CHAPTER III

RESULTS

The main aim of the present study was to investigate the antiurolithiatic activity of two selected plants namely rhizomes of *Bergenia ciliata* and seeds of *Dolichos biflorus* against ethylene glycol-induced urolithiasis in female Wistar rats. Furthermore, *in vitro* anticrystallization property of extracts for calcium oxalate and their antioxidant activity was determined. Phytochemical studies of both the plants were also conducted and HPLC analysis was used to identify and quantify major active components present in the plant extracts. The experiments were carried out in a phased manner and the results obtained in different phases of experiment are presented below:

PART – I

ETHYLENE GLYCOL – INDUCED UROLITHIASIS

Clinical observations

No treatment related clinical signs were observed in control and ethylene glycol - treated animals. However, there was 40% mortality in group 4 rats, administered with 1.0% of ethylene glycol in drinking water.

Body weight

Table 3.1 shows the changes in body weight of untreated control and ethylene glycol -treated rats. No significant change in the body weight was noted in control group (Groups 1). However, ethylene glycol treatment (Groups 2, 3 and 4), caused significant dose – dependent reduction in body weight (LD: 2%; MD: 8%; HD: 18%;
$r^2 = 0.918$; Fig. 3.1) of rats on 14 day as well as on 28 day (LD: 6%; MD: 14%; HD: 25%; $r^2 = 0.983$) as compared to untreated control (Fig. 3.1; Group 1).

**Kidney weight**

Table 3.2 shows the effect of ethylene glycol treatment on absolute and relative weights of kidney in rats. The oral administration of ethylene glycol for 28 days caused significant ($p<0.001$), increase in absolute (LD: 15%; MD: 32%; HD: 51%, Fig. 3.2) as well as relative (LD: 24%; MD: 55%; HD: 88%, Fig. 3.2) kidney weights (Groups 2, 3 and 4) as compared to untreated control (Group 1). The effect was dose-dependent ($r^2 = 0.995$).

**Histopathological changes**

The histopathological examination of kidney sections of untreated control (Plate A; Figs. 1-2) showed no calcifications. The glomeruli, proximal and distal convoluted tubules as well as blood vessels were normal without any inflammatory changes. The administration of low dose of ethylene glycol for 28 days caused mild calcifications with loss of structural arrangement of renal tubules (Plate B; Figs. 1-2). The mid dose administration of ethylene glycol showed mild to moderate calcification in tubular regions with slight inflammation and early degenerative changes in glomeruli (Plate C; Figs. 1-2). However, ethylene glycol administration at higher dose level for 28 days caused severely disrupted renal parenchyma with vacuolar degeneration, focal calcification in glomerulo-tubular structures and interstitial sites with congested blood vessels. The flat epithelial lining and glomerular capsule was also disrupted with severe inflammatory changes (Plate D; Figs. 1-2).
URINARY BIOCHEMICAL ANALYSIS

Effect on calcium and oxalate content

The effect of ethylene glycol treatment on calcium and oxalate excretion in urine is presented in Table 3.3. A significant (p<0.001) dose - and time-dependent increase in urinary calcium and oxalate excretion levels were noted in all ethylene glycol - treated animals (Groups 2, 3 and 4) on 14 day (LD: 34%; MD: 96%; HD: 142%, \(r^2 = 0.989\), Fig. 3.3) as well as on 28 day (LD: 55%; MD: 127%; HD: 200%, \(r^2 = 0.995\), Fig. 3.3) as compared to untreated control (Group 1).

Similarly, there was a significant (p<0.001) increase, as compared to untreated control (Group 1), in urinary oxalate excretion level on 14 day (LD: 32%; MD: 77%; HD: 110%, \(r^2 = 0.990\), Fig. 3.4) as well as on 28 day (LD: 127%; MD: 241%; HD: 334%, \(r^2 = 0.995\), Fig. 3.4) in ethylene glycol-treated rats in a time-dependent manner (Table 3.3).

Effect on phosphate and magnesium content

The effect of ethylene glycol treatment on excretion of phosphate and magnesium in urine of rats is shown in Table 3.4. The ethylene glycol administration caused, as compared to untreated control (Group 2), a significant dose - and time-dependent (p< 0.001) increase in urinary phosphate excretion on 14 day (LD: 161%; MD: 320%; HD: 618%, \(r^2 = 0.973\), Fig. 3.5) as well as on 28 day (LD: 473%; MD: 653%; HD: 912%, \(r^2 = 0.958\), Fig. 3.5) of treatment.

Ethylene glycol administration significantly (p<0.001) decreased urinary magnesium excretion on 14 day (LD: 18%; MD: 42%; HD: 65%, \(r^2 = 0.995\), Fig. 3.6) and 28 day (LD: 37%; MD: 77%; HD: 92%, \(r^2 = 0.968\); Fig. 3.6) of treatment, as compared to untreated control (Group 2) in a dose - and time - dependent manner (Table 3.4).
Effect on total protein

The total protein content in urine of ethylene glycol–treated animals was found to be significantly (p < 0.001) increased when compared to animals of control groups (Table 3.5), in a dose- and time–dependent manner. Fig. 3.7 shows that the increase in protein content by ethylene glycol treatment was 12%, 91% and 260% on 14 day ($r^2 = 0.866$) and by 40%, 180% and 360% on 28 day ($r^2 = 0.936$).

Correlation analysis

Pearson correlation analysis between different parameters in urine were calculated and presented in Table 3.6. A strong positive correlation was observed among urinary excretion of calcium, oxalate, phosphate and total protein ($r > 0.9$). However, magnesium excretion level was found to negatively correlate with all the other parameters ($r > 0.980$).

SERUM BIOCHEMICAL ANALYSISIS

Effect on calcium and phosphate contents

Table 3.7 shows the effect of ethylene glycol treatment on calcium and phosphate contents in the serum of rats. Results revealed that administration of ethylene glycol caused significant (p<0.001) dose- and time- dependent increase in calcium content; LD, MD and HD of the ethylene glycol decreased the calcium content by 20%, 42% and 58% on 14 day ($r^2 = 0.997$) and by 24%, 67% and 85% on 28 day, respectively ($r^2 = 0.952$, Fig. 3.8).

Ethylene glycol administration also caused a significant (p < 0.001) elevation in phosphate content in serum of rats as shown in Table 3.7. All three doses of ethylene glycol had dose - as well as time – dependent (LD: 30%; MD: 75%; HD: 115% on 14 day ($r^2 = 0.994$) and LD: 40%; MD: 134%; HD: 179% on 28 day; $r^2 = 0.973$) increase on phosphate content as compared to untreated control (Fig. 3.9).
**Effect on magnesium content**

Table 3.8 shows the effect of ethylene glycol on magnesium content in serum of rats. As compared with the untreated control rats, ethylene glycol administration caused a significant reduction (p<0.001) in magnesium content on 14 day (LD: 25%; MD: 47%; HD: 77%; $r^2 = 0.995$) as well as on 28 day (LD: 45%; MD: 73%; HD: 84%; $r^2 = 0.929$). The effect was dose- and time-dependent (Fig. 3.10).

