CHAPTER 6

RADIOPROTECTIVE AND CHEMOPROTECTIVE ACTION OF HELICANTHES ELASTICA
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Radiation therapy of cancer depends upon achieving a therapeutic differential between cancer cell cytotoxicity and normal tissue toxicity. The biological consequences of ionizing radiation treatments are expressed by the level of the damage produced and the ability of the cells to circumvent this damage. Chemotherapy along with surgery and radiation therapy is a major modality used in the treatment of cancer. A notable aspect of radiation therapy and cancer chemotherapeutic agents is the lack of tumour specificity, the drugs affect not only tumour tissue but normal tissue as well. The normal tissues affected in radiation as well as in chemotherapy are the gastrointestinal tract, hair follicles and bone marrow and the common side effects encountered are nausea and vomiting, diarrhoea, anorexia, alopecia and haematological problems like bone marrow suppression, leukopenia and anaemia (267). A wide variety of alkylating agents such as cyclophosphamide, nitrosourea, cisplatin etc. are used in cancer chemotherapy and the most extensively used alkylating agent in both experimental and human therapy is cyclophosphamide. CYP has been found to undergo metabolic activation by the liver microsomal P-450 mixed function oxidase system to 4-Hydroxycyclophosphamide which further gets oxidised to the active metabolites, phosphoramidine mustard and acrolein, both are highly cytotoxic (268, 269). The greatest drawback of CYP has been reported as its myelosuppressive potential (270). Compounds that could alleviate the toxicity of radiation and chemotherapy without reducing its activity will be highly beneficial in cancer therapy (271). Sulphydryl
compounds were among the first radioprotectors to be identified (272). Cysteammine, has been shown to protect animals from whole body radiation (273). 2-Mercaptopropionylglycine and WR-2721 have been shown to reduce the toxicity of radiation (274) as well as that of CYP (275, 276) and also have been shown selectively to protect the normal cells (271, 277). The use of immunostimulants such as Bacillus calmette guerin (BCG), Corynebacterium parvum, levamisole, pyran copolymers, isoprinosine (278) and cytokines (279) are often used along with radiation and chemotherapy to enhance the immunological status of the system. Several plant extracts such as Withania somnifera, Asparagus recemosus, Tinospora cordifolia, Codonopsis pilosula and Viscum album were found to enhance the immunity of mice treated with CYP (280, 281) and CYP plus radiation (203, 282). Since, the extract of Helicanthes elastica is nontoxic and possess cytotoxic antitumour and immunomodulatory activity as can be seen from the earlier chapters, investigations were carried out to probe in depth its chemoprotective and radioprotective effect using animal models.

6.1 MATERIALS AND METHODS

6.1.1 Plant Material

The extract of Helicanthes elastica purified by sphadex-G 50 and DE 52 ion exchanger as given in chapter 3a.1.3 was used for all experiments.

6.1.2 Chemotherapeutic agent

Cyclophosphamide (Endoxan) purchased from Khandelwal industries, Bombay, was the chemotherapeutic agent used.
6.1.3 Determination of radioprotective effect of the extract

Experiments were carried out using in-bred strains of female Swiss albino mice (8 weeks) weighing 25-28 g. They were divided into two groups - one group of mice were injected (ip) with the extract of *Helicanthes elastica* (15 µg/dose/animal) and other group received equal volume of PBS. One hour after injection, all the animals were exposed to gamma rays (400 rads) from a Co\(^{60}\) gamma source at the dose rate of 50 rads/minute. The administration of the drug was continued for ten alternate days. Blood was collected from the tail vein before irradiation and on every third day thereafter. The total WBC was counted using a haemocytometer (226) and haemoglobin was determined by cyanmethemoglobin (226) method. The body weight of the animals were monitored before irradiation and every third day thereafter. The bone marrow cellularity, pathological examination of the intestinal villi and the liver ALP and GPT (227, 228) of the treated and untreated mice were carried out on the 5th and 7th day of irradiation.

