Section 6

Effect of treatment with herbal and natural products on LPO and antioxidant enzymes in rats exposed to gamma radiation
Ionizing radiation increases free radicals or reactive oxygen species (ROS) production and causes oxidative stress by compromising the antioxidant defence system. The ROS one of the main reprobate, the other one being RNS is capable of damaging several cellular components such as proteins, lipids and DNA (Koneru et al., 2011). There has been an upsurge of interest in the therapeutic potentials of herbal and natural products as radioprotector. The current research trends are directed towards finding naturally occurring radioprotectors, particularly of herbal and natural origin in reducing free radical induced tissue injury (Ganie et al., 2010a and b; Tuba and Gulcin, 2008). In order to reduce or protect from damaging effect of oxidative stress, cells have evolved an endogenous antioxidant defence mechanism, which includes non-enzymatic entities such as glutathione, ascorbic acid, uric acid and enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), etc. However, in the event of increased production of ROS, the host antioxidant defence mechanisms are overwhelmed, resulting in the oxidative damage of cellular constituents (Wiess and Kumar, 1998).

Considering the fact that oxidative damage is an important mechanism by which gamma irradiation induces cell damage, present study explored the possibility that antioxidant compounds in TCE, WSE, ARE and PE could protect and reduce the effects of gamma irradiation induced oxidative damage on liver and erythrocytes fractions. Therefore, in this study, radioprotection afforded by these herbal and natural was measured by the specific biomarkers of the oxidative stress such as LPO, alterations in activities of antioxidant enzymes i.e., SOD, CAT, GST and glutathione (GSH) content in liver tissues and erythrocytes of rats were studied. Histopathological studies of liver and intestine were also performed.

**Effect of gamma irradiation on LPO, SOD and CAT**

The results of whole body gamma irradiation exposure to rats on LPO and antioxidant enzymes has been presented in Table 8. The MDA level in liver and erythrocytes of gamma irradiated rats (Group 2) was found to be significantly higher (P < 0.001) than the control (Group 1). The results showed that the activities of SOD and CAT
Rats were pretreated intraperitoneally with TCE (80 mg/Kg body wt.), for 5 days prior to whole body gamma irradiation exposure at a dose of 2.0 Gy. Animals were sacrificed after 72 hr of irradiation. Values are mean ± SE of 6 animals.

Groups 2, 3 and 4 as compared to group 1. $^a$P < 0.05; $^c$P < 0.001.

Groups 3 and 4 as compared to group 2. $^y$P < 0.01; $^z$P < 0.001. NS Not significant.

1 = nmoles MDA formed/mg protein for liver and nmoles MDA formed/ml of blood for erythrocytes.

2 = units/mg protein.

3 = $\mu$moles H$_2$O$_2$ decomposed/min/mg protein.

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO$^1$ Liver</th>
<th>LPO$^1$ Erythrocytes</th>
<th>SOD$^2$ Liver</th>
<th>SOD$^2$ Erythrocytes</th>
<th>CAT$^3$ Liver</th>
<th>CAT$^3$ Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.459 ± 0.03</td>
<td>ND</td>
<td>8.51 ± 0.16</td>
<td>21.27 ± 1.08</td>
<td>155.32 ± 2.7</td>
<td>106.62 ± 9.3</td>
</tr>
<tr>
<td>2</td>
<td>1.090 ± 0.17$^c$</td>
<td>23.27 ± 3.4$^c$</td>
<td>5.56 ± 0.22$^c$</td>
<td>10.43 ± 1.09$^c$</td>
<td>97.40 ± 3.1$^c$</td>
<td>54.0 ± 4.2$^c$</td>
</tr>
<tr>
<td>3</td>
<td>0.362 ± 0.11$^z$</td>
<td>ND</td>
<td>9.07 ± 0.23$^z$</td>
<td>23.02 ± 0.32$^z$</td>
<td>170.70 ± 5.7$^{az}$</td>
<td>121.26 ± 5.7$^z$</td>
</tr>
<tr>
<td>4</td>
<td>0.619 ± 0.04$^{az}$</td>
<td>6.54 ± 1.5$^{cz}$</td>
<td>7.67 ± 0.42$^{az}$</td>
<td>17.53 ± 1.09$^{az}$</td>
<td>118.33 ± 3.0$^{cy}$</td>
<td>91.6 ± 6.0$^z$</td>
</tr>
</tbody>
</table>

Table 8. Effect of TCE pretreatment on LPO and antioxidant enzymes in liver and erythrocytes of rats.
in liver and erythrocytes decreased significantly in gamma irradiated rats (Group 2) as compared to control (Group 1).

**Effect of gamma irradiation on GSH and GST activity**

The effect of gamma irradiation on GSH content and GST activity is presented in Table 9. There was no significant change in liver GSH content of gamma irradiated groups (Group 2) as compared to control (Group 1). However, erythrocytes showed significant increase in GSH content of gamma irradiated rats (Group 2) as compared to control (Group 1). A highly significant (P < 0.001) decrease in liver and erythrocytes GST activities was observed in gamma irradiated rats (Group 2) as compared to control (Group 1).

