Observations
Evaluation of \textit{in vitro} antioxidant activity of various types of \textit{Punica granatum} extract

1,1-Diphenyl-2-picrylhydrazyl- radical scavenging activity

In the present study the scavenging capacity of the PGFRE and PGFRA were found to be 86.33\% and 85.81\% with the IC$_{50}$ value 213.53\(\mu\)g/ml and 404.78 \(\mu\)g/ml. Scavenging capacity of PGSM and PGSC were 79.56\% and 71.21\% with the IC$_{50}$ value 381.38 \(\mu\)g/ml and 457.58 \(\mu\)g/ml respectively. The ordered DPPH free radical scavenging activity of different types of extracts of \textit{Punica granatum} are PGFRE > PGSM > PGFRA > PGSC (Table:1; Figure: 7&8).

Lipid peroxidation activity

IC$_{50}$ values of fruit rind extract were 134.69mg/ml for Ethanol extract and 114.60mg/ml for Acetone extract. IC$_{50}$ value of seed extract was 123.315mg/ml and 113.855mg/ml for Methanol and Chloroform extract respectively. Their order was PGFRE > PGSM > PGFRA > PGSC (Table: 2; Figure: 9&10).

Reduced glutathione activity

IC$_{50}$ values of fruit rind extract were 118.8mg/ml for Ethanol extract and 128.38 mg/ml for Acetone extract. IC$_{50}$ values of seed extract were 129.18mg/ml and 95.185mg/ml for Methanol and Chloroform extract respectively. The maximum activity was of PGFRE and the minimum activity was of PGFRA. (Table:3; Figure:11&12).
Table 1: Percentage inhibition of DPPH• Radical by various extracts of *Punica granatum*

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGFRE</td>
</tr>
<tr>
<td>100</td>
<td>37.84</td>
</tr>
<tr>
<td>200</td>
<td>49.99</td>
</tr>
<tr>
<td>300</td>
<td>59.57</td>
</tr>
<tr>
<td>400</td>
<td>62.53</td>
</tr>
<tr>
<td>500</td>
<td>71.21</td>
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<td>600</td>
<td>76.32</td>
</tr>
<tr>
<td>700</td>
<td>79.82</td>
</tr>
<tr>
<td>800</td>
<td>86.33</td>
</tr>
</tbody>
</table>

Table 2: Effect of various extract of *Punica granatum* on ferrous sulphate induced lipid peroxidation in liver homogenate

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Concentration (mg/ml)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGFRE</td>
<td>41.66±1.43</td>
<td>45.92±1.59</td>
<td>51.93±1.44</td>
<td>67.58±1.605</td>
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</tr>
<tr>
<td>PGFRA</td>
<td>28.88±3.85</td>
<td>54.62±1.22</td>
<td>58.66±1.154</td>
<td>64.44±3.84</td>
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<tr>
<td>PGSM</td>
<td>26.44±1.66</td>
<td>38.76±6.27</td>
<td>46.15±6.66</td>
<td>59.77±1.88</td>
<td></td>
</tr>
<tr>
<td>PGSC</td>
<td>22.22±4.81</td>
<td>34.72±2.40</td>
<td>47.43±4.44</td>
<td>71.29±1.605</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Reduced glutathione content in liver homogenate by *Punica granatum* extract

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Concentration (mg/ml)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGFRE</td>
<td>38.90±2.30</td>
<td>45.86±0.461</td>
<td>48.53±1.84</td>
<td>54.40±1.38</td>
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<tr>
<td>PGFRA</td>
<td>72.00±1.38</td>
<td>78.66±2.30</td>
<td>85.06±0.923</td>
<td>90.66±2.30</td>
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<tr>
<td>PGSM</td>
<td>47.98±0.40</td>
<td>59.57±6.14</td>
<td>72.33±4.91</td>
<td>85.81±2.45</td>
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<tr>
<td>PGSC</td>
<td>55.31±1.22</td>
<td>71.15±0.409</td>
<td>76.23±0.617</td>
<td>79.66±0.819</td>
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</table>
Table 4: Free radical scavenging activity of *Punica granatum* extract