**Total protein content**

The effect of ethylene glycol on total protein content in serum is presented in Table 3.9. A significant (p<0.001) increase in total protein (LD: 107%; MD: 229%; HD: 382%; Fig. 3.11) was noted in all ethylene glycol treated animals in a dose-dependent manner ($r^2 = 0.993$).

**Correlation analysis**

The Pearson correlation analysis was done between different parameters in serum and was presented in Table 3.10. A strong negative correlation was observed among serum calcium, phosphate and magnesium ($r > 0.9$). However, serum phosphate level was found to be positively correlated with total protein in serum ($r > 0.980$).

**KIDNEY BIOCHEMICAL ANALYSIS**

**Effect on calcium, oxalate, phosphate and total protein contents**

The effect of ethylene glycol on calcium, oxalate, phosphate and total protein contents in kidney of rats are shown in Table 3.11. Ethylene glycol administration caused significant (p<0.001) increase in calcium content as compared with untreated control (LD: 114%; MD: 266%; HD: 431%). The effect was dose-dependent (Groups 2, 3 and 4; $r^2 = 0.993$; Fig. 3.12).

Ethylene glycol was found to reduce oxalate content significantly (p<0.05) and dose-dependently ($r^2 = 0.990$). As shown in Table 3.11, LD, MD and HD of
ethylene glycol (Groups 2, 3 and 4) reduced oxalate content by 76%, 191% and 273%, respectively (Fig. 3.13).

It was observed that, as compared to untreated control (Group 1; Table 3.13), treatment with ethylene glycol caused a significant increase in phosphate content (LD: 56%; MD: 122%; HD: 191%). The effect dose-dependent manner (Groups 2, 3 and 4; $r^2 = 0.999$; Fig. 3.14).

On the contrary, the administration of ethylene glycol for 28 days caused a significantly ($p<0.05$) and dose-dependently ($r^2 = 0.995$) increase in total protein content. Percent increase in total protein content by LD, MD and HD was 23%, 41% and 71% respectively (Groups 2, 3 and 4; Table 3.11, Fig. 3.15).

**Correlation analysis**

The results of Pearson correlation analysis among different parameters in kidney were depicted in Table 3.12. A strong positive correlation was observed among calcium, oxalate, phosphate and total protein ($r > 0.9800$).

Furthermore, the Pearson correlation analysis of relationship between urinary excretion, serum level and deposition in kidney of different parameters were calculated and presented in Table 3.13. It has been observed that there is strong positive correlations exists between urinary, serum and homogenate contents of each parameter suggesting the dependence of urinary excretion of ions on serum and homogenate concentrations and vice versa.
PART – II

ANTIUROLITHIATIC ACTIVITY OF SELECTED PLANTS AGAINST ETHYLENE GLYCOL - INDUCED UROLITHIASIS

Body weight

Table 3.14 represents the protective effect of cystone/plant extracts on ethylene glycol-induced changes in the body weight of rats. No significant difference in body weight was observed between different control groups (Groups 1 – 4). As compared with untreated control (Group 1), the administration of ethylene glycol (Group 5), caused a significant reduction in body weight by 8% on 14 day and by 14% on 28 day (p<0.001). However, there was a significant protection in body weight by oral administration of standard cystone (CST150: 2%; CST300: 5%) and hydro-alcoholic extracts of BCE (BCE150: 5%; BCE300: 7%) and DBE (DBE150: 3%; DBE300: 6%; Fig. 3.19) on 14 day as well as on 28 day (CST150: 5%; CST300: 9%; BCE150: 9%; BCE300: 14%; DBE150: 8%; DBE300: 13%). Thus, the percentage of protection was highest with B. ciliata followed by D. biflorus and cystone (Fig. 3.16).

Kidney weight

Table 3.15 shows the protective effect of plant extracts/cystone on absolute and relative weights of kidney as compared to ethylene glycol-induced changes. No significant difference in absolute and relative weights of kidney was observed between different control groups (Groups 1 – 4). As compared with untreated control (Group 1), administration of MD of ethylene glycol caused a significant increase the absolute (32%) and relative (50%) kidney weights (p<0.001; Group 5; Fig. 3.17). However, supplementation with cystone/plant extracts along with ethylene glycol significantly restored the changes in absolute kidney weight with percent protection of 9% (CST150) and 24% (CST300) in case of cystone, 11% (BCE150) and 25%
(BCE300) in *B. ciliata* and 13% (DBE150) and 24% (DBE300) in *D. biflorus* treated rats. In the same manner, there was a significant protection in relative kidney weights by oral administration of cystone (CST150: 18%; CST300: 40%) and hydro-alcoholic extracts of BCE (BCE150: 25%; BCE300: 45%) and DBE (DBE150: 25%; DBE300: 42%; Fig. 3.17). Similarly, renoprotective index of cystone/plant extracts were also calculated and presented in Table 3.15. The mitigation was more in the case of *B. ciliata* followed by *D. biflorus* and cystone, as indicated by the percent protection.

**Histopathological changes**

Histopathological studies showed the protective effect of oral administration of cystone and plant extracts on ethylene glycol-induced alterations in kidney architecture. The architectural appearance of the kidneys from the rats in the control group, presented a normal histological appearance with no calcifications with normal glomeruli and Bowman’s capsule, proximal and distal convoluted tubules without any inflammatory changes and normal blood vessels (Plate E; Figs. 1-2). No apparent histopathological changes were observed in the kidney of cystone/plant extracts alone treated rats (Plate F; Figs. 1-2, Plate G; Figs. 1-2, Plate H; Figs. 1-2). Ethylene glycol treatment in rats caused a significant loss in structural arrangement of renal tubules, early degenerative changes in glomeruli and focal calcification in glomerulo-tubular structures (Plate I; Figs. 1-2). Moreover, there was dilation of the renal tubules along with interstitial inflammations and congestions in blood vessels. However, the renal tissue of ethylene glycol along with plant extracts (BCE/DBE) treated rats at a dose of 150 mg/kg b.w./day shows mild to moderate calcification in glomeruli but no calcification in tubular structures (Plate L, Figs. 1-2; Plate N; Figs. 1-2) whereas BCE/DBE at higher dose of 300 mg/kg body weight showed almost complete protection with no areas of calcification in glomeruli and normal tubular structures with no
congestion in blood vessels (Plate M; Figs. 1-2; Plate O; Figs. 1-2). Comparatively, the renal tissue of cystone treated rats still shows moderate calcification in many tubules and few glomeruli (Plate J, Figs. 1-2; Plate K; Figs. 1-2).

