CHAPTER V
SUMMARY AND CONCLUSIONS

The present study was undertaken to examine the antiurolithiatic property of two plants, namely; rhizomes of *Bergenia ciliata* and seeds of *Dolichos biflorus*. Both the selected plants were subjected to scientific evaluation in animal model of urolithiasis and also compared with marketed polyherbal drug, cystone. The major findings from this study are as under:

1. The initial part of the study was devoted in establishing the appropriate dose of ethylene glycol, a commonly used agent to induce urolithiasis in rat model. The ethylene glycol was administered at doses of 0.4% (LD), 0.75% (MD) and 1.0% (HD) in drinking water for 28 days in female Wistar rats to induce urolithiasis. The blood and urine samples were collected on 14 and 28 day of treatment and analyzed for various biochemical parameters. At the end of 28 day of treatment, animals were sacrificed under ether anesthesia; both kidneys were isolated, blotted free of blood and various biochemical and histopathological analysis were done. The ethylene glycol resulted in significant decrease in body weight with concurrent increase in kidney weight as well as urinary excretion of calcium, oxalate, phosphate and total protein. Moreover, there was a significant reduction in serum calcium and magnesium while a significant increase was observed in levels of phosphate and total protein in serum. Similarly, there was significant increase in the deposition of calcium, oxalate, phosphate and total protein in kidney. The effect was dose – and time – dependent.
2. The selected plants were then investigated for their antiurolithiatic property by evaluating various biochemical parameters. The hydro-alcoholic extracts of (30:70, v/v) of rhizomes of *B. ciliata* and seeds of *D. biflorus* was orally administrated simultaneously (150 and 300 mg/kg body weight/day) in nephrolithiasis rats for 28 days along with ethylene glycol (0.75%, v/v) in drinking water. The results were compared with a parallel study conducted with marketed polyherbal drug cystone under identical dosage conditions. The results revealed that the oral administration of plant extracts at a dose regimen of 300 mg/kg body weight along with ethylene glycol evoked the maximum renoprotective response, as indicated by restoration of the body weight, kidney weight and urinary excretions of calcium, oxalate, phosphate, magnesium, total protein, creatinine, uric acid and urea. Also the ethylene glycol treatment results in increased excretion in total and unconjugated bilirubin which was significantly protected by the plant extracts. The effect was time – dependent.

3. The administration of plant extracts along with the lithogen significantly restores the biochemical constituents like calcium, phosphate, magnesium, electrolytes (sodium and potassium), creatinine, uric acid, blood urea nitrogen and total protein levels in serum. The co-treatment of plant extracts with ethylene glycol also reduces the increased activities of ALT and AST. Moreover, the treatment of plant extracts significantly reduces the activity of ADH in liver of ethylene glycol – treated rats.

4. The similar trend was followed by the plant extracts in the levels of these constituents (calcium, oxalate, phosphate and total protein) in the kidney of ethylene glycol – treated rats. The protection was more significant with *B. ciliata* followed by *D. biflorus* and cystone.
5. The ethylene glycol elicited oxidative stress by elevating the lipid peroxidation in serum as well as in kidney of rats. Moreover, ethylene glycol resulted in depletion of antioxidative enzymatic activities (CAT, SOD, GPx and GR) as well as non-enzymatic antioxidants. These changes were effectively protected by the co-administration of plant extracts.

6. The extent of protection for all *in vivo* parameters were calculated and expressed in terms of renoprotective index. *B. ciliata* (BCE) was found to be the most renoprotective plant followed by the *D. biflorus* (DBE) and cystone (CST).

7. The plant extracts were further evaluated for the inhibition of calcium oxalate crystallization *in vitro*. Both the plant extracts have been shown to suppress the nucleation as well as aggregation of calcium oxalate crystals in artificial urine. The percent inhibitions were calculated and maximum inhibition was provided by the extract of *B. ciliata* followed by the *D. biflorus* and cystone. The effect was concentration-dependent.

8. The selected plants were then screened for the presence of various phytochemical constituents. Phytochemical analysis revealed the presence of tannins, saponins, flavonoids and alkaloids in both the plant extracts. Furthermore, both the plants showed significant free radical scavenging activity *in vitro* in a concentration-dependent manner.

9. The plant extracts were also investigated for their antioxidant property in sodium oxalate induced oxidative stress *in vitro* in kidney homogenate of Wistar rats. The sodium oxalate induced a rapid elevation of lipid peroxidation and decrease in the activities of superoxide dismutase and catalase. These changes were also efficiently protected by the plant extracts. The effect was dose-dependent.
10. The standardization of plant extracts is necessary as the observed activity of the plant extracts might be due to the presence of active components in them. These active components act either alone or in synergism with the other components. The HPLC analysis showed the presence of gallic acid and quercetin as major active components in rhizomes of *B. ciliata* and seeds of *D. biflorus*, respectively.

Both the plants were found to be highly effective in resisting the kidney stone formation induced by the ethylene glycol. Also the plant extracts are safe with very high LD$_{50}$ values. Thus, the present study validated the traditional uses of both the plants tested for treatment of urolithiasis.

**FUTURE PROSPECTS AND PLAN OF WORK**

The present study was an attempt towards the exploration of natural resources. It had scientifically validated the therapeutic claims of some known traditional plants and had also phytochemically standardized them. Results revealed a number of interesting facts and findings. However, the study can be extended further in one of the following ways, for the benefit of the human community as kidney protecting alternatives, as phytotherapy has the better potential than the routine surgical methods and synthetic drugs.

- Efficacy studies varying the proportions of these two plant extracts can be carried out.

- Clinical trials, which will prove the effectiveness of these plants in humans, may be initiated.
The antiurolithiatic compounds can be isolated from both the plant extracts, which can be then tested *in vivo* and *in vitro* for their kidney stone protecting property in experimental models. Moreover toxicity testing of the isolated compounds in animal models can be carried out.

The selected plants were shown to possess potent anticrystallization activity. Therefore their isolated compounds can also be tested for inhibition of crystallization in artificial urine.

The effects of these plant extracts and isolated compounds can also be investigated on oxalate-induced cell injury as well as oxidative stress of renal epithelial cells.