CHAPTER VII
MODULATION OF TOXICITY OF THE ANTI-CANCER DRUGS, VINCristine AND CYCLOPHOSPHAMIDE
7.1 INTRODUCTION

The history of the Vinca alkaloids and the story of their discovery are well known (Johnson I S 1968; Johnson et al 1963). Although there is no question that the Vinca alkaloids disrupt microtubules, the biologic mechanisms underlying the antineoplastic activity of these drugs are less contain. In actively proliferating cells, the mechanism of cytotoxicity of the Vinca alkaloids is usually considered to be disruption of the mitotic spindle, resulting in metaphase arrest and ultimately cell death (Malawista SE et al 1968; Bruchovsky N et al 1965; George P et al 1965; Krishan A 1968; Langsfeld A M et al 1980). Among anticancer drugs, Vinca alkaloids are classified as mitotic inhibitors, with their primary site of action being M phase of the cell cycle, although it is by no means certain that mitotic inhibition is the predominant cytotoxic mechanism in vivo. New studies suggest that disruption of the cell cycle may lead to cell death through initiation of programmed cell death pathways, known as apoptosis. (Schrek R 1974; Tuskidate K et al 1993; Stewart B W 1994; Green DR et al 1992; Kerr J F et al 1994; Beck WT 1995).

Since vincristine (VCR), vindesine (VDS) and vinorelbine (VLB) exhibit similar potencies against preparation of tubuline isolated from the same tissue. The potency of VCR relative to VLB can be explained by differences in cellular retention of the two drugs, particularly during drug exposures of limited duration. (Ferguson PJ and Cass CE 1985; Ferguson et al 1984; Gout PW et al 1978; Lengsfeld 1982). VCR and VLB are equitoxic against cultured leukemic cells during continuous exposure, whereas VCR is more potent during exposures of short duration, because cellular retention of VCR is greater than that of VLB. (Ferguson PJ and Cass CE 1985; Gout PW et al 1984). The same can be said for VRLB the chemical change on the catharanthine ring, described above makes VRLB more lipophilic with greater tissue retention and greater affinity for mitotic rather than axonal microtubules. (Hartwell LH and Kastan MB 1994). Because the antitumour effect of VRLB is similar to that of VLB and CVR, this decreased
Chart showing postulated production of hydroxy-cyclophosphamide and a phosphoramidate derivative during the metabolism of cyclophosphamide (CP).
effectiveness of VRLB on axonal microtubules may be a factor in the drug's decreased neurotoxicity. Finally, VFL was shown to have antitumour activity in vivo in mice bearing P388 leukemia, B16 melanoma and in human tumour lung and breast xenografts (Kruczynski A et al 1998), and this activity was found to be superior to that of VRLB (Hill BT et al 1999).

Cyclophosphamide is the drug of choice in the treatment of various solid and haematological malignancies. The alkylating agent, cyclophosphamide (CYP) has been shown to be inactive in vitro but was found to be activated to cytotoxic metabolities by the mixed function oxidase in hepatic microsomes (Foley et al 1960, Cohen and Jao 1970). The high therapeutic index of CYP was shown to be due to the metabolities, 4-hydroxy cyclophosphamide and phosphoramid derivative (Colvin et al 1976). The metabolism of CYP is illustrated in the following chart no.1.

It was shown that free radicals are formed during the activation of CYP and produce tissue injury (Colvin 1982). Toxic syndromes of CYP are suppression of white blood cells (WBC), nausea, vomiting, gonadal atrophy and renal and bladder injury by the cyclophosphamide metabolite. It was previously reported that sulphydryl agents such as mercaptopropionyl glycine (MPG) reduce the toxicity of CYP by preventing free radical accumulation and promoting repair (Philips et al 1983, Bhanumathy et al 1986).

In the present chapter, attempts were made to examine the modulatory effects of *B.racemosa* seed extract on vincristine and cyclophosphamide induced toxicity in normal and tumour bearing mice.

### 7.2 Materials and Methods

#### 7.2.1 Mice:
Inbred strains of Swiss albino mice (males) weighing 25gm were used for the experiment.

#### 7.2.2 Drugs:
Vincristine, Cyclophosphamide and *B.racemosa* seed extract was procured from sources mentioned in chapter 2. The methanol extract
was evaporated to dryness under vacuum and reconstituted in phosphate buffered saline as stated in Materials and methods.

7.2.3 Cyclophosphamide treated normal mice

Four groups of mice were used, 10 mice/group. The first group received CTX at a dose of 50mg/kg i.p., for 5 alternative days. The second group was given 3mg/kg i.p, *B. racemosa* seed extract, 30 minutes before CTX (50mg/kg) i.p., as above. The third group was given *B. racemosa* seed extract (3mg/kg) i.p., without cyclophosphamide. Group four was treated as control, which received the same volume of phosphate buffered saline for the same period.