HISTOCHEMICAL STUDIES

von Kossa staining

Crystal distribution within the kidneys was determined by von Kossa – stained sections. The von Kossa method of staining specifically detects calcium deposition in kidney sections. There were no calcifications found in untreated control rat kidneys (Plate P; Fig. 1). However, a significantly increased number of calcium deposits were found attached to the renal tubules in ethylene glycol – treated rat kidneys (Plate P; Fig. 2). The co – treatment of hydro – alcoholic extracts of BCE/DBE along with ethylene glycol shows only mild calcifications in glomeruli and normal tubular structures (Plate P; Fig. 4; Plate P; Fig. 5) whereas cystone treatment shows moderate calcifications in glomerulo - tubular structures which was comparatively more than plant extracts (Plate P; Fig. 3).

Pizzolato staining

Pizzolato staining for calcium oxalate crystals in kidney sections from untreated control group showed normal appearance of renal tissue with no calcium oxalate depositions (Plate Q; Fig. 1). However, the Pizzolato staining clearly showed black stained calcium oxalate deposits in the glomeruli and tubular region of kidneys of the ethylene glycol - induced urolithiatic rats (Plate Q; Fig. 2). Co – treatment of plant extracts (BCE/ DBE) at 300 mg/kg body weight along with ethylene glycol showed only few calcium oxalate crystals in glomeruli while tubular structures were normal (Plate Q; Fig. 4, Plate Q; Fig. 5). However, comparatively more number of
calcium oxalate crystal deposits were detected in the cystone (300 mg/kg body weight) treated rats (Plate Q; Fig. 3).

**Calcium oxalate crystal deposition in urine**

Microscopic observation revealed that urine of untreated control rats was devoid of any crystal of calcium oxalate (Plate R; Fig. 1). However, numerous aggregated calcium oxalate crystals were observed in the urine of ethylene glycol administrated rats (Plate R; Fig. 2). The oral administration of BCE/DBE at both the dose levels resulted in only few calcium oxalate crystals in their urine (Plate R; Figs. 5-8). The size of the crystals was also visibly reduced by the plant extract co-treatment. There were many calcium oxalate crystals observed in the urine of standard polyherbal drug cystone treated rats at both the dose levels (Plate R; Figs. 3-4).

**URINARY BIOCHEMICAL ANALYSIS**

**Effect on calcium and oxalate content**

No significant difference in calcium and oxalate excretion levels was observed between different control groups (Groups 1 – 4; Table 3.16). Oral administration of ethylene glycol caused a significant increase (p<0.001) in calcium (87% on 14 and 125% on 28 day; Fig. 3.18) excretion levels (Group 5). However, renoprotective index indicates that there was a significant amelioration in calcium excretion level on supplementation with cystone (CST150: 29; CST300: 67) and hydro-alcoholic extracts of BCE (BCE150: 43; BCE300: 81) and DBE (DBE150: 50; DBE300: 85) on 14 day as well as on 28 day (CST150: 35; CST300: 72; BCE150: 52; BCE300: 90; DBE150: 57; DBE300: 94; Table 3.16).

Ethylene glycol also caused a significant increase (p<0.001) in oxalate excretion levels (77% on 14 and 244% on 28 day; Fig. 3.19), as compared with the untreated control rats. Similarly, there was significant reduction in oxalate excretion
level by cystone (CST150: 15%; CST300: 47% on 14 day and CST150: 72%; CST300: 175% on 28 day), hydro-alcoholic extract of *B. ciliata* (BCE150: 32%; BCE300: 62% on 14 day and BCE150: 143%; BCE300: 225% on 28 day) and *D. biflorus* (DBE150: 42%; DBE300: 65% on 14 day and DBE150: 161%; DBE300: 237% on 28 day) in a time-dependent manner (Fig. 3.19). The renoprotective index was highest for DBE (DBE150: 54; DBE300: 84 on 14 day and DBE150: 66; DBE300: 97 on 28 day) followed by BCE (BCE150: 41; BCE300: 80 on 14 day and BCE150: 58; BCE300: 92 on 28 day) and cystone (CST150: 20; CST300: 61 on 14 day and CST150: 30; CST300: 71 on 28 day) at the same dose levels (Table 3.16).

**Effect on phosphate and magnesium content**

The results of ethylene glycol–induced changes in phosphate and magnesium excretion are shown in Table 3.17. No significant change in phosphate and magnesium excretion was noted among different control groups of rats (Groups 1 to 4). However, as compared to untreated control (Group 1), there was a significant (p<0.001) time-dependent increase in phosphate level (301% on 14 day and 650% on 28 day) due to ethylene glycol treatment (Table 3.17; Fig. 3.20). The ethylene glycol–induced changes are significantly reversed, as indicated by the renoprotective index due to the co-treatment of cystone (CST150: 24; CST300: 64) and plant extracts of BCE (BCE150: 51; BCE300: 95) and DBE (DBE150: 42; DBE300: 89) on 14 day, as well as, on 28 day (CST150: 27; CST300: 60; BCE150: 48; BCE300: 93; DBE150: 38; DBE300: 84; Table 3.17).

On the contrary, ethylene glycol caused significant (p<0.001) time-dependent decrease in magnesium excretion level by 42% on 14 day and 77% on 28 day respectively (Fig. 3.21). Oral administration of cystone/plant extracts along with MD of ethylene glycol caused a significant restoration in magnesium level (CST150: 11%;
CST300: 24%; BCE150: 17%; BCE300: 35%; DBE150: 14%; DBE300: 31% on 14 day and CST150: 25%; CST300: 55%; BCE150: 38%; BCE300: 74%; DBE150: 32%; DBE300: 71% on 28 day) in a time – dependent manner at both the dose levels (Fig. 3.21). The renoprotective capacity was highest for BCE followed by DBE and least for cystone at the same dose levels (Table 3.17).

**Effect on total protein**

The ethylene glycol treatment, as compared with untreated control, caused a significant increase (p<0.001) in total protein by 95% (14 day) and 182% (28 day) in a time - dependent manner (Fig. 3.22). No significant alteration in total protein was observed among the different control groups (Groups 1 – 4; Table 3.18). However the oral administration of two doses of cystone/ BCE/ DBE caused a significant decrease in total protein excretion levels as estimated by the renoprotective indices in a time - dependent manner (CST150: 23; CST300: 41; BCE150: 42; BCE300: 95; DBE150: 38; DBE300: 90 on 14 day and CST150: 27; CST300: 61; BCE150: 42; BCE300: 96; DBE150: 39; DBE300: 93 on 28 day; Table 3.18).