<table>
<thead>
<tr>
<th>Activity</th>
<th>Extracts</th>
<th>Equation</th>
<th>r² Values</th>
<th>IC₅₀ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>% inhibition by DPPH assay</td>
<td>Fruit rind ethanol</td>
<td>y = 0.065x + 36.12</td>
<td>0.970</td>
<td>213.53</td>
</tr>
<tr>
<td></td>
<td>Fruit rind acetone</td>
<td>y = 0.092x + 12.76</td>
<td>0.978</td>
<td>404.78</td>
</tr>
<tr>
<td></td>
<td>Seed methanol</td>
<td>y = 0.072x + 22.54</td>
<td>0.990</td>
<td>381.38</td>
</tr>
<tr>
<td></td>
<td>Seed chloroform</td>
<td>y = 0.062x + 21.63</td>
<td>0.992</td>
<td>457.58</td>
</tr>
<tr>
<td>% inhibition by Lipid peroxidation activity</td>
<td>Fruit rind ethanol</td>
<td>y = 1.6754x + 30.83</td>
<td>0.908</td>
<td>114.60</td>
</tr>
<tr>
<td></td>
<td>Fruit rind acetone</td>
<td>y = 2.2144x + 23.97</td>
<td>0.8283</td>
<td>134.69</td>
</tr>
<tr>
<td></td>
<td>Seed methanol</td>
<td>y = 2.1476x + 15.935</td>
<td>0.9886</td>
<td>123.315</td>
</tr>
<tr>
<td></td>
<td>Seed chloroform</td>
<td>y = 3.1984x + 3.935</td>
<td>0.971</td>
<td>163.855</td>
</tr>
<tr>
<td>% inhibition by Reduced glutathione activity</td>
<td>Fruit rind ethanol</td>
<td>y = 1.7834x + 29.63</td>
<td>0.98</td>
<td>118.8</td>
</tr>
<tr>
<td></td>
<td>Fruit rind acetone</td>
<td>y = 1.2476x + 66</td>
<td>0.9985</td>
<td>128.38</td>
</tr>
<tr>
<td></td>
<td>Seed methanol</td>
<td>y = 2.525x + 34.86</td>
<td>0.9989</td>
<td>129.18</td>
</tr>
<tr>
<td></td>
<td>Seed chloroform</td>
<td>y = 0.8826x + 51.055</td>
<td>0.9347</td>
<td>95.185</td>
</tr>
</tbody>
</table>
Figure 7: Percentage inhibition of DPPH$^*$ Radical by various extracts of *Punica granatum* fruit rind

![Graph showing inhibition percentage vs concentration for PGFRE and PGFRA extracts]

\[ y = 0.0652x + 36.12 \]
\[ R^2 = 0.9707 \]

\[ y = 0.0929x + 12.769 \]
\[ R^2 = 0.9787 \]

Figure 8: Percentage inhibition of DPPH$^*$ Radical by various extracts of *Punica granatum* seed

![Graph showing inhibition percentage vs concentration for PGSC and PGSM extracts]

\[ y = 0.0721x + 22.548 \]
\[ R^2 = 0.9906 \]

\[ y = 0.062x + 21.634 \]
\[ R^2 = 0.9924 \]
Figure 9: Effect of extract of *Punica granatum* fruit rind extract on ferrous sulphate induced lipid peroxidation in liver homogenate

![Graph showing the effect of *Punica granatum* fruit rind extract on ferrous sulphate induced lipid peroxidation in liver homogenate. The graph includes linear equations and R² values for the data.](image1)

Figure 10: Effect of extract of *Punica granatum* seed extract on ferrous sulphate induced lipid peroxidation in liver homogenate

![Graph showing the effect of *Punica granatum* seed extract on ferrous sulphate induced lipid peroxidation in liver homogenate. The graph includes linear equations and R² values for the data.](image2)
Figure 11: Reduced glutathione content (GSH) in liver homogenate by *Punica granatum* fruit rind extract

\[ y = 1.7834x + 29.63 \]
\[ R^2 = 0.98 \]

\[ y = 1.2476x + 66 \]
\[ R^2 = 0.9985 \]

