RADIOPROTECTIVE ACTIVITY OF NATURALLY OCCURRING TERPENOIDS

CHAPTER 5

RADIOPROTECTIVE ACTIVITY OF NATURALLY OCCURRING TERPENOIDS
1. INTRODUCTION

Radiotherapy is one of the widely accepted therapeutical approaches for cancer. However whole body radiation with a dose of more than 100 rads cause acute effects including hematopoietic syndrome and gastro intestinal syndromes which involves nausea, vomiting, diarrhea, decreased count of various blood elements such as red blood cells granulocytes lymphocytes and platelets (Manuch et al., 1995). Unfortunately there is no specific drug at present, which can effectively reduce these effects. Development of effective and non toxic radio protectors that are capable of protecting normal tissues without compromising the anti-cancer activity of radiation is still an active area of research (Weiss, 1997).

In the last few decades several compounds that include certain chemicals such as amifostain (Tannehill et al., 1996) natural antioxidants like reduced glutathione (Bump and Brown, 1990), biological response modifiers like cytokine and immunostimulators (Neta, 1986) etc. were found to provide good radioprotection in experimental animal models. But the toxicity produced after repeated administration limited their clinical use.

Natural products are rich source of pharmacologically active compounds in which plant materials deserves an important position. Medicinal plants serve as good source of pharmacological-compounds and the plant based medicinal practices are effectively used in indigenous systems of medicine from time immemorial. Therefore there is an emerging interest during the last several years in plant products for their radioprotective efficacy. Compounds having antioxidant properties are known to provide protection against radiation and their oxidative stress (Agarwal 2002). Two flavonoids, orientin and vicenin, isolated from the leaves of the Indian plant Ocimum sanctum were tested for their radio
protective effect in mice (Uma Devi et al., 2000). Both compounds provided protection against death from gastrointestinal syndrome as well as bone marrow syndrome when injected intraperitoneally (i.p.) prior to whole-body exposure to 11 Gy gamma radiation (Uma Devi et al., 2000). Our recent studies show that commonly used medicinal plants and herbal preparation are good sources of radioprotectors as well as in patients receiving radiotherapy (Rekha et al., 2000, Praveenkumar et al., 1996).

In the present chapter an attempt to investigate the protective effect of some of the terpenoids against sub-lethal dose of gamma radiation-induced damage in mice is investigated.

2. MATERIALS AND METHODS

2.1 Animals

Swiss albino mice (4-6 weeks old male, 20-25 g body wt) and Balb/C mice (5-6 weeks) were used for this study.

2.2 Terpenoid compounds

Carvone, limonene, perilllic acid, ursolic acid, oleanolic acid glycyrrhizic acid, and nomilin

Drug administration: The compounds were suspended in light paraffin oil and intraperitoneally administrated at different concentrations (Carvone and limonene, administered at a concentration of 100 µmoles/kg body wt/dose/animal, perilllic acid, ursolic acid, oleanolic acid and glycyrrhizic were administered at a concentration of 50 µmoles/kg body wt/dose/animal and nomilin 10 µmoles/kg body wt/dose/animal.)
2.3 Radiation treatment and experimental design

Two sets of Swiss albino mice were taken and each set was divided into nine groups (10 mice/group). All animals were treated with a single sub lethal dose of radiation 600 rad (6 Gy). The source of radiation was a $^{60}$Co. Theratron phoniz teletherapy unit (Atomic energy Ltd Canada Ltd). Animals were restrained in specially designed well ventilated cages without anesthesia and exposed to whole body radiation at a rate of 1.40 Gy/min in a field size of 25X25cm$^2$ and at distance of 80 cm from the source. Group I, II, III, IV, V, VI, and VII were treated with 10 doses of carvone, limonene, perillic acid acid, oleanolic acid glycyrrhizic acid, and nomilin respectively. Group VIII was treated with 10 doses of paraffine oil (vehicle control) and group IX was kept as radiation treated control. Drugs were intraperitonially administrated from the same day of radiation and were continued for ten consecutive days for all the experiments. The first set of animals were used to examine hematological parameters and body weight and the second set of animals were used for analyzing intestinal toxicity and bonemarrow cellularity.