**Effect on renal function parameters**

The co-treatment of cystone and plant extracts significantly protected from ethylene glycol – induced changes in urinary excretion of creatinine, uric acid and urea at both the dose levels (Table 3.19). No significant difference in creatinine, uric acid and urea excretion levels were observed between different control groups (Groups 1 – 4). The administration of ethylene glycol (Group 5) for 28 days caused, as compared to untreated control, significant increase in creatinine (706%), uric acid (174%) and urea (176%). However, concurrent administration of ethylene glycol along with two doses of cystone (CST150: 26; CST300: 49), *B. ciliata* (BCE150: 45; BCE300: 87) and *D. biflorus* (DBE150: 42; DBE300: 83) caused significant protective
effect in creatinine levels (Table 3.19). Similarly, in terms of renoprotective index, standard cystone and hydro-alcoholic extracts of plants caused significant protective effect on urinary excretion levels of uric acid (CST150: 35; CST300: 69; BCE150: 49; BCE300: 92; DBE150: 45; DBE300: 88) and urea (CST150: 32; CST300: 77; BCE150: 60; BCE300: 97; DBE150: 57; DBE300: 94; Table 3.19). Percent change of cystone/plant extracts from untreated control were also calculated for creatinine, uric acid and urea and was shown in Figures 3.23-3.25.

**Effect on bilirubin contents**

Table 3.20 represents the result of protective effects of cystone/plant extracts on urinary excretion of total, direct and indirect bilirubin of rats. No significant alteration was observed in these parameters among the different control groups (Groups 1 – 4). However, ethylene glycol treatment caused significant increase (p<0.001) in total (201%), direct (25%) and indirect (540%) bilirubin (Groups 5; Figs. 3.26-3.28). These changes are significantly reversed by the treatment of two doses of cystone (CST150: 31; CST300: 68), BCE (BCE150: 45; BCE300: 86) and DBE (DBE150: 42; DBE300: 82) in total bilirubin excretion, in terms of renoprotective index (Table 3.20).

In the same manner, there is a significant protection by cystone and plant extracts in direct (CST150: 21; CST300: 42; BCE150: 42; BCE300: 67; DBE150: 33; DBE300: 63; Table 3.20) and indirect bilirubin (CST150: 32; CST300: 70; BCE150: 45; BCE300: 88; DBE150: 43; DBE300: 84; Table 3.20).

**Correlation analysis**

Table 3.21 represents the Pearson correlation analysis among excretion of all parameters in urine. It has been found that there is significant positive correlation among all the biochemical parameters in the urine with correlation coefficient (r) in
the range of 0.9468 – 0.9922. However, magnesium is negatively correlated with all the other parameters.

**SERUM BIOCHEMICAL ANALYSIS**

*Effect on calcium and phosphate content*

Table 3.22 represents the result of ethylene glycol - induced changes in calcium and phosphate levels in serum of rats and their amelioration by cystone and plant extracts in a time - dependent manner. No significant alteration was observed in these parameters among the different control groups (Groups 1 – 4). The administration of ethylene glycol caused a significant decrease in serum calcium level by 41% on 14 day and 68% on 28 day respectively (Fig. 3.29). However, the concurrent administration of cystone (CST150: 7%; CST300: 19%), BCE (BCE150: 13%; BCE300: 31%) and DBE (DBE150: 12%; DBE300: 29%) significantly protected ethylene glycol - induced changes in serum calcium level on 14 day and on 28 day (CST150: 15%; CST300: 35%; BCE150: 24%; BCE300: 55%; DBE150: 21%; DBE300: 51%; Fig. 3.29). The renoprotective index calculated for calcium level showed that *B. ciliata* was more potent followed by *D. biflorus* and cystone is least potent at the same dose levels (Table 3.22).

On the contradictory, ethylene glycol treatment resulted in significant increase in serum phosphate level (74% on 14 day and 135% on 28 day; Fig. 3.30). The co – treatment of cystone, BCE and DBE significantly reversed ethylene glycol - induced changes in serum phosphate level at both the doses on 14 day (CST150: 16; CST300: 45; BCE150: 49; BCE300: 86; DBE150: 41; DBE300: 79) as well as, on 28 day (CST150: 26; CST300: 44; BCE150: 53; BCE300: 91; DBE150: 47; DBE300: 84), as depicted by the renoprotective indices (Table 3.22).
Effect on magnesium content

Ethylene glycol - induced a significant decrease in magnesium content by 46% (14 day) and 72% (28 day) in serum of rats in a time – dependent manner (Table 3.23; Fig. 3.31). However, there is a significant mitigation in serum magnesium level as indicated by the percent change, when co – treated with cystone (CST150: 11%; CST300: 25%), BCE (BCE150: 23%; BCE300: 42%) and DBE (DBE150: 18%; DBE300: 40%) on 14 day as well as on 28 day (CST150: 19%; CST300: 44%; BCE150: 32%; BCE300: 69%; DBE150: 30%; DBE300: 65%; Fig. 3. 31). The maximum renoprotection, in terms of renoprotective index, was observed by the *B. ciliata* followed by *D. biflorus* and cystone (Table 3.23). No significant change was observed in magnesium contents in different control groups (Groups 1 – 4).

Effect on electrolytes level

No significant difference was noted in serum sodium and potassium contents in different control groups (Groups 1 – 4). Results presented in Table 3.24 revealed that there was a significant time - dependent decrease in serum sodium level by 14% (14 day) and 21% (28 day) due to ethylene glycol administration in drinking water while there is a significant increase in potassium level in serum by 46% (14 day) and 86% (28 day) respectively (Figs. 3.32-3.33). Conversely, a significant mitigation occurred in serum sodium level by the treatment of standard polyherbal drug, cystone (CST150: 21; CST300: 50), hydro-alcoholic extracts of *B. ciliata* (BCE150: 43; BCE300: 85) and *D. biflorus* (DBE150: 36; DBE300: 64) on 14 day and 28 day (CST150: 20; CST300: 47; BCE150: 43; BCE300: 81; DBE150: 38; DBE300: 62), as calculated by the renoprotective indices (Table 3.24).

Similarly, there was a significant protection in serum potassium level in rats by cystone (CST150: 30; CST300: 56), BCE (BCE150: 48; BCE300: 90) and DBE
(DBE150: 42; DBE300: 81) on 14 day. The similar trend is followed on 28 day also (CST150: 32; CST300: 67; BCE150: 51; BCE300: 95; DBE150: 44; DBE300: 84; Table 3.24). Thus, the results indicated that the kidney protecting property was greatest for *B. ciliata* followed by *D. biflorus* and least for cystone.