Figure 12: Reduced glutathione content (GSH) in liver homogenate by *Punica granatum* seed extract

\[ y = 2.525x + 34.86 \]
\[ R^2 = 0.9989 \]

\[ y = 1.5626x + 51.055 \]
\[ R^2 = 0.8774 \]
**Observations**

### Body weight

Changes in the body weight were recorded in all the groups. It decreased till 3rd post irradiation day in control and all the experimental groups. Maximum weight reduction was recorded on 3rd day after irradiation in control group. Reduction in body weight by 11.49%, 13.74%, 19.67%, 15.29% and 8.69% in control group were recorded on 3hr, 1,3,7 and 14 post irradiation days, respectively, as compared to normal group.

Reduction in body weight of the experimental mice was recorded since day 1 but it was always significantly lesser in comparison to their respective control group. In experimental-1 group (PGFRE+8Gy) a gradual decline in body weight of animals was recorded which was followed by recovery on 7th day. Recovery in body weight by 14.64% and 12.76% was recorded on 7, and 14th post irradiation days, respectively, as compared to those of the control groups. In experimental group-2 (PGFRA+8Gy) decrease in body weight by 10.84%, 7.87%, 9.83%, 13.71%, and 10.08% was recorded on 3 hr, 1,3 and 7 and 14th post irradiation day, respectively as compared to control group. In Experimental-1 group, lesser decrease in body weight was observed at all the post irradiation days as compared to Experimental-2 group but the difference was not statistically significant (Table:5; Figure:13).

In experimental-3 group (PGSM+8Gy), recovery by 13.89%, 9.60% and in experimental-4 group (PGSC+8Gy) 11.89%, 9.38% was recorded on 7, and 14th post irradiation days, respectively, as compared to their respective control groups. In Experimental-3 group, insignificantly higher body weight was observed at all the post irradiation intervals as compared to Experimental-4 group. In only PG fruit rind
Table 5: Variations in the body weight (g) of Co$^{60}$ gamma ray irradiated *Swiss albino* mouse with and without *Punica granatum* pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td></td>
</tr>
</tbody>
</table>
| Control (8Gy)                          | 22.40±0.134 a***               | 21.83±0.17 a***               | 20.33±0.345 a***               | 21.44±0.294 a***               | 23.11±0.485 a***               | ANS
| Experimental -1 (PGFRE + 8Gy)          | 25.50±0.337 b***               | 24.33±0.386 b**               | 22.28±0.358 b**               | 24.58±0.319 b**               | 26.06±0.139 b**               | 26.50±1.37                 |
| PG Rind Ethanol extract only           | 25.83±1.47                       | 26.16±1.47                       | 26.83±0.752                       | 25.83±1.47                       | 25.66±1.63                       | 26.16±1.47               |
| Experimental -2 (PGFRA + 8Gy)          | 24.83±0.632 c* fNS               | 23.55±0.555 c** fNS             | 22.33±0.881 cNS fNS             | 24.38±0.310 c** fNS             | 25.44±0.294 c** fNS             | 26.16±0.752 fNS           |
| PG Rind Acetone extract only           | 25.00±1.09                       | 25.50±1.04                       | 26.16±1.16                       | 26.33±1.03                       | 26.66±1.21                       | 27.16±1.16               |
| Experimental -3 (PGSM +8Gy)            | 25.34±0.280 d***                | 23.83±0.098 d**                 | 22.60±0.247 d**                 | 24.42±0.372 d**                 | 25.53±0.315 d**                 | 26.66±1.03               |
| PG seed Methanol extract only          | 25.33±1.21                       | 26.16±0.983                      | 26.50±1.37                       | 26.83±0.752                      | 27.16±1.16                       | 27.66±1.21               |
| Experimental -4 (PGSC+8Gy)             | 24.05±0.579 e** gNS             | 23.33±0.509 e** gNS             | 21.33±0.509 eNS g*              | 23.99±0.383 e** gNS             | 25.28±0.303 e** gNS             | 25.33±1.21 gNS          |
| PG seed Chloroform extract only        | 24.66±1.41                       | 25.50±1.64                       | 25.66±1.36                       | 25.83±1.47                       | 26.00±1.26                       | 26.25±1.37               |