2.4 Determination of the effect of naturally occurring terpenoids on hematological parameters of mice after radiation

The set I animals were treated with the terpenoid compounds as explained above. Blood was collected from tail vein and parameters such as total WBC count, differential count, body weight and Hb content were recorded prior to the radiation exposure and continued every third day for 30 days.
2.5 Determination of effect of naturally occurring terpenoids on radiation induced gastrointestinal toxicity

After 48h, 7th day and 10th day of radiation treatment six animals from each group of set II were sacrificed by cervical dislocation. Liver was quickly excised and washed with ice-cold saline and used for the estimation of lipid peroxidation (Ohkawa et al., 1979) GPT (Bergmeyer et al., 1974) and GSH levels (Moronet al., 1979) (as explained in Chapter 2)

A portion of the small intestine was taken, washed with ice-cold saline and used for the biochemical analysis and histopathological examination. Intestinal mucosa was collected and used for the estimation of GSH content. Blood was collected for estimating ALP (Kind and King 1954), GPT (Bergmeyer et al., 1974) and lipid per oxidation levels (Ohkawa et al., 1979) (as explained in Chapter 2)

2.6 Determination of the effect of naturally occurring terpenoids on bone marrow cellularity and α-esterase activity

Bone marrow collected from the animals of the previous experiment was made to single cell suspension and cell number was determined using hemocytometer. The number of α-esterase positive cells were determined by azodye coupling method (Bancroff and Cook 1984). A smear of bone marrow cells from the above preparation was made on clean glass slide air dried stained with a naphthyl acetate and para rosiniline hydrochloride and counter stained with hematoxiline. The number of α-esterase positive cells were expressed out of 4000 cells (as explained in Chapter 2).
2.7 Histopathological studies of liver and intestine

The liver and intestinal tissue samples from the previous experiment were fixed in 10% formalin, dehydrated and embedded in paraffine wax. Sections (4μm) were stained with eosine and hematoxiline.

2.8 Determination of the effect of terpenoids on spleen colony formation in irradiated mice.

Inbred strains of Balb/C mice (4-5 weeks) were used for spleen colony assay. The animals were divided into 22 groups (6 animals/group). Group I to VII animals intraperitoneally treated with five doses of carvone, limonene, perilllic acid, ursolic acid, oleanolic acid glycyrrhizic acid, and nomilin on five consecutive days respectively. Group VIII kept as untreated. Animals were sacrificed 24h after the last dose and bonemarrow cells isolated and made into single cell suspension.

Groups IX to XXII animals were exposed to single whole body radiation (6Gy/animal as explained above). It is well known that exposure of mice bone marrow to 6 Gy of γ rays kills most of the hematopoietic cells (Praveen Kumar et al, 1996). Group IX to XVI received bone marrow cells (1x10^6 cells/animal) from normal mice (group VII) through caudal vein. Group X, XI, XII, XIII, XIV, XV and XVI animals received five doses of terpenoids carvone, limonene, perilllic acid, ursolic acid, oleanolic acid, glycyrrhizic acid and nomilin respectively. Group IX, which kept as untreated served as control. Group XVII, to XV animals received the bone marrow cells from terpenoid treated groups (Group I to VII) and the corresponding drugs were intraperitoneally administered for five consecutive days. Maximum numbers of spleen colonies are seen by 7-9 days (Robert, 1989). Hence all the animals were sacrificed on day 7 of radiation exposure and the
number of nodular colonies on the surface of spleen was counted. Each colony formed was derived from a single precursor stem cell designated as Colony Forming Unit Spleen (CFU-Spleen).

