated which showed maximum inhibitory activity against CaOx crystal growth and moderate activity against CaP crystal growth.

3. SFR1 was characterized by Liquid Chromatography- Mass Spectrometry (LC-MS), Nuclear Magnetic Resonance (NMR), Fourier Transfer- Infra Red (FT-IR) and UV-visible spectra. The analysis and interpretation of results confirmed that SFR1 is purified bergenin.

4. At equal concentration, bergenin (BRG) was found to be more effective than BLE and Cystone for inhibiting CaOx crystal growth.

5. In order to understand the mechanism of antilithiatic activity, the antioxidant activity of BRG was established using in vitro methods. The antioxidant potential of BRG was found to be comparable to that of a standard antioxidant (α-tocopherol) in vitro.

6. A rat model of urolithiasis was established by giving 1% NH₄Cl and 0.4% ethylene Glycol in drinking water. Antilithiatic activity of BRG was validated in rat urolithiatic model to evaluate its in vivo efficacy and was found to be comparable with BLE. The levels of oxalate and calcium in urine were found to be normalized in BRG and BLE treated rats with almost equal efficiency.

7. Creatinine levels in serum were managed more effectively if the animals were treated with BRG as compared to BLE, although the urea levels were equally good in both the treatment groups. Creatinine clearance, an important clinical indicator of renal functionality, was normalized significantly in BRG treated urolithiatic animal models, however the rate of creatinine clearance has not shown much elevation in BLE treated animal models.

8. The hyperoxaluric (HYO) rats showed a marked elevation of renal injury marker enzymes viz. alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The enhanced excretion of these renal injury marker enzymes in urolithiatic rats suggests damage to the brush border membrane of the renal tubules which appears
to be associated with the retention and deposition of crystals in the kidney. The excretion of ALP and LDH was found to be significantly reduced in BRG treated HYO rats but the BLE treatment as compared to BRG treatment did not lower the excretion levels of these enzymes.

9. Crystalluria is also an important indicator of hyperoxaluria. In HYO rats the urine samples were found to have abundant crystals which include dumbbell shaped calcium oxalate crystals. Both BRG and BLE treatment showed reduced crystalluria and very few crystals in the urine sample of HYO rats.

10. The results were validated and confirmed by histopathology of kidney. In case of HYO rats the morphology of kidney was found to be deformed. However histological analysis of BRG administered hyperoxaluric rats showed significantly less tissue injury.

11. The mechanistic insight suggests that the antilithiatic potential of BRG might also be due to its ability of being an antioxidant. \textit{In vivo} antioxidant potential of BRG was assessed in hyperoxaluric rats. The lipid oxidation marker (malondialdehyde) was found to be significantly reduced by BRG treatment. A non-enzymatic cellular antioxidant, reduced glutathione (GSH) was measured by using standard methods and the results confirmed the restoration of GSH levels in BRG treated rats.

12. Beside this potential antioxidant, some enzymatic antioxidants like Catalase, Superoxide dismutase and Glutathione peroxidase have important role in maintaining the cellular redox balance. The activity of these antioxidant enzymes in kidney were found to be diminished in HYO rats. Treatment groups, however, were able to significantly enhance the antioxidant activity status of these enzymes.

13. Oxalate toxicity is mediated through generation of reactive oxygen species (ROS) in a process that partly depends upon events that induce mitochondrial damage. The mitochondria serve to utilize redox energy to generate ATP and reducing oxygen to water. The respiratory enzyme complexes activity measurement is a standard approach to assess the mitochondrial well being state. The mitochondria dysfunction was studied by studying the activities of respiratory enzyme
complexes in mitochondrial fraction of renal tissues. In the HYO rats the activities were found to be significantly reduced as compared to healthy animals. Administration of BRG significantly (p<0.05) restored the enzymatic activities of all the four complexes. However, BLE treatment could restore the normal activity of only complex IV.

14. Mitochondrial oxidative stress was also investigated in the isolated mitochondria. HYO rats showed profound increase in MDA levels. BRG administration was not only able to bring down the MDA levels (p<0.05) to near normal value but was also able to significantly (p<0.05) increase the glutathione levels.

15. The investigation of liver suggests the state of oxidative stress in HYO rats. The increase in the levels of MDA was observed in liver tissue in case of HYO rats, however, MDA levels were found to be normalized in BRG treated rats. Both the treatment groups showed significant elevation in levels of antioxidant enzymes also.

In nutshell the present study is an important milestone not only in the understanding of pathophysiology of urolithiasis or emphasising the importance of herbal medicines but in systematic studying the potential of *B. ligulata* rhizome in curtailing the urolithiasis. Also, the mechanistic insight suggesting the role of oxidative stress and mitochondrial dysfunction in pathophysiology of stone diseases was highlighted. Present findings present a direct evidence that hyperoxaluria elicits oxidative damage of renal tissue providing favourable sites for calcium oxalate crystal retention. The isolation of potent biomolecules may direct the pharma sector in uptaking further research collaborations to understand the wider applications in human trials also.