The healthy normal *Swiss albino mouse* without any treatment is = 25.31 ±0.260
Significance level = *P<0.1; ** P <0.05; ***P<0.001; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co$^{60}$ gamma radiation.
Control = The plant extract was given 1hr before irradiation to 8Gy of Co$^{60}$ gamma radiation.
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a;
Control V/s Experimental 1=b; Control V/s Experimental 2= c;
Control V/s Experimental 3=d; Experimental 1 V/s Experimental 2 =f;
Experimental 3 V/s Experimental 4 = g
PGFRE = *Punica granatum* fruit rind Ethanol extract.
PGFRA = *Punica granatum* fruit rind Acetone extract.
PGSM = *Punica granatum* seed Methanol extract.
PGSC = *Punica granatum* seed Chloroform extract.
Figure 13: Variations in the body weight (g) of Co\textsuperscript{60} gamma ray irradiated *Swiss albino mouse* with and without *Punica granatum* fruit rind pretreatment

![Bar chart showing body weight variations](image)

Figure 14: Variations in the body weight (g) of Co\textsuperscript{60} gamma ray irradiated *Swiss albino mouse* with and without *Punica granatum* seed pretreatment

![Bar chart showing body weight variations](image)
and seed extract treated groups, total body weight was not increased till the last interval and remained near to the normal (Table:5; Figure:14).

Amongst all the experimental groups, experimental group 1 (PGFRE +8Gy) had a little more body weight as compared to other experimental groups (2, 3 and 4).

Liver weight

Animals irradiated with 8Gy had highly significant decrease in liver weight (P<0.001). In 8Gy irradiated group decrease in liver weight was observed after 3h, then it started to increase on 1st day which continued to increase till 14th day post irradiation. Reduction in weight of liver by 15.00%, 13.83%, 12.25%, 10.66% and 9.15% in control group was recorded on 3hr, 1, 3, 7 and 14 post irradiation days, respectively, as compared to untreated normal group.

PGFRE and PGFRA pretreated irradiated groups had decrease in weight of liver 3 hr post irradiation but it remained higher than the control group. Then it started to increase and reached to normal till 7th day. In experimental group-1(PGFRE+8Gy) lesser decrease in liver weight was observed in comparison to their respective controls. Liver weight was higher by 04.12%, 4.39%, 4.00%, 4.31% and 3.40% in experimental-1 group and 3.64%, 4.15%, 3.21%, 2.61% and 1.590% in experimental - 2, on 3hr, 1, 3, 7, and 14th post irradiation days, respectively, as compared to those of control groups. In Experimental-1 group, a little higher body weight was observed at all the post irradiation intervals as compared to Experimental-2 group (Table:6; Figure:15).
Table 6: Variations in the weight of liver (g) of Co\(^{60}\) gamma ray irradiated *Swiss albino mouse* with and without *Punica granatum* pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td>1.235±0.018 <strong>a</strong>*</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>1.286±0.006 <em>b</em></td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>1.451±0.011</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>1.280±0.005 <em>c</em> (^{fNS})</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>1.448±0.011</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>1.284±0.007 <em>d</em></td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>1.452±0.015</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>1.278±0.005 <em>e</em> (^{gNS})</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>1.450±0.011</td>
</tr>
</tbody>
</table>

The healthy normal *Swiss albino mouse* without any treatment is = 1.453 ±0.021
Significance level = *P<0.1; ** P <0.05; ***P<0.001; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\(^{60}\) gamma radiation.
Control = Irradiated only to 8Gy of Co\(^{60}\) gamma radiation
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a;  Control V/s Experimental 1=b;  Control V/s Experimental 2=c;  Control V/s Experimental 3=d;  Control V/s Experimental 4=e;  Experimental 1 V/s Experimental 2=f;  Experimental 3 V/s Experimental 4=g

PGFRE = *Punica granatum* fruit rind Ethanol extract.
PGFRA = *Punica granatum* fruit rind Acetone extract.
PGSM = *Punica granatum* seed Methanol extract.
PGSC = *Punica granatum* seed Chloroform extract.
Figure 15: Variations in the weight of liver (g) of Co\textsuperscript{60} gamma ray irradiated \textit{Swiss albino mouse} with and without \textit{Punica granatum} fruit rind pretreatment