**Effect of gamma irradiation on histology of rat liver and intestine**

The histopathological analysis of rat liver and intestine was performed to examine the effect of gamma irradiation on cellular architecture of liver and intestine. The control (Group 1) showed normal cellular characteristics of liver i.e., normal parenchyma, unremarkable hepatocytes, canaliculi, canals of Hering, sinusoids, sinusoidal lining cells, endothelial cells, kupffer cells, pit cells, space of Disse, stellate cells and reticulin fibrils (Fig. 44 A). The liver histology of the rats exposed with gamma irradiation at a dose of 2.0 Gy (Group 2), revealed degenerative changes such as feathering of tissue, subintimal edema, haemorrhage involving small hepatic veins, patchy sinusoidal congestion and focal areas of necrosis (Fig. 44 B).

Histopathological analysis of small jejunal portion of the intestine was also performed on the animals exposed to 10.0 Gy of whole body gamma irradiations. The control animals (Group 1) showed normal architecture of intestinal linings i.e., normal crypts, clear ciliary border, villi of normal height and normal filial structure (Fig. 44 C) while, gamma irradiated animals (Group 2) showed severe damage to crypts, fusion of villi, blunting of edges, lumen widening, shrunken mucosa, shorter villi dead cells and damaged ciliary border (Fig. 44 D). Histopathological studies showed damage to the intestine because of its susceptibility to the lethal radiation dose.
Table 9. Effect of TCE pretreatment on GSH content and GST activity in liver and erythrocytes of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH$^1$</th>
<th>GST$^2$</th>
<th>GSH$^1$</th>
<th>GST$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Erythrocytes</td>
<td>Liver</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>1</td>
<td>0.031 ± 0.04</td>
<td>2.77 ± 1.2</td>
<td>0.762 ± 0.02</td>
<td>0.260 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.033 ± 0.05$^{NS}$</td>
<td>3.81 ± 0.6$^c$</td>
<td>0.276 ± 0.01$^c$</td>
<td>0.126 ± 0.10$^c$</td>
</tr>
<tr>
<td>3</td>
<td>0.031 ± 0.02$^{NS}$</td>
<td>2.84 ± 0.2$x$</td>
<td>0.820 ± 0.11$^z$</td>
<td>0.284 ± 0.03$^z$</td>
</tr>
<tr>
<td>4</td>
<td>0.031 ± 0.01$^{NS}$</td>
<td>3.47 ± 0.5$^b$</td>
<td>0.552 ± 0.04$^{cz}$</td>
<td>0.088 ± 0.05$^c$</td>
</tr>
</tbody>
</table>

Experimental details were as shown in Table 8.
Values are mean ± SE of 6 animals.
Groups 2, 3 and 4 as compared to group 1. $^b$P < 0.01; $^c$P < 0.001.
Groups 3 and 4 as compared to group 2. $^x$P < 0.05; $^z$P < 0.001. $^{NS}$Not significant.
1 = µmoles DTNB conjugated/mg protein for liver and µmoles DTNB conjugated /ml of blood for erythrocytes. 2 = µmoles GSH conjugated/min/mg protein.
Fig. 44 A. H and E stained section of liver from control rats.

Fig. 44 B. H and E stained section of liver showing degenerative changes in architecture such as subintimal edema, haemorrhage involving small hepatic veins, patchy sinusoidal congestion and focal areas of necrosis in rat exposed with 2.0 Gy of whole body gamma irradiation.
Fig. 44 C. H and E stained section of intestine from control rats.

Fig. 44 D. H and E stained section of intestine showing degenerative changes in architecture, damage to crypts, fusion of villi, blunting of edges, lumen widening, shrunken mucosa, shorter villi dead cells and damaged ciliary border in rat exposed with 10.0 Gy of whole body gamma irradiation.
**Effect of TCE pretreatment on LPO and antioxidant enzymes**

The effect of TCE pretreatment on LPO and antioxidant enzymes in gamma irradiated rats has been presented in Table 8. The pretreatment with TCE followed by gamma irradiation exposure (Group 4) showed significant increase in liver and erythrocytes LPO as compared to control (Group 1). However, a significant (P < 0.001) decrease in liver and erythrocytes LPO was observed in rats pretreated with TCE prior to gamma irradiation exposure (Groups 4) as compared to gamma irradiated rats (Group 2).

TCE pretreatment (Group 3) showed no significant change in liver and erythrocytes SOD activity as compared to control (Group 1). TCE pretreatment prior to gamma irradiation (Group 4) showed significant decrease in liver and erythrocytes SOD activity as compared to control (Group 1), while a significant increase was observed as compared to gamma irradiated rats (Group 2).