7.2.4 Cyclophosphamide treated tumour bearing mice

One million DLA cells were injected i.p., and drug treatment was started one day after the tumour inoculum. To the first group was injected with CTX (50mg/kg i.p.) for five alternative days, group second received *B. racemosa* seed extract, 3mg/kg i.p, 30 minutes before CTX (50mg/kg i.p.) as above, the third group received *B. racemosa* seed extract (3mg/kg) for five alternative days while the fourth group served as control.

7.2.5 Vincristine treated normal mice

Four groups of mice were used, 12 mice/group. The first group received Vincristine at a dose of 0.5mg/kg i.p. for 10 alternative days, the second group received *B. racemosa* seed extract 3mg/kg i.p, 30 minutes before Vincristine (0.5mg/kg i.p). The third group received *B. racemosa* seed extract (3mg/kg) and the fourth group used as control.

7.2.6 Vincristine treated tumour bearing mice

One million tumour cells (DLA) were injected i.p. and drug treatment started one day after the tumour inoculum. To the first group was injected with Vincristine (0.5mg/kg, i.p.) for 10 alternative days. The second group received *B. racemosa* seed extract (3mg/kg, i.p.) 30 minutes before
Vincristine (0.5mg/kg, i.p.). The third group was given only *B. racemosa* seed extract (3mg/kg, i.p.) and fourth group was used as control.

7.2.7 Haematological and biochemical parameters

Peripheral blood for various biochemical and haematological studies was collected at different intervals of time. All the biochemical and haematological parameters were done by the standard protocol. Detailed procedure is mentioned in section 2.9.5.1 and 2.9.5.6.

7.3 Result

7.3.1 Cyclophosphamide treated normal mice

This experiment was carried out with the intention of finding out whether our drug can reduce the toxicity associated with chemotherapeutic drugs like cyclophosphamide (CYP) and vincristine (VCR). The group of animals receiving CYP alone, showed a much greater decrease in body weight, as compared to animal receiving CYP along with *B. racemosa* seed extract (Table-21). In the case of normal mice, the blood urea was found to be 21 while it was gone upto 58 during 9 days of treatment and this was come down to 35 when our drugs was given along with CYP (Fig:4). This is the result of 10 days treatment, which shows a remarkable reduction in blood urea level causing the reversal of toxic symptoms. CYP administration also increased serum alkaline phosphatase (SAKP), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels in mice, which is indicative of hepatic damage. However, *B. racemosa* seed extract normalized to a large extent of SGOT, SGPT and SKAP levels (Fig: 5, 6, 7), indicating hepatoprotection by the drug. There is no much significant difference between WBC, RBC, Hb, HCT, MCHC and platelets of CYP alone treated group and CYP + *B. racemosa* treated group (Table-22, 23, 24, 25, 26 & 27).
Table - 21

Effect of *B. racemosa* seed extract on the body weight of cyclophosphamide (CYP) treated normal mice

<table>
<thead>
<tr>
<th>Concentration of <em>B. racemosa</em> seed extract</th>
<th>Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Days</td>
</tr>
<tr>
<td>Control</td>
<td>24.3±0.81</td>
</tr>
<tr>
<td>CTX (50mg/kg)</td>
<td>24.5±0.54</td>
</tr>
<tr>
<td><em>B. racemosa</em> seed Extract (3mg/kg)</td>
<td>24.6±0.51</td>
</tr>
<tr>
<td>CYP (50mg/kg)+ <em>B. racemosa</em> seed Extract (3mg/kg)</td>
<td>25±0.63</td>
</tr>
</tbody>
</table>

Values represent the mean±S.D , n=12

Values marked with asterisk are statistically significant (** P< 0.001) as compared to CYP control. CYP (50mg/kg) and *B. racemosa* seed extract (3mg/kg) were administered for 5 alternative days (i.p.).
Table- 22

Total WBC ($10^9$/mm$^3$) counts of normal mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
<th>9 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>2.25±0.88</td>
<td>3.05±0.46**</td>
<td>1.8±0.58</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1.7±0.52</td>
<td>1.42±0.26</td>
<td>2.0±0.43</td>
</tr>
<tr>
<td>BR</td>
<td>9.5±0.53</td>
<td>8.175±0.45</td>
<td>8.4±0.69</td>
</tr>
<tr>
<td>Control</td>
<td>7.95±0.7</td>
<td>8.05±0.55</td>
<td>8.05±0.89</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), **P<0.001