3 RESULTS

3.1 Effect of naturally occurring terpenoids on hematological parameters

The effect of naturally occurring terpenoids on total WBC count is shown in figure 5.1. The number of WBC in control animals maximally decreased to 1030 cells/cmm after 9th day of radiation treatment and gradually increased up to 4945 cells/cmm by 27 days. On 9th day the total WBC count in carvone, glycyrrhizic acid, oleanolic acid, perillic acid and limonene treated animals was 2970, 2588, 2200, 2188 and 2094 cells/cmm respectively and then these values increased steadily and attained 6381,6177,6100, 5098 and 6100 cells/cmm blood after 30 days. Other terpenoids also increase the total WBC. Where as in the radiation alone treated control group have only the total WBC count was only 4945-cells/cmm even after 30 days. There was no significant change in the differential count of terpenoid treated and untreated control animals. Administration of terpenoid compounds did not have a prominent effect on the Hb levels of radiation-exposed animals.

3.2 Effect of naturally occurring terpenoids on bone marrow cellularity and α-esterase activity in radiation treated mice.

The effect of naturally occurring terpenoid compounds on bone marrow cellularity and α-esterase positive cell is shown in figure 5.2 and 5.3. The number of bone marrow and α esterase positive cells was decreased drastically in the irradiated animals. Administration of the terpenoid compounds could enhance the bone marrow cellularity as
Fig 5.1 Effect of terpenoids on total WBC counts of radiation treated animals

Days after the radiation treatment

- Carvone
- Perillic acid
- Limonene
- Control

Days after the radiation treatment

- Nomilin
- Glycyrrhizic acid
- Ursolic acid
- Oleanolic acid
- Control
well as the number of α-esterase positive cells. In control animals after 48th hour of irradiation there was a drastic reduction in the number of bone marrow cells (4X10^6 cells/femur) and α-esterase positive cells (35.1 α-esterase positive cells /4000 bone marrow cells) compared to the normal animals. Even 10 days after radiation exposure the bone marrow cells (7X10^6 bone marrow cells/femur) and α-esterase positive cell number (161 /4000 bone marrow cells) did not reach back to the normal levels (17.9X10^6 bone marrow cells/femur and 1063 α-esterase positive cells/4000 bone marrow cells). Administration of terpenes could protect the animals from this drastic reduction in the number of bone marrow cells. In the case of carvone, limonene and perillic acid treated animals the bone marrow cellularity was 6.22 X 10^6 cells/femur, 6.76 X 10^6 cells/femur and 8.22 X10^6 cells/femur respectively after 48h of the radiation treatment. On 10th day carvone enhanced bone marrow cell numbers up to 14.6X10^6 cells/femur. By 10th day of radiation exposure carvone treatment could enhance the cell number (14.6X10^6 cells/femur) to normal level. Where as bone marrow cellularity of limonene and perillic acid treated animals on 10th day was 9.5X10^6 cells/femur and 11.8X10^6 cells/femur respectively.

3.3 Effect of naturally occurring terpenoids on gastrointestinal toxicity

The effect of terpenoids in the formation of lipid peroxides in serum and liver of irradiated mice is shown in table 5.1. The whole body radiation elevated the level of lipid peroxides in liver and serum. Administration of terpenoids clearly inhibited the production of lipid peroxides. The maximum inhibition of serum lipid peroxide production after 48h of radiation was obtained in the oleanolic acid and glycyrrhizic acid treated groups (1.8 and 1.9 n mol/ml). Where as after 7th day of radiation was obtained in the perillic acid and
Fig 5.2 Effect of terpenoids on bone marrow cellularity of mice after irradiation
Fig 5.3 Effect of terpenoids on esterase positive cells of mice after irradiation

- Carvone
- Limonene
- Perillic acid
- Glycyrrhizic acid
- Ursolic acid
- Oleanolic acid
- Nomilin
- Control
- Normal

estrase positive cells /4000 bone marrow cells

- 48h
- 7thDay
- 10thDay
All animals were treated with a single sub lethal dose of radiation 600 rad (6 Gy). Terpenoids were administrated intraperitoneally to various animal groups. After 48h, 7th day and 10th day of radiation treatment six animals from each group were sacrificed by cervical dislocation. Liver was quickly excised, blood collected by heat pucturing and serum seperated and used for the estimation of lipid peroxidation (*P<0.01 Compared with radiation alone treated control.)