Liver CAT activity was found to be increased with TCE pretreatment without gamma irradiation (Group 3) as compared to control (Group 1) and gamma irradiated rats (Group 2) (Table 8). TCE pretreatment with gamma irradiation (Group 4) showed decrease in liver CAT activity as compared to control (Group 1), however the liver CAT activity increased significantly as compared to gamma irradiated rats (Group 2). The results showed a significant increase in erythrocytes CAT activity in rats pretreated with TCE prior to gamma irradiation exposure (Group 4) as compared to gamma irradiated rats (Group 2).

**Effect of TCE pretreatment on GSH and GST activity**

TCE pretreatment without or with gamma irradiation exposure (Groups 3 and 4) showed no significant change in GSH content of liver as compared to control or gamma irradiated rats (Groups 1 and 2) (Table 9). The erythrocytes GSH content in TCE pretreated rats without gamma irradiation exposure (Group 3) decreased as compared to gamma irradiated rats (Group 2) and was similar to control (Group 1). However, GSH content in erythrocytes of TCE pretreated rats prior to gamma irradiation (Group 4) showed no significant change as compared to gamma irradiated rats (Group 2).
Liver and erythrocytes GST activity in TCE pretreated rats (Group 3) showed no significant change as compared to control (Group 1), while it increased significantly (P < 0.001) as compared to gamma irradiated rats (Group 2) (Table 9). TCE pretreatment prior to gamma irradiation (Group 4) showed decreased liver GST activity as compared to control (Group 1), while it was higher than gamma irradiated rats (Group 2). TCE pretreatment (Group 3) showed higher erythrocytes GST activity as compared to gamma irradiated rats (Group 2). Erythrocytes GST activity in TCE pretreated rats prior to gamma irradiation exposure (Group 4) was significantly decreased as compared to control (Group 1) and gamma irradiated rats (Group 2).

**Effect of TCE on histology of rat liver and intestine**

The liver histology of rats administered TCE (80 mg/kg body wt.) for 5 days without or with gamma irradiation (Groups 3 and 4) showed normal cellular architecture at a dose of 2.0 Gy (Figure 45 A and 45 B) in comparison with the extent of liver damage revealed in gamma irradiated rats (Group 2).

Intestinal histology revealed that rats pretreated with TCE prior without or with gamma irradiation gamma irradiation (10.0 Gy) (Groups 3 and 4) showed less damage to crypts and cellular structure as compared to gamma irradiated (Group 2) (Figure 45 C and 45 D).

**Effect of WSE pretreatment on LPO and antioxidant enzymes**

The effect of WSE pretreatment on LPO and antioxidant enzymes in liver and erythrocytes of gamma irradiated rats is presented in Table 10. Pretreatment with WSE without gamma irradiation exposure (Groups 3) showed decrease in liver LPO while no change in LPO of erythrocytes was observed as compared to control (Group 1). Rats pretreated with WSE followed by gamma irradiation (Group 4) showed no significant change in liver LPO, while increase in erythrocytes LPO was observed as compared to control (Group 1). However, in rats pretreated with WSE without or with gamma irradiation exposure (Groups 3 and 4) liver and erythrocytes LPO decreased significantly (P < 0.001) as compared to gamma irradiated rats (Group 2).
Fig. 45 A. H and E stained section of liver showing normal cellular structure in rats treated with TCE (80 mg/kg body wt).

Fig. 45 B. H and E stained section of liver showing reduced effect of radiation in rats pretreated with TCE (80 mg/kg body wt) prior to whole body gamma irradiation exposure at a dose of 2.0 Gy.
Fig. 45 C. H and E stained section of intestine showing normal cellular structure in rats administered TCE (80 mg/kg body wt).

Fig. 45 D. H and E stained section of intestine showing reduced effect of radiation in rats pretreated with TCE (80 mg/kg body wt) and prior to whole body gamma irradiation exposure at a dose of 10.0 Gy.
Table 10. Effect of WSE pretreatment on LPO and antioxidant enzymes in liver and erythrocytes of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO$^1$ (Liver)</th>
<th>LPO$^1$ (Erythrocytes)</th>
<th>SOD$^2$ (Liver)</th>
<th>SOD$^2$ (Erythrocytes)</th>
<th>CAT$^3$ (Liver)</th>
<th>CAT$^3$ (Erythrocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.448 ± 0.07</td>
<td>ND</td>
<td>9.01 ± 0.20</td>
<td>22.92 ± 1.80</td>
<td>164.40 ± 2.5</td>
<td>112.42 ± 3.5</td>
</tr>
<tr>
<td>2</td>
<td>1.083 ± 0.20$^c$</td>
<td>25.90 ± 1.8$^c$</td>
<td>5.78 ± 0.13$^c$</td>
<td>11.22 ± 0.80$^c$</td>
<td>102.48 ± 2.2$^c$</td>
<td>60.02 ± 3.6$^c$</td>
</tr>
<tr>
<td>3</td>
<td>0.256 ± 0.05$^{az}$</td>
<td>ND</td>
<td>8.95 ± 0.22$^x$</td>
<td>23.70 ± 1.50$^z$</td>
<td>181.82 ± 8.3$^z$</td>
<td>168.96 ± 5.0$^{cz}$</td>
</tr>
<tr>
<td>4</td>
<td>0.552 ± 0.12$^z$</td>
<td>5.68 ± 0.9$^{cz}$</td>
<td>7.50 ± 0.18$^{hz}$</td>
<td>16.33 ± 0.85$^{az}$</td>
<td>129.90 ± 6.9$^x$</td>
<td>82.84 ± 4.9$^{ay}$</td>
</tr>
</tbody>
</table>

Rats were pretreated intraperitonially with WSE (20 mg/Kg body wt.), for 5 days prior to whole body gamma irradiation exposure at a dose of 2.0 Gy. Animals were sacrificed after 72 hr of irradiation. Values are mean ± SE of 6 animals.