![Graph showing liver weight variations][1]

Figure 16: Variations in the weight of liver (g) of Co\textsuperscript{60} gamma ray irradiated \textit{Swiss albino mouse} with and without \textit{Punica granatum} seed pretreatment

![Graph showing liver weight variations][2]
In experimental group-3(PGSM+8Gy) lesser decrease in liver weight was observed in comparison to their respective controls. It was more by 3.96%, 4.31%, 3.37%, 3.08% and 2.27% in experimental-3 group and 3.48%, 4.07%, 2.98%, 2.00% and 1.51% in experimental group -4 (PGSC+8Gy), recorded on 3hr, 1, 3, 7, and 14th post irradiation days, respectively, as compared to those of the control groups. In Experimental-3 group, a small increase in liver weight was observed at all the post irradiation days as compared to Experimental-4 group. In groups treated with PG extracts only, no significant change in liver weight was observed till the last interval (Table:6; Figure:16).

Amongst all the experimental groups, experimental group 1 (PGFRE +8Gy) had a little more increase as compared to other experimental groups which was not significant statistically (2, 3 and 4).

The liver weight was correlated to the body weight in the irradiated animals. The co-efficient of correlation calculated was 0.999 for 8Gy for control, 0.998 for PGFRE, 0.999 for PGFRA, PGSM and PGSC pretreated irradiated animals separately. In the only plant extract (PGFRE, PGFRA, PGSM and PGSC) treated group it is also 0.999.

**Histopathology of Liver**

**Histopathology of liver in normal mouse**

The liver of mouse is the largest internal organ, anatomically centrally located and surrounded by other radio-sensitive tissues (small intestine, stomach, heart, kidneys and lungs), occupying the anterior one third part of the abdominal cavity. The anterior surface lies against the arch of diaphragm and the concave portion fits
Figure 17 : Normal architecture of mouse liver showing uniform arrangement of hepatic cords, binucleated cells. (X400)

Figure 18 and 19 : Liver of control mouse 3 hrs after exposure to 8Gy of gamma rays showing cytoplasmic degranulation along with vacuolation, pyknotic nuclei, some enucleated cells and kupffer cells. (X 200 and X 400)
Observations

over and partly covers the stomach and duodenum. The entire gland consists of five lobes joined dorsally. The large median lobe is subdivided into right and left portions by a deep bifurcation the undivided left lateral lobe, the right lateral lobe divided horizontally into anterior and posterior portion, and a caudal lobe consisting of two leaf like parts, dorsal and ventral to the oesophagus.

Liver is enclosed by a firm smooth layer of connective tissue, the Glisson’s capsule. The capsule also forms a sheath around the portal vein, hepatic artery and bile duct. The hepatic parenchyma is made up of innumerable small lobules with a shape of pyramidal hexagon. In the intralobular area branches of the hepatic artery, hepatic portal vein and bile duct are present. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into capillaries, which then lead to a lobule. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells. Lobules are the functional units of the liver. Lobules consist of hepatocytes and the spaces between them. In between the cords sinusoids are present with phagocytic stellate cells or Von Kupffer cells. The hepatocytes are polyhedral in shape with large central nuclei and one or two nucleoli. Binucleate cells are commonly seen. The bile capillaries are located between the adjoining faces of the cells, the opposite surface being in contact with sinusoids. (Figure: 17)

Following histopathological changes were observed in control and experimental groups after exposure to 8Gy gamma radiation.
Figure 20 and 21: Liver of control mouse 1 days after exposure to 8Gy of gamma rays showing distorted hepatic cords, cytoplasmic vacuolation, kupffer cells, crenated and shrunken nuclei, binucleated cells, pyknotic nuclei, enucleated cells and condensed chromatin material. (X200 and X400)

Figure 22 and 23: Liver of control mouse 3 days after exposure to 8Gy of gamma rays showing cytoplasmic vacuolation, lymphocytic infiltration, reduced nuclear size, wider sinusoidal spaces, kupffer cells. (X200 and X400)
3hrs

Histopathological changes like cytoplasmic degranulation along with vacuolation, pyknotic nuclei and some enucleated cells were observed. Kupffer cells appeared prominent. (Figure : 18 and 19)

1st day

At this interval histopathological changes became severe. Hepatic cords looked highly distorted. The cytoplasmic vacuolation became pronounced and increased number of kupffer cells, crenated and shrunken nuclei, binucleated cells, large number of pyknotic nuclei, enucleated cells and condensed chromatin material were also observed (Figure : 20 and 21).