<table>
<thead>
<tr>
<th></th>
<th>Serum lipid (nmol/mg protein)</th>
<th>Liver lipid (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48\textsuperscript{th} h</td>
<td>7\textsuperscript{th} Day</td>
</tr>
<tr>
<td>Normal</td>
<td>1.5±0.03</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.9±0.08</td>
<td>2.5±0.05</td>
</tr>
<tr>
<td>Carvone</td>
<td>2.4±0.01*</td>
<td>2.1±0.06*</td>
</tr>
<tr>
<td>Limonene</td>
<td>2.1±0.02*</td>
<td>2.0±0.06*</td>
</tr>
<tr>
<td>Perillic acid</td>
<td>2.4±0.09*</td>
<td>1.7±0.04*</td>
</tr>
<tr>
<td>Glycyrrhizic acid</td>
<td>1.9±0.08*</td>
<td>1.6±0.05*</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>2.1±0.05*</td>
<td>2.0±0.03*</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>1.8±0.09*</td>
<td>1.8±0.05*</td>
</tr>
<tr>
<td>Nomilin</td>
<td>2.3±0.09*</td>
<td>1.9±0.04*</td>
</tr>
</tbody>
</table>
glycyrrhizic acid treated groups (1.7 and 1.6 nmol /ml). 10\textsuperscript{th} after day of radiation treatment all the terpenoid treated groups tend to attain normal value (1.5 n mol /ml) where as radiation alone treated control group shown the peroxide production was 2.3 n mol /ml. Inhibition of liver lipid peroxide production was negligible after 48h of radiation exposure. But 7\textsuperscript{th} day after irradiation limonene treated groups were showed maximum inhibition (2.1 nmol/mg protein). On the 10\textsuperscript{th} day of irradiation limonene (1.6 nmol/mg protein) ursolic acid (1.7 nmol/mg protein), perillic acid (1.8 nmol/mg protein) and oleanolic acid 1.9 (nmol/mg protein) treated groups were shown the maximum inhibition. Where as in radiation alone treated groups the value was 2.9 nmol/mg protein.

The effect of terpenoids in the formation of alkaline phosphate levels (ALP) after radiation exposure is shown in table 5.2. Administration of various terpenoids lowered the elevated levels of ALP in radiation treated mice. After 48h serum ALP production in radiation alone treated animals was 23.9 KA units where as in carvone, limonene, perillic acid and nomilin treated mice it was only 8.2, 10.1, 11.1 and 10 KA units respectively. Glycyrrhizic acid and ursolic acid treated groups did not show any appreciable inhibition (20.7 and 22.8 respectively). On 10 day after irradiation all the terpenoid treated groups showed normal levels of ALP production (6.1 KA units). After 48h liver ALP production in radiation alone treated animals was enhanced to 17.7 KA units. The maximum inhibition in the production of liver ALP after 48h of radiation exposure was obtained in the case of ursolic acid treated groups (9.7 KA units) followed by limonene (11.5 KA units), glycyrrhizic acid (11.6 KA units), perillic acid (12.2 KA units), nomilin (12.5 KA units) and carvone (12.7 KA units) treated groups. After 10 day of irradiation all the
Table 5.2 Effect of terpenoids on the alkaline phosphatase levels of serum and liver of irradiated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum ALP (KA units)</th>
<th>Liver ALP (KA units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48&lt;sup&gt;th&lt;/sup&gt; h</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; Day 10&lt;sup&gt;th&lt;/sup&gt; Day</td>
</tr>
<tr>
<td>Normal</td>
<td>6.1±0.4</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>Control</td>
<td>23.9±1.0</td>
<td>14.4±0.7* 11.2±0.7* 17.7±0.8* 16.7±0.9* 13.3±0.5*</td>
</tr>
<tr>
<td>Carvone</td>
<td>8.2±0.1*</td>
<td>10±0.2* 8.1±0.8* 12.7±0.5* 8.2±0.5* 7.2±0.3*</td>
</tr>
<tr>
<td>Limonene</td>
<td>10.1±0.4*</td>
<td>8.2±0.5* 7±0.4* 11.5±0.7* 7.8±0.5* 6.8±0.5*</td>
</tr>
<tr>
<td>Perillic acid</td>
<td>11.1±0.5*</td>
<td>10±0.5* 9.4±0.5* 12.2±0.7* 7.6±0.6* 6.4±0.6*</td>
</tr>
<tr>
<td>Glycyrrhizic acid</td>
<td>20.7±0.8*</td>
<td>11.6±0.4* 7.1±0.3* 11.6±0.8* 7.4±0.3* 6.6±0.4*</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>22.8±0.7**</td>
<td>12±0.6* 6.2±0.4* 9.7±0.7* 7.5±0.3* 6.5±0.6*</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>18.6±0.8*</td>
<td>13.1±0.7* 6.4±0.4* 13.1±0.5* 6.7±1.0* 6.3±0.7*</td>
</tr>
<tr>
<td>Nomilin</td>
<td>10±0.6*</td>
<td>8.2±0.8* 7.5±0.7* 12.5±0.7* 7.5±0.9* 6.9±0.5*</td>
</tr>
</tbody>
</table>