Table- 23

Total RBC ($10^6$/mm$^3$) counts of normal mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
<th>9 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>6.87±0.35</td>
<td>5.55±0.86</td>
<td>5.82±1.07</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>6.87±0.29</td>
<td>6.03±0.37</td>
<td>6.45±0.86</td>
</tr>
<tr>
<td>BR</td>
<td>5.92±0.27</td>
<td>5.9±0.73</td>
<td>5.55±0.7</td>
</tr>
<tr>
<td>Control</td>
<td>6.07±0.27</td>
<td>6.0±0.40</td>
<td>6.5±0.59</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values are not statistically significant from CYP control (50mg/kg)
Table- 24

Total Hb (gm%) counts of normal mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
<th>9 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>12.47±0.68</td>
<td>10.6±0.58***</td>
<td>10.6±0.98</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>11.87±0.96</td>
<td>12.3±0.39</td>
<td>11.07±0.63</td>
</tr>
<tr>
<td>BR</td>
<td>9.65±0.44</td>
<td>10.9±0.52</td>
<td>10.55±0.59</td>
</tr>
<tr>
<td>Control</td>
<td>12.82±1.38</td>
<td>12.22±0.92</td>
<td>12.65±0.38</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), ***P<0.003

Table- 25

Total HCT (%) counts of normal mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
<th>9 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>36.75±2.56</td>
<td>30.9±2.18***</td>
<td>31.7±3.07</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>34.6±3.29</td>
<td>36.45±1.61</td>
<td>32.3±1.90</td>
</tr>
<tr>
<td>BR</td>
<td>28.95±1.77</td>
<td>32.85±1.73</td>
<td>31.07±2.56</td>
</tr>
<tr>
<td>Control</td>
<td>38.7±3.42</td>
<td>36.9±3.34</td>
<td>38.7±1.96</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50mg/kg), ***P<0.006
Table – 26
Total MCHC (gm/dl) counts of normal mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
<th>9 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>35.27±3.13</td>
<td>43.75±0.85</td>
<td>34.17±2.25</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>37.4±1.41</td>
<td>43.27±0.42</td>
<td>34.75±1.57</td>
</tr>
<tr>
<td>BR</td>
<td>30.47±3.22</td>
<td>28.35±0.66</td>
<td>33.82±1.15</td>
</tr>
<tr>
<td>Control</td>
<td>29.47±0.68</td>
<td>29.17±0.79</td>
<td>29.95±1.19</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values are not statistically significant as compared to CYP control (50mg/kg)

Table – 27
Total PLT (10⁴/mm³) counts of normal mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
<th>9 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>713.75±176</td>
<td>820.5±172</td>
<td>194.89±221**</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>908.75±158</td>
<td>813.0±90</td>
<td>940.0±82</td>
</tr>
<tr>
<td>BR</td>
<td>224.25±56</td>
<td>235.0±16</td>
<td>307.5±33</td>
</tr>
<tr>
<td>Control</td>
<td>175.5±48</td>
<td>201.25±35</td>
<td>228.25±53</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), **P<0.001
Fig. 4 Urea of normal mice treated with treated cyclophosphamide (50 mg/kg) with or without *B.* *racemosa* (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice/group

*P < 0.0001, **P < 0.001
Fig. 5 SGOT of normal mice treated with cyclophosphamide (50mg/kg) with or without *B. racemosa* (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice/group

*e P < 0.01, † P < 0.02*
Fig. 6 SGPT of normal mice treated with cyclophosphamide (50 mg/kg) with or without *B. racemosa* (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice /group

Values are not statistically significant from CYP control
Fig. 7 SKAP of normal mice treated with Cyclophosphamide (50mg/kg) with or without *B. racemosa* (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice / group

\[ b \ P < 0.005, \ f \ P < 0.02, \ g \ P < 0.03 \]
7.3.2 CYP treated tumour bearing mice

The mean survival time of untreated tumour bearing mice was 22.8 ± 0.75 days while that for the CYP alone treated mice was 36.6 ± 3.07 days. The mean survival time of animals receiving combination treatment was increased to 58.5 ± 13.5 as compared to CYP alone treated group (Table-28). In the case of urea there is a significant differences in toxicity in the CYP alone treated group and the group with CYP + B.racemosa treated group (Fig:8). The increase in alkaline phosphatase, SGPT and SGOT values by CYP was also checked by the combined administration of CYP and B.racemosa (Br) seed extract indicating hepatoprotection (Fig: 9,10,11). There is no significant relationship in the parameters of WBC, RBC, Hb, HCT, MCHC and platelets between the CYP alone and CYP with B.racemosa seed extract treated groups. (Table-29, 30,31,32,33&34)