6.1.4 Determination of the chemoprotective effect of the extract against cyclophosphamide toxicity.

In-bred strains of female Swiss albino mice (8 weeks) weighing 25-28 g were used for the experiments. Animals were divided into two groups (10 animals/group). CYP at a level of 50 mg/kg body wt. being the chronic lethal dosage, was given to all the animals on 14 alternate days. To one group of animals, 14 doses of the extract (15 µg/dose/animal) was administered (ip) on alternate days. Weight of the animals were recorded before and every 3rd day after CYP treatment. Blood
was collected from the tail vein before and after every 3rd day of the experiment, and total WBC was counted using a haemocytometer (226), haemoglobin was determined by cyanmethemoglobin method (226) and ratio of polymorphonuclear cells was determined after staining with Leishman's stain. The days in which animals died of CYP toxicity in each group was noted and average life span was calculated.

6.2 RESULTS

6.2.1 Effect of the extract on radiation induced damage

6.2.1.1 Haematological parameters

As indicated in fig. 6.1., the total number of leukocytes dropped gradually from the day of irradiation and on 6th day it was 2000 crnm and 2,200 crnm for the untreated and treated groups respectively. Subsequently the leukocyte count of the treated group of animals was found to increase much faster when compared to the untreated irradiated mice and on 27th day, the value was 6800 crnm and 3600 crnm in the treated and untreated groups respectively. Haemoglobin level was decreased (fig. 6.2) in both groups after irradiation, however it was better stabilised in the treated group. On 27th day, the haemoglobin level was 9.9 and 11.5 g/dl in the untreated and treated group of mice, respectively.

6.2.1.2 Bone marrow cellularity

Data given in Table 6.1 indicate a remarkable increase in the number of bone marrow cells of the mice treated with the extract. On the 7th day of irradiation,
FIG. 6.1 Effect of partially purified extract of Helicanthes elastica on WBC count of irradiated mice (400 rads)
FIG. 6.2 Effect of partially purified extract of *Helicanthes elastica* on the haemoglobin level of irradiated mice (400 rads)
Table 6.1

Effect of partially purified extract of *Helicanthes elastica* on bone marrow cellularity of irradiated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bone marrow cells per femur (x 10^5)</th>
<th>Days after radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5th</td>
</tr>
<tr>
<td>Normal un-treated</td>
<td>15.17 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>Irradiated mice</td>
<td>10.78 ± 1.4</td>
<td>8.85 ± 0.49</td>
</tr>
<tr>
<td>Irradiated mice treated with extract</td>
<td>14.05 ± 0.75</td>
<td>12.79 ± 1.0</td>
</tr>
</tbody>
</table>

Values are average of 3 animals / group and expressed as number of cells / femur
the BM cellularity was $12.79 \times 10^6$ and $8.85 \times 10^6$ respectively in the treated and untreated group of animals.

### 6.2.1.3 Body Weight

There was a gradual decrease of body weight in mice treated with gamma rays from a Co$^{60}$ unit. When the animals were exposed to 400 rads, the untreated animals showed an average decrease of 4.9 g per animal on 16th day while the mean weight change of the treated group of animals was 2.8 g (fig. 6.3).

### 6.2.1.4 Liver enzymes

Table 6.2 illustrates the effect of the extract on the liver GPT and ALP values of the irradiated mice on 5th and 7th day respectively. On 5th day, the value of liver GPT of the irradiated control mice was 1416 units while that of the treated mice was 1250 units and this value had coincided with 1277 units of the liver GPT of normal mice. Similarly on 5th day the liver ALP values of the normal animals, irradiated control animals and irradiated treated animals were 1.22, 1.95 and 1.20 KA units respectively.