**Effect on renal function parameters**

Table 3.25 depicts the effect of plant extracts on ethylene glycol - induced changes in renal function parameters. No significant difference was noted in creatinine, uric acid and urea in serum in different control groups (Groups 1 – 4). However, a significant elevation was observed in creatinine (102%), uric acid (237%) and urea (75%) in serum of ethylene glycol - treated animals (Group 5; Figs. 3.34-3.36). Renoprotective index calculated revealed that there was a significant amelioration in the creatinine (CST150: 21; CST300: 57; BCE150: 40; BCE300: 91; DBE150: 34; DBE300: 81; Table 3.25), uric acid (CST150: 23; CST300: 53; BCE150: 48; BCE300: 92; DBE150: 42; DBE300: 86; Table 3.25) and urea (CST150: 30; CST300: 57; BCE150: 45; BCE300: 94; DBE150: 43; DBE300: 88; Table 3.25) by cystone and plant extracts (BCE and DBE). The effects were more prominent in case of BCE as compared with DBE and cystone.

**Effect on protein content**

Table 3.26 represents the protective effect of cystone/ plant extracts on ethylene glycol - induced changes in total protein content in serum of rats. The administration of ethylene glycol in drinking water caused a significant (p<0.001; Group 5) increase in the serum level of total protein (148%; Fig. 3.37). However, oral administration of cystone/ plant extracts along with ethylene glycol caused, as compared with ethylene glycol treated, a significant amelioration in the level of serum total protein (CST150: 33%; CST300: 67%; BCE150: 47%; BCE300: 89%; DBE150:
percent decrease is highest when administered with *B. ciliata* followed by *D. biflorus* and cystone (Fig. 3.37).

**Effect on oxidant – antioxidant balance in serum**

Table 3.28 presents the results of mitigatory effects of cystone and plant extracts on ethylene glycol - induced changes in serum LPO of rats. Ethylene glycol - induced a significant increase in serum LPO level by 413% (Fig. 3.38) which was significantly protected by oral administration of cystone (CST150: 22; CST300: 48), BCE (BCE150: 40; BCE300: 93) and DBE (DBE150: 38; DBE300: 90), as shown by the renoprotective index (Table 3.28).

Contradictorily, activity of superoxide dismutase (SOD) and total antioxidant capacity (TAC) of serum decreased significantly by 54% and 71% due to ethylene glycol treatment (Figs. 3.39-3.40). These changes were significantly mitigated by co – administration of cystone (CST150: 14%; CST300: 31%), BCE (BCE150: 24%; BCE300: 52%) and DBE (DBE150: 22%; DBE300: 49%; Fig. 3.39) in case of SOD and similarly in TAC (CST150: 17%; CST300: 37%; BCE150: 36%; BCE300: 69%; DBE150: 35%; DBE300: 67%; Fig. 3.40). The renoprotective index in case of SOD (CST150: 26; CST300: 57; BCE150: 45; BCE300: 97; DBE150: 41; DBE300: 91; Table 3.28) and TAC (CST150: 24; CST300: 52; BCE150: 50; BCE300: 97; DBE150: 49; DBE300: 94; Table 3.28) was found to be highest for BCE followed by DBE and cystone. Results also revealed that there was no significant alteration in these parameters between different control groups (Groups 1 – 4).

**Effect on serum enzymatic activities**

The results obtained showed, as compared with untreated control, a significant increase in activities of ALT (77%) and AST (38%) due to ethylene glycol treatment in
rats (Table 3.30; Figs. 3.41-3.42). No significant alteration was observed in the activities of these enzymes among the different control groups (Groups 1 – 4). As compared with ethylene glycol – treated animals (Group 5), oral administration of cystone/ plant extracts along with ethylene glycol for 28 days caused a significant decrease in activity of ALT (CST150: 29%; CST300: 58%; BCE150: 41%; BCE300: 74%; DBE150: 34%; DBE300: 70%; Fig. 3.41) and concurrent decrease in AST activity (CST150: 16%; CST300: 24%; BCE150: 18%; BCE300: 36%; DBE150: 6%; DBE300: 34%; Fig. 3.42) in rats. The effect was more pronounced with *B. ciliata* followed by *D. biflorus* and cysone.

**Correlation analysis**

Table 3.27 represents the Pearson correlation analysis among all the experimental parameters in the serum. It has been observed that there was significant positive correlation among phosphate, potassium, creatinine, uric acid and total protein with correlation coefficient (r) value in the range of 0.9522 – 0.9959. However, calcium, sodium and magnesium levels in serum were negatively correlated with all the other parameters.

Moreover the Table 3.29 depicts the Pearson correlation analysis between increase in serum LPO and corresponding depletion in antioxidative enzyme (SOD) and total antioxidative capacity of serum of rats. There was a significant negative correlation between serum LPO and other parameters.

**Effect on alcohol dehydrogenase activity in liver**

The activity of alcohol dehydrogenase in liver of rats was found to be severely affected by ethylene glycol and was reversed by the co – treatment of cystone and hydro - alcoholic extracts of plants (Table 3.31). The activity of alcohol dehydrogenase in liver was significantly increased by 55% (Fig. 3.43) on ethylene glycol treatment
whereas it was significantly mitigated by cystone (CST150: 20%; CST300: 41%), BCE (BCE150: 15%; BCE300: 52%) and DBE (DBE150: 23%; DBE300: 50%; Fig. 3.43). No significant difference was noted in the different control groups (Groups 1 – 4).

**KIDNEY BIOCHEMICAL ANALYSIS**

*Effect on calcium, oxalate and phosphate contents*

The changes in levels of calcium, oxalate and phosphate in kidney of rats administered with ethylene glycol and reversal of these changes by cystone/ plant extracts were shown in Table 3.32. No significant difference in levels of calcium, oxalate and phosphate in kidney were observed among the different control groups (Groups 1 – 4). Ethylene glycol - induced a significant increase in the level of calcium (266%) in kidney of rat (Group 5; Fig. 3.44). However, concurrent administration of ethylene glycol along with two doses of cystone (CST150: 93%; CST300: 176%), BCE (BCE150: 142%; BCE300: 250%) and DBE (DBE150: 125%; DBE300: 236%) significantly restored the calcium level (Fig. 3.44). The renoprotective index was calculated and it was observed that *B. ciliata* (BCE150: 53; BCE300: 94) was most potent followed by *D. biflorus* (DBE150: 47; DBE300: 88) and cystone (CST150: 34; CST300: 66; Table 3.32).