All animals were treated with a single sub lethal dose of radiation 600 rad (6 Gy).
Terpenoids were administrated intraperitoneally to various animal groups. After 48h, 7th day and 10th day of radiation treatment six animals from each group were sacrificed by cervical dislocation. Liver was quickly excised, blood collected by heart pucturing and serum seperated and used for the estimation of alkaline phosphatase levels
(*P<0.01, **P<0.05 Compared with radiation alone treated control)
treated animal groups showed normal levels of ALP production (5.8 KA units) except radiation alone treated control group.

The effect of terpenoids on serum and liver GPT levels in irradiated mice is shown in Table 5.3. The whole body radiation elevated the GPT levels in liver and serum. The maximum inhibition in serum GPT levels, 48th h after irradiation was found to be obtained in nomilin (20.5 U/ml) treated animals followed by ursolic acid (21.2 U/ml), glycyrrhizic acid (21.4 U/ml), perillic acid (22.1 U/ml), oleanolic acid (22.3 U/ml) and limonene (23.9 U/ml). After 48th h of irradiation serum GPT levels of carvone treated group was 27 U/ml where as in radiation alone treated control group the value was 36.6 U/ml. 48th h after irradiation the liver GPT level was highly elevated in radiation alone treated animals (76.3 U/ml). Administrations of various terpenoids clearly reduced the elevated liver GPT levels. In glycyrrhizic acid and oleanolic acid treated groups liver GPT levels were only 54.2 U/ml and 55.2 U/ml respectively. Intraperitoneal administration of other terpenoids also reduced the elevated levels of GPT (Carvone (64.6 U/ml), limonene (68.2 U/ml) perillic acid (63.1 U/ml) ursolic acid (65.5 U/ml) and nomilin (58.3 U/ml)). On 10th day after irradiation radiation alone treated control animals alone have elevated levels of liver GPT (69.6 U/ml) where as terpenoid treated groups attained almost normal values (48.9 U/ml).

The change in the GSH levels in liver and intestine is shown in figure 5.4 and 5.5. Administration of terpenoids enhanced the GSH level in liver as well as intestine of radiation exposed animals. In control animals the levels in the intestine and liver were 5.5 and 5.8 n mol/mg protein respectively after 48h of radiation exposure. But in carvone treated animals the GSH levels were enhanced to 7.0 and 5.8 nmol/mg protein in intestine
Table 5.3 Effect of terpenoids on glutamate pyruvate transaminase (GPT) level in serum and liver of irradiated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum GPT (U/ml)</th>
<th>Liver GPT(U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48th h</td>
<td>7th Day</td>
</tr>
<tr>
<td>Normal</td>
<td>13.5±0.7</td>
<td>48.9±0.4</td>
</tr>
<tr>
<td>Control</td>
<td>36.6±1.4</td>
<td>36.4±0.5</td>
</tr>
<tr>
<td>Carvone</td>
<td>27±1.1*</td>
<td>28.9±0.9*</td>
</tr>
<tr>
<td>Limonene</td>
<td>23.9±1.2*</td>
<td>25.4±0-7*</td>
</tr>
<tr>
<td>Perillc acid</td>
<td>22.1±0.9*</td>
<td>23.1±0.5*</td>
</tr>
<tr>
<td>Glycyrhhizic acid</td>
<td>21.4±0.9*</td>
<td>17.2±1.1*</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>21.2±1.5*</td>
<td>21.5±1.5*</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>22.3±0.8*</td>
<td>22.5±0.7*</td>
</tr>
<tr>
<td>Nomilin</td>
<td>20.5±0.5*</td>
<td>20.8±0.9*</td>
</tr>
</tbody>
</table>