Table - 28

Effect of B.racemosa seed extract on the life span of cyclophosphamide (CYP) treated tumour bearing mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean survival time (MST) Days</th>
<th>Increase in life span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>22.8±0.75</td>
<td>-</td>
</tr>
<tr>
<td>CYP (50mg/kg)</td>
<td>36.6±3.07</td>
<td>60</td>
</tr>
<tr>
<td>CYP (50mg/kg)+B.r (3mg/kg)</td>
<td>** 58.5±13.5</td>
<td>156</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to control (50 mg/kg), ** P < 0.001
Table – 29

Total WBC (10³/mm³) counts of DLA Tumour bearing mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>2.75±0.34*</td>
<td>1.25±0.20**</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>2.15±0.28</td>
<td>2.35±0.26</td>
</tr>
<tr>
<td>BR</td>
<td>19.7±1.21</td>
<td>26.2±3.87</td>
</tr>
<tr>
<td>Control</td>
<td>28.05±5.42</td>
<td>36.875±6.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), *P<0.01; **P<0.001

Table – 30

Total RBC (10⁶/mm³) counts of DLA Tumour bearing mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>8.58±0.64*</td>
<td>6.07±0.25**</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>7.37±0.38</td>
<td>7.02±0.47</td>
</tr>
<tr>
<td>BR</td>
<td>8.15±1.03</td>
<td>6.55±0.44</td>
</tr>
<tr>
<td>Control</td>
<td>7.17±0.27</td>
<td>7.44±0.50</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), *P<0.01; **P<0.001
Table – 31
Total Hb (gm%) counts of DLA Tumour bearing mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>10.45±1.49</td>
<td>9.65±0.42***</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>10.7±0.41</td>
<td>10.75±0.36</td>
</tr>
<tr>
<td>BR</td>
<td>11.82±0.62</td>
<td>12.25±0.73</td>
</tr>
<tr>
<td>Control</td>
<td>13.0±1.31</td>
<td>13.8±0.42</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), ***P<0.008

Table – 32
Total HCT (%) counts of DLA Tumour bearing mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>32.12±4.98</td>
<td>29.45±1.33*</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>33.35±0.89</td>
<td>32.52±1.24</td>
</tr>
<tr>
<td>BR</td>
<td>35.37±1.44</td>
<td>34.27±2.19</td>
</tr>
<tr>
<td>Control</td>
<td>38.25±3.57</td>
<td>40.02±3.58</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to CYP control (50 mg/kg), *P<0.01
Table - 33

Total MCHC (gm/dl) counts of DLA Tumour bearing mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>25.97±1.31*</td>
<td>31.55±0.97</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>28.5±0.82</td>
<td>29.87±2.42</td>
</tr>
<tr>
<td>BR</td>
<td>32.7±1.33</td>
<td>30.5±1.29</td>
</tr>
<tr>
<td>Control</td>
<td>38.85±1.17</td>
<td>34.9±1.47</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant compared to CYP control (50 mg/kg), *P<0.01

Table- 34

Total PLT (10^4/mm^3) counts of DLA Tumour bearing mice treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 Days</th>
<th>6 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide+BR</td>
<td>1001.75±138.81</td>
<td>1087.5±134.02</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1023.0±84.0</td>
<td>1305.75±179.74</td>
</tr>
<tr>
<td>BR</td>
<td>404.5±88.98</td>
<td>531.0±213.31</td>
</tr>
<tr>
<td>Control</td>
<td>350.5±87.32</td>
<td>525.0±139.52</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values are not statistically significant as compared to CYP control (50mg/kg)
Fig. 8 UREA of DLA tumour bearing mice treated with cyclophosphamide (50 mg/kg) with or without *B.racemosa* (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice/group

* P < 0.0001, ** P < 0.001
Fig. 9 SKAP of DLA tumour bearing mice treated with cyclophosphamide (50 mg/kg) with or without *B. racemosa* (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice /group

Values are not statistically significant from CYP control
Fig. 10  SGPT of DLA tumour mice treated with cyclophosphamide (50mg/kg) with or without *B. racemosa* (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice/group
Fig. 11  SGOT of DLA tumour bearing mice treated with cyclophosphamide (50 mg/kg) with or without B.racemosa (3mg/kg)

Values are mean ± SD from 3 experiments using 12 mice/group

\[ ^d P < 0.007, \quad ^c P < 0.006, \quad ^f P < 0.02 \]
7.3.3 Vincristine treated normal mice