Similarly, ethylene glycol administration also caused a significant increase in the level of oxalate (191%) and phosphate (122%) in the in kidney of rats (Group 5; Fig. 3.45-3.46). However, the treatment of cystone/ plant extracts significantly ameliorate these changes as calculated by the percent change from ethylene glycol treatment (CST150: 64%; CST300: 122%; BCE150: 102%; BCE300: 182%; DBE150: 84%; DBE300: 172%; Fig. 3. 45). The renoprotective index indicates that the maximum protection was provided by the extract of *B. ciliata* (BCE150: 54; BCE300: 95).
followed by the extract of *D. biflorus* (DBE150: 44; DBE300: 90) and cystone (CST150: 33; CST300: 64; Table 3.32).

In the same manner, there was a significant protection in the kidney phosphate level by cystone (CST150: 34%; CST300: 114%), *B. ciliata* (BCE150: 63%; BCE300: 113%) and *D. biflorus* (DBE150: 58%; DBE300: 109%; Fig. 3.46). Renoprotective index revealed that the maximum protection was provided by the extract of *B. ciliata* (BCE150: 52; BCE300: 92) followed by the extract of *D. biflorus* (DBE150: 47; DBE300: 89) and cystone (CST150: 28; CST300: 57; Table 3.32).

**Effect on lipid peroxidation (LPO) and total protein content**

The changes in levels of LPO and total protein contents in kidney of rats administered with ethylene glycol along with cystone/plant extracts were shown in Table 3.34. No significant difference in levels of these parameters in kidney was observed among the different control groups (Groups 1 – 4). Ethylene glycol-induced a significant increase in the level of LPO (220%) and total protein content (41%) in kidney of rat (Group 5; Figs. 3.47-3.48). However, the treatment of ethylene glycol along with standard cystone (CST150: 32; CST300: 68) and plant extracts (BCE150: 67; BCE300: 98; DBE150: 63; DBE300: 96) significantly mitigated the ethylene glycol-induced changes in LPO contents in kidney of rats as depicted by the renoprotective index (Table 3.34). In a similar way, cystone (CST150: 34; CST300: 71), *B. ciliata* (BCE150: 50; BCE300: 92) and *D. biflorus* (DBE150: 47; DBE300: 88; Table 3.34) significantly ameliorated the ethylene glycol-induced changes in total protein contents in kidney of rats. As shown by the renoprotective indices, *B. ciliata* was more potent than *D. biflorus* and cystone.
**Effect on non-enzymatic antioxidants**

The protective effects of cystone and plant products on ethylene glycol-induced changes in the GSH and TAA levels are presented in Table 3.35. No significant differences were noted in the levels of these parameters in different control groups (Groups 1 – 4). The oral administration of standard cystone (CST150: 21%; CST300: 35%) and plant extracts (BCE150: 36%; BCE300: 62%; DBE150: 32%; DBE300: 57%; Fig. 3.49) significantly restored the GSH content in the kidney of rats which was reduced by 63% with ethylene glycol treatment as compared with untreated control (Group 1).

Similarly, another non-enzymatic antioxidant that is TAA content was also found to be significantly reduced (83%) by ethylene glycol treatment which was potently prevented by cystone (CST150: 30%; CST300: 58%) and plant extracts (BCE150: 41%; BCE300: 79%; DBE150: 39%; DBE300: 75%; Fig. 3.50). Renoprotective index calculated for GSH (CST150: 33; CST300: 55; BCE150: 57; BCE300: 98; DBE150: 51; DBE300: 91; Table 3.35) and TAA contents (CST150: 36; CST300: 70; BCE150: 49; BCE300: 95; DBE150: 46; DBE300: 90; Table 3.35) were found to be more potent in case of BCE as compared with DBE and standard cystone at the same dose levels (Table 3.35).

**Effect on enzymatic antioxidants**

Table 3.36 presents the mitigatory effect of co-treatment with cystone and plant extracts at both the dose levels, on ethylene glycol-induced changes in enzymatic antioxidants in the kidney of rats. No significant difference was noted in the activity of these enzymes in different control groups (Groups 1 – 4). However, ethylene glycol treatment caused a significant reduction in activities of CAT (63%), SOD (72%), GPx (70%) and GR (61%) in kidney as compared to untreated control.
(Group 1; Figs. 3.51-3.54). It was revealed by the renoprotective index that the co-treatment of cystone (CST150: 24; CST300: 64) and plant extracts (BCE150: 49; BCE300: 97; DBE150: 43; DBE300: 90; Fig. 3.51) significantly (p<0.001) ameliorated the ethylene glycol induced changes in CAT activity in kidney of rats.

Similarly, the ethylene glycol - induced decrease in the activity of SOD in kidney of rats was also mitigated by the administration of cystone/ plant extracts. The renoprotective index showed that the *B. ciliata* (BCE150: 45; BCE300: 94) caused most potent increase in the SOD activity followed by *D. biflorus* (DBE150: 40; DBE300: 85) and cystone (CST150: 23; CST300: 51; Table 3.36).

In the similar manner, both the plant extracts and cystone significantly increased the activity of GPx which was reduced by 30% by ethylene glycol treatment in kidney of rats (Fig. 3.53). The mitigation in the activity of GPx was maximum with *B. ciliata* (BCE150: 46; BCE300: 94) followed by *D. biflorus* (DBE150: 41; DBE300: 87) and cystone (CST150: 28; CST300: 54; Table 3.36), as indicated by the renoprotective index.

And finally in case of GR activity in kidney, calculated renoprotective indices indicated that the co –treatment of cystone (CST150: 37; CST300: 62), *B. ciliata* (BCE150: 53; BCE300: 95) and *D. biflorus* (DBE150: 51; DBE300: 90; Table 3.36) significantly elevated the ethylene glycol - induced reduction in activity of GR in kidney. The renoprotection index was more for *B. ciliata* as compared to *D. biflorus* and cystone.

**Correlation analysis**

Table 3.33 represents the Pearson correlation analysis between all the experimental parameters in kidney of rats. The results indicates that there is significant positive correlation among all the biochemical parameters with correlation
The coefficient (r) value was in the range of 0.9536 – 0.9991. Similarly, the results of the Pearson correlation analysis between increase in LPO and depletion of enzymatic and non-enzymatic antioxidants in kidney of rats were shown in Table 3.37. There is significant negative correlation between elevation in LPO and antioxidants present in the kidney.

PART III

ANTICRYSTALLIZATION ACTIVITY OF EXTRACTS

In this study, an in vitro inhibitory effect of hydro-alcoholic extracts of B. ciliata and D. biflorus on various phases of calcium oxalate crystallization was determined by time course measurement of turbidity in the synthetic urine at graded concentrations of 1, 2, 5, 7.5 and 10 mg/mL and compared with standard polyherbal drug cystone.