Groups 2, 3 and 4 as compared to group 1. $^a$P < 0.05; $^b$P < 0.01; $^c$P < 0.001.

Groups 3 and 4 as compared to group 2. $^x$P < 0.05; $^y$P < 0.01; $^z$P < 0.001.

1 = nmoles MDA formed/mg protein for liver and nmoles MDA formed/ml of blood for erythrocytes.
2 = units/mg protein.
3 = μmoles H$_2$O$_2$ decomposed/min/mg protein.
WSE pretreated animals without gamma irradiation (Groups 3) showed no significant change in liver and erythrocytes SOD activity and was similar to that of control (Group 1) (Table 10). Pretreatment with WSE prior to gamma irradiation (Groups 4) showed decreased SOD activity in liver and erythrocytes as compared to control (Group 1), while it was higher than gamma irradiated rats (Group 2).

Liver CAT activity in rats pretreated with WSE without gamma irradiation (Groups 3) was statistically similar to control (Group 1) but significantly higher than gamma irradiated group (Group 2) (Table 10). However, WSE pretreatment (Group 3) significantly enhance the erythrocytes CAT activity as compared to control (Group 1). Liver CAT activity in WSE pretreated rats prior to gamma irradiation (Groups 4) was decreased as compared to control (Group 1) but it was significantly higher (P < 0.05) than gamma irradiated rats (Group 2). Results showed that erythrocytes CAT activity in WSE pretreatment prior to gamma irradiation exposure (Group 4) was significantly increased as compared to gamma irradiated rats (Group 2).

**Effect of WSE on GSH and GST activity of liver and erythrocytes**

WSE pretreatment without gamma irradiation exposure (Groups 3) showed significant decrease in liver GSH content as compared to control and gamma irradiated rats (Groups 1 and 2) (Table 11), while WSE pretreatment prior to gamma irradiation exposure (Group 4) showed significant increase in liver GSH content as compared to control and gamma irradiated rats (Groups 1 and 2). However, erythrocytes GSH content in WSE pretreated without or with gamma irradiation exposure (Groups 3 and 4) increased as compared to control (Group 1) and was not significantly altered as compared to gamma irradiated rats (Group 2).

WSE pretreatment without gamma irradiation (Group 3) showed no significant change in liver GST activity as compared to control (Group 1) but was significantly higher (P < 0.001) than gamma irradiated rats (Group 2) (Table 11). WSE pretreatment prior to gamma irradiation (Group 4) showed decreased liver GST activity as compared to control (Group 1), while it was significantly higher (P < 0.001) than gamma irradiated rats (Group 2). Erythrocytes GST activity significantly decreased in rats pretreated with WSE without
Table 11. Effect of WSE pretreatment on GSH content and GST activity in liver and erythrocytes of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH $^1$</th>
<th>GST $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>1</td>
<td>0.032 ± 0.01</td>
<td>2.62 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.033 ± 0.02 $^{NS}$</td>
<td>3.53 ± 0.5$^c$</td>
</tr>
<tr>
<td>3</td>
<td>0.028 ± 0.03$^{ax}$</td>
<td>3.06 ± 0.8$^a$</td>
</tr>
<tr>
<td>4</td>
<td>0.037 ± 0.02$^{bx}$</td>
<td>3.39 ± 0.4$^b$</td>
</tr>
</tbody>
</table>

Experimental details were as shown in Table 10.
Values are mean ± SE of 6 animals.
Groups 2, 3 and 4 as compared to group 1. $^a$ P < 0.05; $^b$ P < 0.01; $^c$ P < 0.001.
Groups 3 and 4 as compared to group 2. $^x$ P < 0.05; $^z$ P < 0.001. $^{NS}$ Not significant.
1 = µmoles DTNB conjugated/mg protein for liver and µmoles DTNB conjugated/ml of blood for erythrocytes.
2 = µmoles GSH conjugated/min/mg protein.
or with gamma irradiation exposure (Group 3 and 4) as compared to control (Group 1) and was statistically similar to gamma irradiated rats (Group 2).

**Effect of WSE on histology of rat liver and intestine**

The histology of liver of rats administered WSE (20 mg/kg body wt.) for 5 days without or with gamma irradiation showed normal cellular architecture (Fig. 46 A and 46 B) in comparison with the extent of damage revealed in gamma irradiated rats (Group 2).