3rd day

The degree of damage was higher on 3rd day. Cytoplasmic vacuolation and lymphocytic infiltration were observed. Cord like arrangement of hepatocytes was completely lost. Reduced nuclear size, wider sinusoidal spaces, large number of kupffer cells and a few giant cells were also observed (Figure : 22 and 23).

7th Day

Signs of recovery in some hepatic lobules with increased nuclear size and cytoplasmic regranulation were recorded. Pronounced vacuolation of cytoplasm, enucleated hepatocytes, binucleated cells and a few giant nuclei were seen (Figure:24).

14th Day

Signs of recovery were visible but spaces between hepatic cords were more than the normal. Regranulation of cytoplasm, large number of kupffer cells, enucleated hepatocytes and some pyknotic nuclei were also noted. (Figure : 25)
Figure 24: Liver of control mouse 7 days after exposure to 8Gy of gamma rays showing cytoplasmic regranulation, pronounced vacuolation of cytoplasm, enucleated hepatocytes and binucleated cells. (X400)

Figure 25: Liver of control mouse 14 days after exposure to 8Gy of gamma rays showing increased spaces between hepatic cords, regranulation of cytoplasm, large number of kupffer cells, enucleated hepatocytes and some pyknotic nuclei. (X200)

Figure 26 and 27: Liver of experimental-1 mouse (PGFRE+8Gy) 14 days after exposure to 8Gy of gamma rays showing near normal architecture, regranulation of cytoplasm, some pyknotic nuclei, binucleated cells, and kupffer cells. (X100 and X400)
Figure 28 : Liver of experimental-2 mouse (PGFRA+8Gy) 14 days after exposure to 8Gy of gamma rays showing normal architecture, less kupffer cells, crenated nuclei, chromatin condensation and enucleated cells. (X200)

Figure 29 : Liver of experimental-3 mouse (PGSM+8Gy) 14 days after exposure to 8Gy of gamma rays showing normal hepatocytes, enucleated hepatocytes, some kupffer cells and crenated nuclei. (X400)

Figure 30 and 31 : Liver of experimental-4 mouse (PGSC+8Gy) 14 days after exposure to 8Gy of gamma rays showing large number of nuclei with condensed chromatin, binucleated cells, few enucleated cells, dividing cells and large number of kupffer cells. (X200 and X400)
In experimental-1 (PGFRE+8Gy) group, near normal histology was observed. Nuclei appeared in their normal size, regranulation of cytoplasm, some pyknotic nuclei, binucleated cells and kupffer cells were observed. (Figure : 26 and 27)

In experimental-2 (PGFRA+ 8Gy) group, recovery was observed. Normal arrangement of hepatocytes and well organized nuclei were seen. Number of kupffer cells was comparatively less, crenated nuclei, chromatin condensation and enucleated cells were also visible in less quantity. (Figure : 28)

In experimental-3(PGSM+8Gy) group also signs of recovery were observed. Normal hepatocytes, enucleated hepatocytes, some kupffer cells and creanted nuclei were visible in sufficient number. (Figure : 29)

In experimental-4(PGSC+8Gy) group, the tissue was seen approaching to the normal condition. Large number of nuclei with condensed chromatin were present. Number of binucleated cells was remarkable. A few enucleated cells, dividing cells and large number of kupffer cells were also observed. (Figure : 30 and 31)

28th day

Animals of control group (8Gy gamma irradiated only) were not survived till 28th day. In Experimental-1 (PGFRE+8Gy) group, recovery was observed. Normal arrangement of hepatocytes and well organized nuclei were seen. (Figure : 32)