The results revealed that addition of sodium oxalate (10 – 50 mM) in artificial urine induced the formation of calcium oxalate crystals by significantly increasing the nucleation and aggregation rate of formation of calcium oxalate crystals in a concentration- and time-dependent manner (Table 3.38). The significant initial increase in absorbance till 30 min of incubation of sodium oxalate with artificial urine corresponds to the nucleation of the calcium oxalate crystals and then followed by a decrease in absorbance indicating the aggregation rate of the crystals (Fig. 3.55).

Table 3.38 presents the mitigatory effect of both the plant extracts at various concentrations on sodium oxalate-induced crystallization in artificial urine.

Nucleation assay

In nucleation assay (0 - 30 min), the number of crystals formed was estimated in terms of the turbidity of the solution. The absorbance of control recorded was subtracted from that obtained with the plant extracts. There is a steep decrease in the
absorbance with the increase in the concentration of the plant extracts and cystone when incubated along with sodium oxalate (50 mM). The percent inhibition of the extract of rhizomes of *B. ciliata* was in the range of 32 – 92% whereas in case of extract of seeds of *D. biflorus*, percent inhibition was in the range of 27 – 85% respectively. However, cystone showed the percent inhibition in the range of 17 – 56% (Table 3.38; Figs. 3.56-3.58). There was a dose dependent increase of percent inhibition of nucleation by the extracts of *B. ciliata* ($r^2 = 0.965$), *D. biflorus* ($r^2 = 0.982$) and cystone ($r^2 = 0.990$). The IC$_{50}$ value of the *B. ciliata* was found to be 2.3 mg/mL which was more potent than *D. biflorus* (IC$_{50}$ = 3.5 mg/mL) as compared with the cystone with IC$_{50}$ value of 8.1 mg/mL.

**Aggregation assay**

Similarly, in aggregation assay, *B. ciliata* and *D. biflorus* showed more significant concentration-dependent inhibition of the aggregation of crystals of CaOx when compared with cystone (Table 3.38). However, cystone also showed inhibitory activity on crystal aggregation but it is comparatively less potent than the plant extracts at the same concentration range. The percent inhibitions (360 min) of *B. ciliata* were found to be in the range of 43 – 90% (Fig. 3.57) whereas extract of *D. biflorus* showed 32 – 81% of inhibition (Fig. 3.58). The percent inhibitions at 360 min of marketed drug, cystone was observed to be in the range of 19 - 67% with an IC$_{50}$ value of 3.03 mg/mL (Fig. 3.56). The IC$_{50}$ value of the *B. ciliata* and *D. biflorus* was found to be 0.9 and 1.107 mg/mL respectively. The coefficient of regression $r^2$ was obtained by linear regression. All results exhibited coefficient of regression with $r^2 > 0.9$ ($p < 0.01$).
Microscopic analysis

The light microscopic photographs taken at 360 min of sodium oxalate (50 mM) treatment in artificial urine showed formation of both types of CaOx crystals, oval shaped calcium oxalate monohydrate (COM) and bipyramidal shaped calcium oxalate dehydrate (COD) with significant aggregations (Fig. 3.59A). However, *B. ciliata* at the highest concentration of 10 mg/mL caused inhibition in the CaOx crystal formation with no COM crystals (Fig. 3.59C). Similarly, *D. biflorus* showed significantly less number of COM crystals at the concentration of 10 mg/mL. Moreover the number of COD crystals was also less with the plant extracts (Fig. 3.59D). Furthermore, the results of the marketed drug cystone were less significant as compared with both the plant extracts with numerous COM and COD crystals at the same concentration level (Fig. 3.59B). Thus, the extract of *B. ciliata* was found to most potent than *D. biflorus* and cystone as depicted by the IC\textsubscript{50} values in nucleation and aggregation assays as well as light microscopy photographs.

PART IV

PHYTOCHEMICAL EVALUATION OF PLANT EXTRACTS

Qualitative analysis

Table 3.39 depicts the results of phytochemical analysis of hydro – alcoholic extracts of both the selected plants. The extract yields were calculated and presented. The qualitative analysis of extracts indicated the presence of tannins, saponins, flavonoids and alkaloids.

Quantitative analysis

*Total phenolic content (TPC)*

The total phenolic content of both the extracts were estimated by plotting gallic acid standard curve. The extract of *B. ciliata* was found to contain 135.07 mg
gallic acid equivalent/gm dry weight. Whereas, hydro – alcoholic extract of *D. biflorus* was found to contain 112.30 mg gallic acid equivalent/gm dry weight. Therefore, the results indicated that the *B. ciliata* contained more phenolic compounds than *D. biflorus* (Table 3.39).

**Flavonoid content**

The standard curve of quercetin was plotted to calculate flavonoid contents of both the plant extracts. The flavonoid content of the extract of *B. ciliata* was observed to be 14.52 mg of quercetin equivalent/gm dry weight which was found to be less than the extract of *D. biflorus* which contained 32.08 mg of quercetin equivalent/gm dry weight (Table 3.39).

**Tannin content**

The tannin contents of both the plant extracts was calculated by plotting standard curve of rutin at various concentrations. The tannin content of the extract of *B. ciliata* was found to be 41.09 mg rutin equivalent/gm dry weight. Comparatively, the extract of *D. biflorus* contained 4.71 mg rutin equivalent/gm dry weight which is significantly lesser than the extract of *B. ciliata* (Table 3.39).

**Ascorbic acid content**

Ascorbic acid content of extract of *B. ciliata* was found to be 5.30 mg % dry weight whereas the extract of *D. biflorus* contained 3.20 mg % dry weight of ascorbic acid (Table 3.39).

**IN VITRO ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS/CYSTONE**

1. **Radical scavenging capacity of plant extracts**

*Reducing power assay*

The reducing capacity of the plant extracts to transform Fe$^{3+}$ to Fe$^{2+}$ was measured at various concentrations and compared with polyherbal drug cystone. A
greater absorbance of the reaction mixture corresponds with the greater reducing ability of the plant extracts. The results revealed concentration – dependent increase in reducing power with maximum activity attained at maximum dose of 250 µg/mL by BCE and followed by DBE and cystone (Fig. 3.60).

*Superoxide radical scavenging assay*

The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the cystone/ plant extracts and the reference compound ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture. Results indicated a concentration – dependent increase in the superoxide radical scavenging activity. The IC$_{50}$ values of the plant extracts (BCE: 7.97 µg/mL; $r^2 = 0.970$; DBE: 13.25 µg/mL; $r^2 = 0.999$; Fig. 3.61) was lower than that of the ascorbic acid (19.99 µg/mL; $r^2 = 0.991$). However, the IC$_{50}$ value of the cystone was more than that of the ascorbic acid (35.50 µg/mL; $r^2 = 0.997$).