### 6.2.1.5 Pathology

The histological changes of intestine after irradiation are illustrated by Fig. 6.4 (a - d). During irradiation the intestinal villi continue to lose cells and progressively shrink and the shrinkage of villi is reflected in the loss of height. After 3 days of irradiation, the proximal part of the villus is covered by large misshapen cells and
FIG. 6.3 Effect of partially purified extract of *Helianthes elastica* on the body weight of irradiated mice (400 rads)
Table 6.2

Effect of the extract on liver GPT and ALP of the irradiated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GPT (U/mg protein)</th>
<th>ALP (KA units / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5th day</td>
<td>7th day</td>
</tr>
<tr>
<td>Normal un-treated</td>
<td>1277 ± 14.0</td>
<td>1277 ± 14.0</td>
</tr>
<tr>
<td>Irradiated mice</td>
<td>1416 ± 38.8</td>
<td>1694 ± 19.09</td>
</tr>
<tr>
<td>Treated irradiated mice</td>
<td>1250 ± 0\textsuperscript{a}</td>
<td>1375 ± 58.6\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Partially purified extract of *Helianthus elastica* (15 µg / dose / animal) was injected to the irradiated mice (400 rads).
Values are average of ten animals per group with standard deviation.
Significance from un-treated \( ^{a} \) \( P < 0.001 \)
Figure 6.4 Pathological studies on intestine of irradiated mice with and without treatment of the extract of *Helicanthes elastica* (a) normal (b) 5th day after irradiation (c) 7th day after irradiation (d) radiation protection with *Helicanthes elastica* extract.
the villus is considerably shrunken in size and finally collapsed into small mounds.

The radiation protection by the administration of *Helicanthes elastica* is illustrated in fig (6.4.d)

6.2.2 Effect of the extract on CYP induced toxicity

6.2.2.1 Haematological parameters

The effect of the extract on WBC count of CYP treated animals is shown in fig 6.5. Although there was an initial drop of WBC in both the groups, there was a subsequent enhancement in the total number of leukocytes in the treated group of animals. On 35th day after the completion of CYP treatment, the WBC count of the treated animals was enhanced significantly to 14,000 cmm while in the untreated animals it was only 5500 cmm. Similarly CYP treatment produced a gradual decline in the number of polymorphonuclear cells (fig 6.6) in both the treated and untreated animals. However, the withdrawal of CYP enhanced the proportion of polymorphonuclear cells in total WBC of the treated mice considerably. A decrease in the haemoglobin level of the treated and untreated mice was observed following the CYP administration (fig. 6.7). In the treated group of animals upon termination of CYP, on 31st day the haemoglobin level was 8.6 while in the untreated animals it was 7.8 g/100 ml.

6.2.2.2 Life span

Administration of the extract along with the CYP treatment considerably increased the life span of animals when compared with untreated animals (Table
**FIG. 6.5** Effect of partially purified extract of *Helicanthes elastica* on WBC count of animals treated with cyclophosphamide
FIG. 6.6 Effect of partially purified extract of *Helianthes elastica* on the polymorpho nuclear cells of animals treated with cyclophosphamide.
FIG. 6.7 Effect of partially purified extract of *Helicanthes elastica* on haemoglobin level of mice treated with cyclophosphamide
Table 6.3

Effect of partially purified extract of *Helicanthes elastica* on the survival of animals treated with cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of animals survived</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Un-treated CYP alone</td>
<td>100</td>
</tr>
<tr>
<td>Treatment + CYP</td>
<td>100</td>
</tr>
</tbody>
</table>

Ten animals / group were injected with cyclophosphamide (50 mg / kg body weight) on 14 alternate days.

The extract of *Helicanthes elastica* (15 μg / dose / animal) was injected on 14 alternate days.
6.3). On 27th day, only 40 per cent of animals were alive in the untreated group while 80 per cent were alive in the treated group.

6.2.2.3 Body Weight

There was a gradual decrease in the body weight of animals of both groups treated with CYP. However, the loss of weight was more pronounced in the untreated group. As indicated in Table 6.4, on 19th day, the control group of animals suffered an average reduction of 3 g in body weight while in the treated group, the average decrease was only 2.2 g.