Vincristine treated groups exhibited toxicological symptoms. Table indicates the body weight vincristine treated normal mice. At 20 days, the body weight was 19.5±1 for vincristine treated group. Whereas it was increased upto 22.8±0.75 at the same days for vincristine along with our drug treated group. (Table-35). Vincristine alone treated group showed an increase in the level of urea during the 5 days of treatment was 44 ± 4.32 even though this level was decreased and come down to normal level during the subsequent days. Whereas this initial increase in urea was prevented by the administration of *B. racemosa* seed extract along with vincristine. (Fig:12). There are no much significant differences between alkaline phosphatase, SGPT and SGOT levels of vincristine alone treated group and vincristine with B.r treated group (Fig: 13,14,15). It also did not exhibit any significance differences in the parameters of WBC, RBC, Hb, HCT, MCHC and platelets between the VCR alone treated group and VCR+ Br. treated group (Table-36, 37, 38, 39, 40 & 41).
Fig. 12  UREA of normal mice treated with vincristine (0.5 mg/kg) with or without B. racemosa (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice / group

** P < 0.001, *** P < 0.002
Fig. 13  SKAP of normal mice treated with vincristine (0.5 mg/kg) with or without *B. racemosa* (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice /group

Values are not statistically significant as compared to VCR control
Fig. 14 SGPT of normal mice treated with vincristine (0.5 mg/kg) with or without B. racemosa (3 mg/kg)
Values are mean ± SD from 3 experiments using 12 mice/group

**P < 0.001, ***P < 0.002
Fig. 15. SGOT of normal mice treated with vincristine (0.5mg/kg) with or without B. racemosa (3mg/kg).

Values are mean ± SD from 3 experiments using 12 mice/group.

- **Control**
- **VCR**
- **BR**
- **VCR+BR**

*P < 0.01, †P < 0.02
Table - 35
Effect of *B. racemosa* seed extract on the body weight of vincristine (Vcr) treated normal mice

<table>
<thead>
<tr>
<th>Concentration of <em>B. racemosa</em> seed extract</th>
<th>Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Days</td>
</tr>
<tr>
<td>Control</td>
<td>23.3±0.81</td>
</tr>
<tr>
<td>Vincristine (0.5mg/kg)</td>
<td>22.6±0.81</td>
</tr>
<tr>
<td><em>B. racemosa</em> seed Extract (3mg/kg)</td>
<td>23±0.89</td>
</tr>
<tr>
<td>VCR (0.5mg/kg)+ <em>B. racemosa</em> seed extract (3mg/kg)</td>
<td>23.1±0.75</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.D, n=12, Values marked with asterisk are statistically significant (**P < 0.001) as compared to vincristine control. Vincristine (0.5mg/kg) and *B. racemosa* (3mg/kg) were administered for 10 alternative days.

Table – 36
Total WBC (10^3/mm^3) counts of normal mice treated with vincristine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>5.42±0.17</td>
<td>3.9±0.83</td>
<td>3.85±0.66***</td>
</tr>
<tr>
<td>VCR</td>
<td>6.07±0.54</td>
<td>3.8±0.29</td>
<td>5.7±0.64</td>
</tr>
<tr>
<td>BR</td>
<td>9.5±0.53</td>
<td>8.2±0.0</td>
<td>8.4±0.69</td>
</tr>
<tr>
<td>Control</td>
<td>7.95±0.7</td>
<td>8.05±0.55</td>
<td>8.05±0.89</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12, Values marked with asterisk are statistically significant compared to VCR control (0.5 mg/kg), ***P<0.007
# Table- 37

**Total RBC (10^6/mm^3) counts of normal mice treated with vincristine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcr+BR</td>
<td>7.55±0.48</td>
<td>5.91±0.43*</td>
<td>2.63±0.64</td>
</tr>
<tr>
<td>Vcr</td>
<td>6.88±0.61</td>
<td>4.02±1.03</td>
<td>3.56±1.01</td>
</tr>
<tr>
<td>BR</td>
<td>5.92±0.27</td>
<td>5.9±0.73</td>
<td>5.55±0.70</td>
</tr>
<tr>
<td>Control</td>
<td>6.07±0.27</td>
<td>6.0±0.40</td>
<td>6.5±0.59</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant compared to VCR control (0.5 mg/kg), *P<0.01

# Table- 38

**Total Hb (gm%) counts of normal mice treated with vincristine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>12.4±0.77****</td>
<td>9.67±0.73***</td>
<td>4.25±0.47*</td>
</tr>
<tr>
<td>VCR</td>
<td>10.9±0.70</td>
<td>5.8±1.13</td>
<td>6.1±0.84</td>
</tr>
<tr>
<td>BR</td>
<td>9.6±0.44</td>
<td>10.9±0.52</td>
<td>10.55±0.59</td>
</tr>
<tr>
<td>Control</td>
<td>12.82±1.38</td>
<td>12.22±0.92</td>
<td>12.65±0.38</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant compared to VCR control (0.5 mg/kg), *P<0.01; ****P<0.002
Table - 39