*DPPH radical scavenging assay*

The DPPH radical scavenging activity of cystone/ plant extracts (BCE and DBE) at different concentrations is shown in Fig. 3.62 and compared with reference compound ascorbic acid. The radical scavenging activity was highest for extract of *B. ciliata* (IC$_{50}$ value = 40.25 µg/mL) followed by *D. biflorus* extract with an IC$_{50}$ value of 47.50 µg/mL. Moreover, the ascorbic acid showed less scavenging activity than plant extracts with an IC$_{50}$ value of 54.76 µg/mL. The least ability was shown by the polyherbal drug cystone with an IC$_{50}$ value of 71.40 µg/mL. The effect was concentration – dependent with coefficient of regression value of $r^2 > 0.9$. 
**Hydrogen peroxide scavenging assay**

The hydrogen peroxide scavenging activity of various concentrations of plant extracts was found to be statistically more significant than ascorbic acid and cystone. The percent inhibition of hydrogen peroxide induced radicals significantly increased with increasing concentrations with maximum inhibition at 150 µg/mL. The hydro-alcoholic extract of *B. ciliata* was observed to be most potent with lowest IC$_{50}$ value of 35.28 µg/mL. This was followed by extract of *D. biflorus* (IC$_{50}$ value = 49.09 µg/mL), ascorbic acid (IC$_{50}$ value = 67.10 µg/mL) and least for cystone (IC$_{50}$ value = 103.83 µg/mL; Fig. 3.63).

**Nitric oxide radical scavenging assay**

In the present study, the nitric oxide radical quenching activity of the plant extracts was detected and compared with ascorbic acid and cystone. The percent inhibitions of both the plant extracts (BCE/DBE) are significantly higher than the ascorbic acid and increases in a concentration-dependent manner ($r^2 > 0.950$). The highest scavenging activity of extracts of *B. ciliata* and *D. biflorus* was found to be 105.3% and 92.08% respectively whereas in case of ascorbic acid and cystone, it was observed to be only 71.5% and 83.2% respectively at the highest concentration of 250 µg/mL. IC$_{50}$ values for nitric oxide radical scavenging activity were 56.33, 79.57, 105.2 and 125.4 µg/mL for *B. ciliata*, *D. biflorus*, ascorbic acid and cystone respectively (Fig. 3.64).

2. **Effect of sodium oxalate and plant extracts in rat kidney homogenate**

**Lipid peroxidation**

During lipid peroxidation, low molecular-weight end products, probably malonaldehyde (MDA), are formed by oxidation of polyunsaturated fatty acids that can be reacted with two molecules of thiobarbituric acid to give a pinkish red
The incubation of kidney homogenate of rats with sodium oxalate (100 – 500 µg/mL) caused a significant increase in LPO level in a concentration – dependent manner \( (r^2 = 0.9890; \ p < 0.001; \ \text{Table 3.40}; \ \text{Fig. 3.65}) \). The maximum increase has been attained at a dose of 500 µg/mL.

However, the co – administration of cystone and plant extracts (BCE/ DBE) at various concentrations (50, 100, 150, 200 and 250 µg/mL) resulted in a significant reduction in sodium oxalate (500 µg/mL) induced increase in LPO. The hydro – alcoholic extract of \( B. \ ciliata \) \( (r^2 = 0.881) \), resulted in maximum reduction in LPO followed by the extract of \( D. \ biflorus \) \( (r^2 = 0.980) \) and cystone \( (r^2 = 0.932) \) in a dose – dependent manner in kidney homogenate of rats (Fig. 3.66).

**Superoxide dismutase activity**

The results shown in Table 3.40 also indicates that the incubation of sodium oxalate with kidney homogenate of rats resulted in significant decrease in antioxidative enzyme that is superoxide dismutase in a concentration – dependent manner \( (r^2 = 0.996; \ \text{Fig. 3.67}) \). However, when cystone/ plant extracts were added along with the highest dose of sodium oxalate (500 µg/mL), there was a significant increase in SOD activity in kidney homogenate of rats. The maximum percent change was found to be 94% which was achieved by \( B. \ ciliata \) extract at a dose of 250 µg/mL \( (r^2 = 0.997) \). This was followed by the extract of \( D. \ biflorus \) \( (r^2 = 0.999) \) and then least for cystone \( (r^2 = 0.994) \) in a dose – dependent manner (Fig. 3.68).

**Catalase activity**

The catalase activity in kidney homogenate of rats was also significantly reduced by the exposure of sodium oxalate at various concentrations (100 – 500 µg/mL; Table 3.40). The sodium oxalate at doses of 100, 200, 300, 400 and µg/mL
reduced the catalase activity by 88%, 75%, 64%, 51% and 37% respectively ($r^2 = 0.999$; Fig. 3.69).

However, the co–treatment of cystone and plant extracts (BCE and DBE) at the graded concentrations of 50, 100, 150, 200 and 250 mg/mL had significantly improved the catalase activity in a dose – dependent manner. The treatment of *B. ciliata* extract had maximum potency with highest percent change of 97% at 250 mg/mL ($r^2 = 0.998$; Fig. 3.70). This was followed by the extract of *D. biflorus* which showed maximum percent change of 93% in a dose – dependent manner ($r^2 = 0.992$). Comparatively, the least percent change was obtained by the cystone with highest change of only 81% ($r^2 = 0.979$; Fig. 3.71).

**PART V**

**IDENTIFICATION AND QUANTIFICATION OF ACTIVE COMPONENTS OF PLANT EXTRACTS**

The results obtained in part – II, III and IV concluded that the plant extracts have higher significant potency than polyherbal drug cystone in terms of antiurolithiatic activity. Therefore, the standardization of plant extracts was done by quantifying the major active components of both the plant extracts. The major active components present in these two plants namely gallic acid from *B. ciliata* and quercetin from *D. biflorus* were separated from other constituents by reverse-phase HPLC analysis.

The extract of rhizomes of *B. ciliata* in methanol: water (70:30, v/v) showed the presence of gallic acid as the highest peak with retention factor of 2.77 (Fig. 3.72) which is comparable with the retention factor of pure standard gallic acid with 2.78 (Fig. 3.71). The results were further confirmed by the spiking of extract of *B. ciliata*
with standard gallic acid which showed the same peak with retention factor of 2.77 (Fig. 3.73).

In the similar way, the presence of quercetin has been obtained as the major peak with retention factor of 5.327 (Fig. 3.75) in extract of *D. biflorus* in methanol: water (70:30, v/v) which is comparable with the retention factor of pure standard quercetin with 5.35 (Fig. 3.74). The extract of *D. biflorus* was then further spiked with standard quercetin and the chromatogram of these showed the rise in the major peak with the retention factor of 5.249 which concludes that this peak corresponds to the peak of quercetin (Fig. 3.76).