Intestinal histology revealed that rats pretreated with WSE without or with gamma irradiation gamma irradiation at a dose of 10.0 Gy (Groups 3 and 4) showed less damage to crypts and cellular structure as compared to gamma irradiated rats (Group 2) (Fig. 46 C and 46 D).

**Effect of ARE pretreatment on LPO and antioxidant enzymes**

The effect of ARE pretreatment on LPO and antioxidant enzymes in gamma irradiated rats has been presented in Table 12. Pretreatment with ARE without gamma irradiation exposure (Groups 3) showed decreased liver LPO as compared to control and gamma irradiated rats (Group 1 and 2). Rats pretreated with ARE followed by gamma irradiation (Group 4) showed significant increase in liver and erythrocytes LPO as compared to control (Group 1). However, pretreatment with ARE with gamma irradiation exposure (Groups 4) showed significant decrease in erythrocytes LPO as compared to gamma irradiated rats (Group 2).

ARE pretreated animals without gamma irradiation (Groups 3) showed no significant change in liver and erythrocytes SOD activity as compared to control (Group 1) but was higher than gamma irradiated rats (Group 2). Pretreatment with ARE prior to gamma irradiation (Groups 4) showed decreased SOD activity in both liver and erythrocytes as compared to control (Group 1), while a significant increase was found as compared to gamma irradiated rats (Group 2).

Liver and erythrocytes CAT activity in rats pretreated with ARE without gamma irradiation (Groups 3) was similar to control (Group 1), while it was significantly higher than gamma irradiated rats (Group 2). ARE pretreatment prior to gamma irradiation
Fig. 46 A. H and E stained section of liver showing normal cellular structure in rats treated with WSE (20 mg/kg body wt).

Fig. 46 B. H and E stained section of liver showing reduced effect of radiation in rats pretreated with WSE (20 mg/kg body wt) prior to whole body gamma irradiation exposure at a dose of 2.0 Gy.
Fig. 46 C. H and E stained section of intestine showing normal cellular structure in rats administered WSE (20 mg/kg body wt).

Fig. 46 D. H and E stained section of intestine showing reduced effect of radiation in rats pretreated with WSE (20 mg/kg body wt) and prior to whole body gamma irradiation exposure at a dose of 10.0 Gy.
Table 12. Effect of ARE pretreatment on LPO and antioxidant enzymes in liver and erythrocytes of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO(^1) Liver</th>
<th>LPO(^1) Erythrocytes</th>
<th>SOD(^2) Liver</th>
<th>SOD(^2) Erythrocytes</th>
<th>CAT(^3) Liver</th>
<th>CAT(^3) Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.463 ± 0.03</td>
<td>ND</td>
<td>8.12 ± 0.24</td>
<td>21.89 ± 1.60</td>
<td>159.26 ± 2.2</td>
<td>109.31 ± 2.8</td>
</tr>
<tr>
<td>2</td>
<td>1.094 ± 0.12(^c)</td>
<td>22.38 ± 2.1(^c)</td>
<td>5.29 ± 0.31(^c)</td>
<td>10.78 ± 1.13(^c)</td>
<td>103.04 ± 3.3(^c)</td>
<td>56.69 ± 1.4(^c)</td>
</tr>
<tr>
<td>3</td>
<td>0.365 ± 0.05(^ay)</td>
<td>ND</td>
<td>8.27 ± 0.32(^z)</td>
<td>22.65 ± 0.62(^z)</td>
<td>161.57 ± 7.3(^z)</td>
<td>110.60 ± 3.6(^z)</td>
</tr>
<tr>
<td>4</td>
<td>0.724 ± 0.04(^ax)</td>
<td>14.75 ± 0.8(^cy)</td>
<td>7.16 ± 0.19(^ay)</td>
<td>17.45 ± 1.18(^ay)</td>
<td>101.16 ± 2.6(^c)</td>
<td>76.49 ± 5.0(^bz)</td>
</tr>
</tbody>
</table>

Rats were pretreated intraperitonially with ARE (50 mg/Kg body wt.), for 5 days prior to whole body gamma irradiation exposure at a dose of 2.0 Gy. Animals were sacrificed after 72 hr of irradiation.

Values are mean ± SE of 6 animals.

Groups 2, 3 and 4 as compared to group 1. \(^a\) P < 0.05; \(^b\) P < 0.01; \(^c\) P < 0.001.

Groups 3 and 4 as compared to group 2. \(^x\) P < 0.05; \(^y\) P < 0.01; \(^z\) P < 0.001. \(^{NS}\) Not significant.

1 = nmol MDA formed/mg protein for liver and nmol MDA formed/ ml of blood for erythrocytes.

2 = units/mg protein.

3 = µmol H\(_2\)O\(_2\) decomposed/min/mg protein.
(Groups 4) showed significant decrease in liver CAT activity as compared to control (Group 1) but was similar to irradiated rats (Group 2). ARE pretreatment prior to gamma irradiation (Group 4) showed increased erythrocytes CAT activity as compared to gamma irradiated rats (Group 2), while it was reduced as compared to control (Group 1).