**Total HCT (%) counts of normal mice treated with vincristine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcr+BR</td>
<td>36.02±2.27</td>
<td>27.72±2.90**</td>
<td>11.42±1.42****</td>
</tr>
<tr>
<td>Vcr</td>
<td>32.62±3.63</td>
<td>19.12±1.76</td>
<td>18.57±2.52</td>
</tr>
<tr>
<td>BR</td>
<td>28.95±1.77</td>
<td>32.85±1.73</td>
<td>31.07±2.5</td>
</tr>
<tr>
<td>Control</td>
<td>38.70±3.42</td>
<td>36.9±3.34</td>
<td>38.7±1.96</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant compared to VCR control (0.5 mg/kg), **P<0.002; ****P<0.005

Table - 40

**Total MCHC (gm/dl) counts of normal mice treated with vincristine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>34.95±0.33</td>
<td>36.07±1.15</td>
<td>47.8±3.15</td>
</tr>
<tr>
<td>VCR</td>
<td>33±1.76</td>
<td>29.82±4.99</td>
<td>41.32±7.37</td>
</tr>
<tr>
<td>BR</td>
<td>30.47±3.22</td>
<td>28.35±0.66</td>
<td>33.82±1.15</td>
</tr>
<tr>
<td>Control</td>
<td>29.47±0.68</td>
<td>29.17±0.79</td>
<td>29.95±1.19</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values are not statistically significant from VCR control (0.5 mg/kg)
Table – 41

Total PLT (10⁴/mm³) counts of normal mice treated with vincristine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>1090.75±213*</td>
<td>808.25±154</td>
<td>812.75±29.39</td>
</tr>
<tr>
<td>VCR</td>
<td>494.5±291</td>
<td>631.75±309</td>
<td>585.5±352</td>
</tr>
<tr>
<td>BR</td>
<td>224.25±56.16</td>
<td>235.0±16.87</td>
<td>307.5±33.04</td>
</tr>
<tr>
<td>Control</td>
<td>175.5±48.99</td>
<td>201.25±35.88</td>
<td>228.25±53.93</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to VCR control (0.5 mg/kg), *P<0.01

7.3.4 Vincristine treated tumour bearing mice

An increase in the life span of tumour bearing mice treated with *B. racemosa* seed extract (3mg /kg) in combination with vincristine (0.5mg/kg) was observed 238% as compared to those treated with vincristine alone (179%) (Table-42). The vincristine along with B.r. showed a strong a significance in the level of urea. In the case of tumour control, the level of urea was 52.6 ± 2.74 during 20 days. However, this level was not changed when it was given vincristine during the same days. Whereas this increased level was brought down to 30.6 ± 4.2 when it was given vincristine along with our drug. This is of highly significant because the high value of urea was reduced about to half with the treatment of vincristine along with B.r (Fig:16).

Serum alkaline phosphatase was also increased to 262 ± 21 during 20 days when it was given with vincristine alone. Whereas this value was
come down to 124 ± 41 during the same days when it was given vincristine along with B.r. (Fig:17). There is no much significant differences between the parameters of SGOT & SGPT of vincristine alone treated group and vincristine ± B.r treated group (Fig:18,19). Another significant change was observed in the platelet count. In the tumour control it was 894± 80 during 20 days and it rose to 1420 ± 321 during the same days when it was given vincristine alone. Whereas this increased level was 654 ± 360 in the case of VCR along with B.r treated group (Table-48). There is no significant difference between the parameters of WBC, RBC,HB,HCD and MCHC (Table-43,44,45,46&47).

Table - 42
Effect of B. Racemosa seed extract on the life span of vincristine (VCR) treated tumour bearing mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean survival time (MST)</th>
<th>Increase in life span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.8 ± 1.47</td>
<td>–</td>
</tr>
<tr>
<td>Vincristine (0.5mg/kg)</td>
<td>63.8 ± 28.79</td>
<td>179</td>
</tr>
<tr>
<td>Vincristine (0.5mg/kg) + B.r (3 mg/kg)</td>
<td><strong>77.1± 22.78</strong></td>
<td>238</td>
</tr>
</tbody>
</table>

Values are mean ± SD n=12

Values marked with asterisk are statistically significant as compared to control. *P<0.001
Table – 43