All animals were treated with a single sub lethal dose of radiation 600 rad (6 Gy). Terpenoids were administrated intraperitoneally to various animal groups. After 48h, 7th day and 10th day of radiation treatment six animals from each group were sacrificed by cervical dislocation. Liver was quickly excised, blood collected by heat pucturing and serum seperated and used for the estimation of glutamate pyruvate transaminase (GPT) levels

(*P<0.01 Compared with radiation alone treated control)
Fig 5.4 Effect of terpenoids in liver GSH production of mice after irradiation

- Carvone
- Limonene
- Perillic acid
- Glycyrrhizic acid
- Ursolic acid
- Oleanolic acid
- Nomolin
- Control
- Normal

GSH (μmol/mg protein)

48h 7thDay 10thDay
Fig 5.5 Effect of terpenoids in intestinal GSH production of mice after irradiation
and liver respectively. After 48h of radiation exposure liver GSH content of perillic acid and limonene treated animals were 6.6 and 5.8 nmol/mg respectively. On 10th day after radiation treatment the GSH values of terpenoid treated animals attained nearly equal to normal values (16.6 and 6.1 n mol /mg protein in intestine and liver respectively) where as in control animals the values were 5.6 and 7.1 n mol /mg protein in intestine and liver respectively.

3.4 Effect of naturally occurring terpenoids on the histopathology of liver and intestine of irradiated mice.

Histopathological studies show damage to the intestine and liver because of its susceptibility to radiation. A severe damage to intestinal villi and crypts could be seen in control animals. Control animals showed maximum damage to intestinal mucosa and maximum damage was seen after 48h of irradiation. The maximum liver toxicity was obtained in control animals after 48h of radiation treatment. The number of globlet and dead cells were also found to be increased in control animals. The increase in the number of globlet cells and dead cells was found to be much less in terpenoid treated group.

3.5 Effect of terpenoids on spleen colony formation in irradiated mice.

Effect of terpenoids compounds on spleen colony formation is shown in figure 5.7. On 7th day after the irradiation, untreated bonemarrow administrated mice have the least number of spleen colonies (the total number of spleen colonies were only 2.5). Animals which received the bonemarrow from terpenoid treated animals after whole body irradiation followed by terpenoid treatment showed maximum number of spleen colonies. Among these groups maximum spleen colonies were observed in perillic acid treated group (13.25 spleen colonies) followed by oleanolic acid (10.5 spleen colonies). The
5.6 Effect of terpenoids on spleen colony formation in irradiated mice

<table>
<thead>
<tr>
<th>Drug treated bone marrow alone</th>
<th>Non treated bone marrow + Drug</th>
<th>Drug treated bone marrow + Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Carvone</td>
<td>Limonene</td>
</tr>
<tr>
<td>Perillic acid</td>
<td>Ursolic acid</td>
<td>Oleanolic acid</td>
</tr>
<tr>
<td>Glycyrrhizinic acid</td>
<td></td>
<td>Nomilin</td>
</tr>
</tbody>
</table>
Figure 5.7 Effect of naturally occurring terpenoids on the histopathology of intestine of irradiated mice (48 h)

Figure 5.7a  Radiation alone treated control animals
Figure 5.7b  Carvone treated
Figure 5.7c  Limonene treated
Figure 5.7d  Perillic acid treated
Figure 5.7e  Ursolic acid treated
Figure 5.7f  Oleanolic acid treated
Figure 5.7g  Glycyrrhizic acid treated
Figure 5.7h  Nomilin treated
Figure 5.7i  Normal animal
Fig 5.7 Effect of naturally occurring terpenoids on the histopathology of intestine of irradiated mice.