6.3 DISCUSSION

The present result indicate the usefulness of the extract of Helicanthes elastica in reducing the cyclophosphamide and radiation induced toxicity in mice. The extract did not have any noticeable effect on radiation induced leukocyte depletion but provided a significant protection at a later period when the replenishment of leucocyte is expected for protection of the haematopoietic function. This is also reflected in the enhancement of bone marrow cellularity upon injection of the extract after irradiation. The repair mechanism induced by the extract is also evidenced by the pathological examination of the intestinal villai after irradiation. The enzymatic studies on liver GPT and ALP also revealed the extent of reduction of tissue injury on administration of the extract after irradiation. The results obtained for CYP treatment studies showed the chemoprotective effect of the extract. In the treated group upon completion of the CYP treatment, the total leukocyte count was enhanced rapidly indicating the regenerative capacity. The H.E. extract also increased the life span of the ani-
### Table 6.4

**Effect of partially purified extract of *Helicanthes elastica* on the body weight of animals treated with cyclophosphamide**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 day</th>
<th>3rd day</th>
<th>7th day</th>
<th>11th day</th>
<th>15th day</th>
<th>19th day</th>
<th>23rd day</th>
<th>27th day</th>
<th>31st day</th>
<th>35th day</th>
<th>39th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-treated</td>
<td>29.2 ± 3.1</td>
<td>28.8 ± 3.2</td>
<td>27.8 ± 5.7</td>
<td>27.08 ± 5.7</td>
<td>26.6 ± 3.2</td>
<td>26.2 ± 1.1</td>
<td>26.3 ± 1.0</td>
<td>26.8 ± 3.2</td>
<td>26.5 ± 3.5</td>
<td>26.3 ± 1.1</td>
<td>26.5 ± 2.2</td>
</tr>
<tr>
<td>CYP alone</td>
<td>30.4 ± 3.2</td>
<td>29.7 ± 4.2</td>
<td>28.8 ± 2.2</td>
<td>28.6 ± 1.1</td>
<td>28.4 ± 2.3</td>
<td>28.2 ± 3.2</td>
<td>28.5 ± 1.0</td>
<td>28.3 ± 1.2</td>
<td>28.5 ± 1.1</td>
<td>28.6 ± 1.2</td>
<td>28.5 ± 1.2</td>
</tr>
<tr>
<td>Treatment + CYP</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ten animals / group were injected with CYP (50 mg / kg) on alternate days.

The partially purified extract of *Helicanthes elastica* (15 µg/dose/animal) was injected on 14 alternate days.
mals. The increased production of leukocytes may be one of the mechanisms by which the extract protects the animals from the drastic haematological changes induced by the toxicity of CYP.

The H.E. extract has also been shown to protect the animals from weight loss due to the toxicity of radiation or CYP treatment and it could also restore the haemoglobin level in both treatments. The protection effected by radioprotectors in mice are either by a direct effect on the cellular targets of radiation or by altering the physiological functions that interfere with the effects of radiation such as vasoconstriction and hypoxia or by enhancing the recovery or repair of normal tissues (283).

Hematopoietic cytokines have been investigated as radioprotectors and chemoprotectors (279, 284). The mechanism of action of these compounds have been reported to be different from sulphhydryl compounds because they are believed to protect quickly by restoring hematopoietic function and shorten the duration of bone marrow depletion (284). IL-1, GM-CSF or cytokine inducers have been shown to protect mice from lethality of whole body radiation (279, 285) or CYP induced toxicity (284). Even though, cytokines are normal physiological mediators, they have been reported to have side effects (284). Hence, stimulations of cytokines by immunopotentiating non toxic extract of *Helicanthes elastica* can minimise the toxicity of radiation and chemotherapy. The mechanism of protection effected by the extract of *Helicanthes elastica* may be due to the stimulation of the immune system by the enhancement of stem cell proliferation and maturation of leukocytes. The extract has been shown to produce thymic proliferation and hence it can be expected to show an increase
in T-cell population as reported earlier (268). The extract of *Helicanthes elastica*

is nontoxic and is having cytotoxic, antitumour (Chapter 3) and immunodulatory
activity (Chapter 5). The present study indicates the usefulness of the extract as
a chemoprotector and radiation protector in cancer therapy.