Total WBC (10^3/mm^3) counts of DLA tumour bearing mice treated with vincristine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcr+BR</td>
<td>1.37±0.35***</td>
<td>8.87±0.58</td>
<td>28.65±4.57</td>
</tr>
<tr>
<td>Vcr</td>
<td>7.3±3.08</td>
<td>12.67±6.10</td>
<td>26.25±2.68</td>
</tr>
<tr>
<td>BR</td>
<td>19.7±1.21</td>
<td>26.2±3.87</td>
<td>34.82±5.47</td>
</tr>
<tr>
<td>Control</td>
<td>28.05±5.42</td>
<td>37.37±12.31</td>
<td>44.82±14.94</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to VCR control (0.5 mg/kg), ***P<0.009

Table – 44

Total RBC (10^6/mm^3) counts of DLA tumour bearing mice treated with vincristine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>5.15±0.55</td>
<td>5.01±0.66</td>
<td>6.05±0.31</td>
</tr>
<tr>
<td>VCR</td>
<td>6.03±0.59</td>
<td>5.54±0.77</td>
<td>6.54±0.86</td>
</tr>
<tr>
<td>BR</td>
<td>8.15±1.03</td>
<td>6.55±0.44</td>
<td>6.55±0.62</td>
</tr>
<tr>
<td>Control</td>
<td>7.177±0.27</td>
<td>7.44±0.50</td>
<td>5.52±0.63</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values are not statistically significant as compared to VCR control (0.5mg/kg)
**Table - 45**

**Total Hb (gm%) counts of DLA tumour bearing mice treated with vincristine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>7.45±1.05***</td>
<td>8.15±1.18</td>
<td>10.75±0.34</td>
</tr>
<tr>
<td>VCR</td>
<td>10.62±0.84</td>
<td>9.27±1.55</td>
<td>10.37±0.47</td>
</tr>
<tr>
<td>BR</td>
<td>11.82±0.62</td>
<td>12.25±0.73</td>
<td>10.05±0.7</td>
</tr>
<tr>
<td>Control</td>
<td>13.0±1.31</td>
<td>13.8±0.42</td>
<td>11.02±1.51</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to VCR control (0.5 mg/kg), ***P <0.003

**Table- 46**

**Total HCT (%) counts of DLA tumour bearing mice treated with vincristine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>22.22±3.20***</td>
<td>25.9±3.23</td>
<td>32.02±1.62</td>
</tr>
<tr>
<td>VCR</td>
<td>31.5±2.39</td>
<td>27.35±3.65</td>
<td>30.35±1.33</td>
</tr>
<tr>
<td>BR</td>
<td>35.37±1.44</td>
<td>34.27±2.19</td>
<td>30.27±1.75</td>
</tr>
<tr>
<td>Control</td>
<td>38.25±3.57</td>
<td>39.84±3.93</td>
<td>32.75±4.87</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12
Values marked with asterisk are statistically significant as compared to VCR control (0.5 mg/kg), ***P<0.003
Table – 47

Total MCHC (gm/dl) counts of DLA tumour bearing mice treated with vincristine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCR+BR</td>
<td>30.52±6.10</td>
<td>35.25±0.78</td>
<td>34.47±0.81</td>
</tr>
<tr>
<td>VCR</td>
<td>36.2±0.78</td>
<td>34.5±0.94</td>
<td>33.05±1.28</td>
</tr>
<tr>
<td>BR</td>
<td>32.7±1.33</td>
<td>30.5±0.29</td>
<td>28.5±1.29</td>
</tr>
<tr>
<td>Control</td>
<td>38.85±1.17</td>
<td>34.9±1.47</td>
<td>34.575±1.06</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12

Values are not statistically significant as compared to VCR control (0.5mg/kg)

Table – 48

Total PLT (10⁴/mm³) counts of DLA tumour bearing mice treated with vincristine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 Days</th>
<th>10 Days</th>
<th>20 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vcr+BR</td>
<td>526±92.76***</td>
<td>996.5±91.17*</td>
<td>654.5±360.70*</td>
</tr>
<tr>
<td>Vcr</td>
<td>1374.5±337.48</td>
<td>1595.5±318.04</td>
<td>1420.0±321.72</td>
</tr>
<tr>
<td>BR</td>
<td>404.5±88.98</td>
<td>531.0±213.31</td>
<td>77.5±125.79</td>
</tr>
<tr>
<td>Control</td>
<td>350.5±87.32</td>
<td>525.0±139.52</td>
<td>894.75±80.17</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 12

Values marked with asterisk are statistically significant as compared to VCR control (0.5 mg/kg), *P<0.01; ***P<0.003
Fig. 16 UREA of DLA tumour bearing mice treated with vincristine (0.5 mg/kg) with or without *B. racemosa* (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice/group