**Effect of ARE pretreatment on GSH and GST activity**

ARE pretreatment without or with gamma irradiation exposure (Groups 3 and 4) showed no significant change in GSH content of liver as compared to control or gamma irradiated rats (Groups 1 and 2) (Table 13). The erythrocytes GSH content in ARE pretreated rats (Group 3) decreased as compared to gamma irradiated rats (Group 2) but was similar to control (Group 1). However, erythrocytes GSH content in ARE pretreated rats prior to gamma irradiation (Group 4) showed no significant change as compared to gamma irradiated rats (Group 2).

Liver and erythrocytes GST activity in ARE pretreated rats (Group 3) increased significantly (P < 0.001) as compared to gamma irradiated rats (Group 2), while it was statistically similar to control (Group 1) (Table 13). ARE pretreatment with gamma irradiation (Group 4) showed higher liver GST activity as compared to gamma irradiated rats (Group 2) and was less than control (Group 1). No significant change in erythrocytes GST activity was observed in ARE pretreatment prior to gamma irradiation (Group 4) as compared to gamma irradiated rats (Group 2).

**Effect of ARE pretreatment on histology of rat liver and intestine**

The histology of liver of rats administered ARE (50 mg/kg body wt.) for 5 days without or with gamma irradiation (Group 3 and 4) showed normal cellular architecture (Fig. 47 A and 47 B) and revealed recovery of damaged tissue to a certain extent as compared to gamma irradiated rats at 2.0 Gy (Group 2).

Rats pretreated with ARE without or with gamma irradiation (Groups 3 and 4) showed normal cellular architecture of intestine (Fig. 47 C and 47 D) as compared to the damage revealed by gamma irradiated tissue at 10.0 Gy (Group 2).
<table>
<thead>
<tr>
<th>Group</th>
<th>GSH&lt;sup&gt;1&lt;/sup&gt;</th>
<th>GST&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>1</td>
<td>0.031 ± 0.02</td>
<td>2.83 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.032 ± 0.01&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.69 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.030 ± 0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.90 ± 1.3&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.032 ± 0.01&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.61 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Experimental details were as shown in Table 12. Values are mean ± SE of 6 animals.

Groups 2, 3 and 4 as compared to group 1. <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.001.

Groups 3 and 4 as compared to group 2. <sup>x</sup>P < 0.05; <sup>y</sup>P < 0.01; <sup>z</sup>P < 0.001. <sup>NS</sup> Not significant.

1 = μmoles DTNB conjugated/mg protein for liver and μmoles DTNB conjugated /ml of blood for erythrocytes.
2 = μmoles GSH conjugated/min/mg protein.
Fig. 47 A. H and E stained section of liver showing normal cellular structure in rats treated with ARE (50 mg/kg body wt).

Fig. 47 B. H and E stained section of liver showing reduced effect of radiation in rats pretreated with ARE (50 mg/kg body wt) and prior to whole body gamma irradiation exposure at a dose of 2.0 Gy.
Fig. 47 C. H and E stained section of intestine showing normal cellular structure in rats administered ARE (50 mg/kg body wt).

Fig. 47 D. H and E stained section of intestine showing reduced effect of radiation in rats pretreated with ARE (50 mg/kg body wt) and prior to whole body gamma irradiation exposure at a dose of 10.0 Gy.
**Effect of PE pretreatment on LPO and antioxidant enzymes**

The effect of PE pretreatment on LPO and antioxidant enzymes in liver and erythrocytes of gamma irradiated rats are presented in Table 14. Pretreatment with PE without gamma irradiation exposure (Groups 3) showed decreased liver LPO as compared to control (Group 1). Rats pretreated with PE prior to gamma irradiation (Group 4) showed significant increase in liver and erythrocytes LPO as compared to control (Group 1). However, pretreatment with PE without or with gamma irradiation exposure (Groups 3 and 4) showed significant decrease in liver and erythrocytes LPO as compared to gamma irradiated rats (Group 2).

PE pretreated animals without gamma irradiation (Groups 3) showed no significant change in liver and erythrocytes SOD activity as compared to control (Group 1) (Table 14). Pretreatment with PE prior to gamma irradiation (Groups 4) showed decreased SOD activity in both liver and erythrocytes as compared to control (Group 1), while it was significantly higher than gamma irradiated rats (Group 2).

Liver CAT activity was found to be increased with PE pretreatment without gamma irradiation (Group 3) as compared to control (Group 1) and gamma irradiated rats (Group 2). The results showed that PE pretreatment prior to gamma irradiation exposure (Group 4) showed decrease in liver and erythrocyte CAT activity as compared to control (Group 1), but was significantly higher than gamma irradiated rats (Group 2).