Fig 5.7a

Fig 5.7b

Fig 5.7c

Fig 5.7d

Fig 5.7e

Fig 5.7f

Fig 5.7g

Fig 5.7h

Fig 5.7i
Figure 5.8 Effect of terpenoids on spleen nodule formation in irradiated mice

Figure 5.8a Control animals
Figure 5.8b Carvone treated
Figure 5.8c Limonene treated
Figure 5.8d Perillic acid treated
Figure 5.8e Ursolic acid treated
Figure 5.8f Oleanolic acid treated
Figure 5.8g Glycyrrhizic acid treated
Figure 5.8h Nomilin treated
Figure 5.8i Normal animal
Fig 5.8 Effect of terpenoids on spleen nodule formation in irradiated mice

Fig 5.8a

Fig 5.8b

Fig 5.8c

Fig 5.8d

Fig 5.8e

Fig 5.8f

Fig 5.8g

Fig 5.8h

Fig 5.8i
relative spleen weights also assessed. The maximum relative spleen weight was observed in animals which received the bonemarrow from oleanolic acid treated animals after whole body irradiation followed by oleanolic acid treatment (0.47g).

4 DISCUSSION

Ionizing radiation is toxic to organism since it induces deleterious structural changes in essential macromolecules (Bond et al., 1965; Jagetia et al., 2003). Radiation therapy mainly aims the proliferative cells. It also targets normal cells that are in mitotic phase in addition to highly proliferative tumour cells (Shaheen and Hassan, 1990). The main side effects of radiation therapy is due to the tissue damage is mainly by cell depletion of target renewal tissues. These effects depend on the balance between cell killing and compensatory replication of stem cell and proliferative cells.

Whole body radiation result in a reduction in total WBC count, bone marrow cellularity and α-esterase positive cell. Bone marrow serves as major source of hemopoetic stem cells. Enhanced number of bone marrow and α-esterase positive cells clearly indicate the effect of these compounds on stem cell proliferation in irradiated mice and its protective activity against radiation induced hemopoetic syndrome.

Ionizing radiation causes damage to living tissue through a series of molecular events depending on the radiation energy. Since human tissue contains 80% water the major radiation damage is due to the aqueous free radical generated by the action of radiation on water. These free radicals react with cellular macromolecule such as DNA, RNA, proteins membrane etc. and cause cell dysfunction and mortality (Moller and Wallin, 1998). Free radicals increase membrane lipid peroxidation, which in turn can alter
the integrity of membrane structure leading to inactivation of membrane, bound enzymes, loss of permeability of membrane and decrease in membrane fluidity. Whole body irradiation increased the level of lipid peroxidation in liver and serum. But administration of terpenoids could effectively inhibit the lipid peroxidation in irradiated mice.

The multiple physiological and metabolic functions of GSH include thiol transfer reactions that protect cell membrane and protein. GSH participate in reaction that destroy hydrogen peroxide, organic peroxide, free radicals and certain compounds (Rana et al., 2002). Administration of terpenoids increased the GSH content in intestine as well as liver in irradiated mice. Intestine is most susceptible to radiation leading to the damage of intestinal villii and crypts. Enhanced level of GSH could prevent the tissue damage in intestine. Higher GSH content in liver of terpenes treated mice could protect the tissue from radiation-induced damage. This observation was also confirmed by histopathological analysis of liver and small intestine. In irradiated mice treated with terpenoid compounds normal liver and intestinal architecture was observed

Serum alkaline phosphates levels also indicate tissue damage. Terpenoid administration showed to decrease the level of ALP levels in irradiated mice, which shows the protective activity of terpenes against radiation induced toxicity.

This study showed that terpenoids could protect damages produced after radiation exposure and also prevent the peroxide damage of cell membrane. These compounds are nontoxic nutritive ingredients present in various fruits and essential oils consumed by man. A detailed clinical study must be carried out to exploit the potential of terpenoids as good radio- protectors.