****P < 0.003, *P < 0.004,  P < 0.01,  P < 0.03
Fig. 17  SKAP of DLA tumour bearing mice treated with vincristine (0.5mg/kg) with or without *B.racemosa* (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice /group

* P < 0.0001,  ** P < 0.001,  **** P < 0.003,
Fig. 18 SGOT of DLA tumour bearing mice treated with vincristine (0.5 mg/kg) with or without *B. racemosa* (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice / group

Values are not statistically significant from VCR control
Fig. 19  SGPT of DLA tumour bearing mie treated with vincristine (0.5 mg/kg) with or without *B. racemosa* (3 mg/kg)

Values are mean ± SD from 3 experiments using 12 mice / group

**** P < 0.003,  d P < 0.007
7.4 Discussion

Chemotherapy affects non cancerous cells to a certain extent. Most of the chemotherapeutic agents used against malignant disease interfere with cell production by damaging mitotic or cell compartments of the marrow or by slowing cell division (Wintrobe, 1976). Most of the toxic reactions in cancer therapy are manifested by the gastrointestinal epithelium and bone marrow (Evans et al. 1984). The concentration of the chemotherapeutic agents cannot be increased for optimum activity, since they produce adverse side effects. Several groups are working chemo protective agents, attempting to find a preferential protection of normal tissues during cancer treatment (Yuhas et al. 1980).

The main symptom in CYP therapy is urotoxicity, due to certain compounds produced by metabolism of CYP. TLC studies by Nair (1991) on urine samples of mice have shown the absence of certain compounds in the CYP and plant extract treated mice as compared to CYP control, thus the toxicity was overcome by giving the combined treatment. The B. racemosa seed extract was also found to reduce toxicity induced by CYP, another anticancer drug used in this study. The general health characters of B. racemosa seed extract and CYP treated group were better than CYP alone treated group. The combination treatment of CYP and B. r extract showed a remarkable reduction in the blood urea level in normal as well as tumour bearing animal indicating protection from the acute and chronic intrinsic renal failure. The combination treatment (CYP + B. r) also increased the life span of tumour bearing animal compared to CYP alone treated group. Reduced serum SGPT, SGOT and SAKP levels in B. racemosa treated groups in comparison to CYP treated group revealed protection by the active fraction against liver necrosis in normal as well as tumour bearing animals. SGOT
and SAKP are the most sensitive tests used to detect hepatic disease (Harpar 1973). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes of liver (Drotman and Lawhorm, 1978). Due to liver injury, there is disturbance in transport function of hepatocytes, resulting in leakage due to changed permeability of membranes. Similar results were obtained by Unnikrishnan et al (1990) using garlic extract has a chemoprotector with CYP.

The present study reveals for the first time the protective effects of *B.racemosa* seed extract against vincristine induced toxicity in normal and tumour bearing mice. Treatment with the *B.racemosa* seed extract partially prevented the decrease in body weight and significantly prolonged the life span of normal mice receiving vincristine. The elevated blood urea level after vincristine treatment was significantly reduced by co-administration of vincristine along with *B.racemosa* seed extract. However, the combined treatment of vincristine and B.r. seed extract also increased the life span of tumour bearing animal by 238% as compared to VCR alone treated group. The blood urea level was restored to near normal values by combination treatment with vincristine and B.r. seed extract. From this result it becomes clear that partial protection is afforded by combination treatment with vincristine and B.r. seed extract. *B.racemosa* seed extract itself has tumour reducing properties and therefore it helped to reduce the tumour burden, besides protecting the normal cells against chemotoxicity. Therefore, co-administration of *B.racemosa* seed extract with vincristine could considerably help to reduce its toxicity, thus serving as an adjuvant in vincristine therapy.

Vinca alkaloids have been incorporated in to combination chemotherapy protocols based not only on their lack of cross-resistance with the drugs that alkylate DNA but also on their different mechanism of action.
VCR has the added advantage that its limiting toxic effects peripheral neuropathy whereas VLB may cause additive myelosuppression with other myelosuppressive agents. The use of VCR and VLB in combination therapy extents beyond the spectrum of cancers for which definitive activity has been demonstrated. VCR is approved as a component of combination therapy for using Hodgkin’s lymphoma, non-Hodgkin’s lymphomas (including lymphocytes, mixed cell, histiocytic, undifferentiated, nodular and diffuse). Rhabdomyosarcoma of childhood neuroblestomas and Wilms tumour (nephroblestoma). VLB has a similar spectrum of activity for Hodgkin’s and non-Hodgkin’s lymphomas and has been used in advanced mycosis, fungoides, advanced testicular carcinoma, Kaposis sarcoma and histiocytosis (Houwen B et al 1981; Osterlind K et al 1982; Retsas et al 1979).