**Effect of PE pretreatment on GSH and GST activity**

PE pretreatment without or with gamma irradiation exposure (Groups 3 and 4) showed no significant change in liver GSH content as compared to control or gamma irradiated rats (Groups 1 and 2) (Table 15). Erythrocytes GSH content in PE pretreated rats (Group 3) was decreased as compared to gamma irradiated (Group 2) and was statistically similar to that of control (Group 1). PE pretreated with gamma irradiation (Group 4) showed higher erythrocytes GSH content as compared to control (Group 1) but was not significantly altered as compared to gamma irradiated rats (Group 2).
Table 14. Effect of PE pretreatment on LPO and antioxidant enzymes in liver and erythrocytes of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (^1)</th>
<th>SOD (^2)</th>
<th>CAT (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Erythrocytes</td>
<td>Liver</td>
</tr>
<tr>
<td>1</td>
<td>0.440 ± 0.05</td>
<td>ND</td>
<td>8.42 ± 0.10</td>
</tr>
<tr>
<td>2</td>
<td>1.078 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.07 ± 3.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.62 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.221 ± 0.05&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>ND</td>
<td>8.72 ± 0.20&lt;sup&gt;z&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.637 ± 0.04&lt;sup&gt;az&lt;/sup&gt;</td>
<td>8.27 ± 0.72&lt;sup&gt;cz&lt;/sup&gt;</td>
<td>7.54 ± 0.20&lt;sup&gt;az&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Rats were pretreated intraperitonially with PE (100 mg/Kg body wt.), for 5 days prior to whole body gamma irradiation exposure at a dose of 2.0 Gy. Animals were sacrificed after 72 hr of irradiation.

Values are mean ± SE of 6 animals.

Groups 2, 3 and 4 as compared to group 1. \(^aP < 0.05; ^bP < 0.01; ^cP < 0.001.\)

Groups 3 and 4 as compared to group 2. \(^azP < 0.05; ^bzP < 0.001.\)

1 = nmoles MDA formed/mg protein for liver and nmoles MDA formed/ml of blood for erythrocytes.
2 = units/mg protein.
3 = µmoles H\(_2\)O\(_2\) decomposed/min/mg protein.
Table 15. Effect of Propolis pretreatment on GSH content and GST activity in liver and erythrocytes of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH$^1$ (µmol/mg protein)</th>
<th>GST$^2$ (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>1</td>
<td>0.031 ± 0.01</td>
<td>2.68 ± 0.40</td>
</tr>
<tr>
<td>2</td>
<td>0.033 ± 0.02$^\text{NS}$</td>
<td>3.84 ± 0.10$^c$</td>
</tr>
<tr>
<td>3</td>
<td>0.029 ± 0.02$^\text{NS}$</td>
<td>2.78 ± 0.70$^y$</td>
</tr>
<tr>
<td>4</td>
<td>0.031 ± 0.01$^\text{NS}$</td>
<td>3.57 ± 0.19$^c$</td>
</tr>
</tbody>
</table>

Experimental details were similar as shown in Table 14. Values are mean ± SE of 6 animals.

Groups 2, 3 and 4 as compared to group 1. $^b$P < 0.01; $^c$P < 0.001.

Groups 3 and 4 as compared to group 2. $^y$P < 0.01; $^z$P < 0.001 $^\text{NS}$ Not significant.

1 = µmoles DTNB conjugated/mg protein for liver and µmoles DTNB conjugated /ml of blood for erythrocytes. 2 = µmoles GSH conjugated/min/mg protein.
In PE treated rats (Group 3) liver and erythrocytes GST activity was significantly increased as compared to gamma irradiated rats (Group 2) and was similar to that of control (Group 1) (Table 15). PE pretreatment with gamma irradiation (Group 4) showed decrease in liver GST activity as compared to control (Group 1) but was significantly higher than gamma irradiated rats (Group 2). The result showed that erythrocytes GST activity in PE pretreated rats prior to gamma irradiation (Group 4) was reduced as compared to control (Group 1), while it was statistically similar to that of gamma irradiated rats (Group 2).

**Effect of PE on histology of rat liver and intestine**

The histology of liver of rats administered PE (100 mg/kg body wt.) for 5 days without or with gamma irradiation (Group 3 and 4) showed normal cellular architecture (Fig. 48 A and 48 B) and revealed recovery of damaged tissue to a certain extent as compared to gamma irradiated rats at 2.0 Gy (Group 2).

Rats pretreated with PE without or with gamma irradiation (Groups 3 and 4) showed normal cellular architecture of intestine as compared to the damage revealed by gamma irradiated tissue at 10.0 Gy (Group 2) (Fig. 48 C and 48 D).

**Discussion**

Increase in oxidative stress as a result of an imbalance between oxidant attack and antioxidant defense has been well documented in literature (Lands et al., 1999; Vecchiet et al., 2003). Thus, treatments that reverse oxidative reactions may be acting through mechanisms that scavenge ROS and other free radicals. Nature has endowed us with endogenous cellular defense mechanisms in the form of enzymatic and non enzymatic antioxidants to eliminate ROS. These antioxidants defense mechanisms become weaker during chronic fatigue and other disease conditions. Similarly, exposure to lethal dose of ionizing radiation, severely increases the oxidative burden on the body and endogenous antioxidant defense mechanism cannot cope with increased oxidative stress (Lata et al., 2009). The exogenous antioxidants from herbal and natural products act directly or indirectly with endogenous antioxidants to form a cooperative network of cellular antioxidants to defend oxidative stress. Therefore, the present study was designed to evaluate the effects of gamma irradiation on the antioxidant defense system and the radioprotection afforded by TCE, WSE, ARE and PE.
Fig. 48 A. H and E stained section of liver showing normal cellular structure in rats treated with PE (100 mg/kg body wt).

Fig. 48 B. H and E stained section of liver showing reduced effect of radiation in rats pretreated with PE (100 mg/kg body wt) prior to whole body gamma irradiation exposure at a dose of 2.0 Gy.
Fig. 48 C. H and E stained section of intestine showing normal cellular structure in rats administered PE (100 mg/kg body wt).

Fig. 48 D. H and E stained section of intestine showing reduced effect of radiation in rats pretreated with PE (100 mg/kg body wt) and prior to whole body gamma irradiation exposure at a dose of 10.0 Gy.
The results showed that gamma irradiation exposure to rats caused a marked increase in MDA levels in liver and erythrocytes, which indicates increased oxidative stress. However, a decrease in LPO was observed in rats pretreated with TCE, WSE, ARE and PE prior to gamma irradiation. As LPO in vivo can affect the structural and functional integrity of cell membranes and impair cell function by reacting with various macromolecules including proteins and nucleic acids (Rice-Evans and Burdon, 1993), it is suggested that pretreatment with TCE, WSE, ARE and PE prior to gamma irradiation strengthen the antioxidant defense system and prepared the animals to withstand the damaging effects of irradiation. SOD, one of the first antioxidant enzymes in the line of defence against the deleterious effects of oxygen radicals in the cells, scavenges ROS by catalyzing the dismutation of superoxide to hydrogen peroxide (H₂O₂). In the present study, the SOD activity significantly reduced in liver and erythrocytes of gamma irradiated rats. The SOD activity was returned to near normal levels with the TCE, WSE, ARE and PE extracts. Catalase acts as a preventive antioxidant and plays an important role in the protection against the deleterious effects of lipid hydroperoxide. A decreased activity of CAT was as a result of oxidative stress caused by gamma irradiation. Presumably, a decrease in CAT activity could be attributed to cross-linking and inactivation of the enzyme protein. The CAT activity was restored to normal after treatment with extracts, which shows the antioxidant property of the extracts against oxidative stress. The earlier studies in which the animals were exposed to gamma irradiation also showed marked increase in LPO and inhibition of SOD, CAT and GST activity (Prasad et al., 2006, Devipriya et al., 2008; Srinivasan et al., 2009; Kalpana et al., 2009, Dixit et al., 2012a and b ; 2013).

GSH is a tripeptide (γ-L-Glutamyl-L-cysteinyl-glycine), an antioxidant and a powerful nucleophile, critical for cellular protection, such as detoxification of ROS, conjugation and excretion of toxic molecules and control of inflammatory cytokine cascade (Brown et al., 2004). Gamma irradiation resulted in a significant increase in the levels of GSH in erythrocytes. However, gamma irradiation decreased the GST activity in liver and erythrocytes of rats. The results showed that liver and erythrocytes GST activity was significantly increased in extract pretreated animals as compared to gamma irradiated rats, suggesting that pretreatment with TCE, WSE, ARE and PE facilitates the removal of free radicals and enhanced the post irradiation repair of endogenous antioxidant system.
The histological studies also suggest that pretreatment with TCE (80 mg/Kg body wt), WSE (20 mg/Kg body wt), ARE (50 mg/Kg body wt) and PE (100 mg/Kg body wt) for 5 days (i.p.), prior to gamma irradiation reduced the extent of hepatocellular radiation damage and gastrointestinal damage, thereby accelerated the recovery process against gamma irradiation induced oxidative damages, depending on the susceptibility of the tissues towards irradiations. These protective effects may be attributed due to the principle constituents such as polyphenols, flavonoids, triterpenoids etc. in the herbal and natural extracts (Harborne et al., 1999). These active constituents may contribute for the various antioxidant and radioprotective mechanisms of the extracts (Gandhi and Nair, 2005; Naik et al., 2003; 2004). The results of the present study indicate that there was recovery from oxidative damage caused by gamma irradiation in extract pretreated groups.

In conclusion, the results of the present study confirms the effectiveness of T. chebula, W. somnifera, A. racemosus and propolis extracts as antioxidants, capable of neutralizing damage caused by ROS generated during radiation exposure. This was confirmed by decreased liver and erythrocytes LPO, enhanced antioxidant enzyme activities and histopathology. The different radioprotective and antioxidant activities of these natural and herbal extracts may be assigned to the different biologically active constituents.