In Experimental-2 (PGFRA+8Gy) group, signs of recovery were observed. Few binucleated cells, enucleated cells and kupffer cells were also observed. (Figure: 33)
Figure 32: Liver of experimental-1 mouse (PGFRE+8Gy) 28 days after exposure to 8Gy of gamma rays showing normal architecture of hepatocytes and well organized nuclei. (X200)

Figure 33: Liver of experimental-2 mouse (PGFRA+8Gy) 28 days after exposure to 8Gy of gamma rays showing few binucleated, enucleated and kupffer cells. (X400)

Figure 34: Liver of experimental-3 mouse (PGSM+8Gy) 28 days after exposure to 8Gy of gamma rays showing normal arrangement of hepatocytes, some kupffer and binucleated cells. (X200)

Figure 35: Liver of experimental-4 mouse (PGSC+8Gy) 28 days after exposure to 8Gy of gamma rays showing normal architecture, less kupffer cells. (X200)
In Experimental-3 (PGSM+8Gy) group, Normal arrangement of hepatocytes, some kupffer cells and a few binucleated cells were observed. (Figure : 34)

In Experimental-4 (PGSC+8Gy) group, recovery was observed. Normal hepatocytes and well organised nuclei were seen. A few binucleated and kupffer cells were observed. (Figure :35)

**Biochemical studies**

The biochemical changes recorded in the liver of *Swiss albino mouse* after exposure to 8Gy gamma rays at different post irradiation intervals are as follows.

**DNA Content**

A sharp decline in control group was recorded up to 3rd day followed by a slight recovery till 14th day. Reduction in DNA content by 45.26%, 47.63%, 50.59%, 36.39% and 33.43% in control group was recorded on 3hr, 1st, 3rd, 7th and 14th post irradiation days, respectively, as compared to that of the normal groups.

A decrease in DNA content in all the experimental groups was also observed on 3hr, 1st and 3rd day but it was higher than the controls. Recovery was seen on 7th day to 28th day (P<0.001). In experimental group-1(PGFRE+8Gy) lesser decrease in DNA content was observed in comparison to their respective controls. Statistically a significant difference by 70.27%, 54.80%, 70.05%, 38.60% and 34.22% in DNA content in experimental-1 group and 64.86%, 36.72%, 58.08%, 26.51% and 30.66% in experimental group -2, was recorded on 3hr, 1,3,7, and 14th post irradiation days, respectively, as compared to their respective control groups. In Experimental-1 group, higher DNA content was observed at all the post irradiation days as compared to Experimental-2 group (Table:7; Figure:36).
Table 7: Variations in the DNA content (mg/g tissue) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td>1.85±0.124\textsuperscript{a***}</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>3.15±0.049\textsuperscript{b***}</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>3.23±0.005</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>3.05±0.063\textsuperscript{c*** f\textsuperscript{NS}}</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>3.26±0.009</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>3.10±0.057\textsuperscript{d***}</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>3.31±0.015</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>2.92±0.04\textsuperscript{e*** g*}</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>3.23±0.005</td>
</tr>
</tbody>
</table>

The healthy normal Swiss albino mouse without any treatment is = 3.38±0.088
Significance level = *P<0.1; **P<0.05; ***P<0.001; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
Control = Irradiated only to 8Gy of Co\textsuperscript{60} gamma radiation
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1= b; Control V/s Experimental 2= c; Control V/s Experimental 3= d; Control V/s Experimental 4= e; Experimental 1 V/s Experimental 2 =f; Experimental 3 V/s Experimental 4 = g
PGFRE = Punica granatum fruit rind Ethanol extract.  PGFRA = Punica granatum fruit rind Acetone extract.
PGSM = Punica granatum seed Methanol extract.  PGSC = Punica granatum seed Chloroform extract
Figure 36: Variations in the DNA content (mg/g tissue) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum fruit rind pretreatment

Figure 37: Variations in the DNA content (mg/g tissue) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum seed pretreatment
In experimental group-3 (PGSM+8Gy) DNA was more by 67.56%, 61.58%, 74.25%, 38.60% and 34.66% whereas in experimental -2 group 57.83%, 50.28%, 72.45%, 37.20% and 34.66% on 3hr, 1, 3, 7, and 14th post irradiation days, respectively, as compared to control groups. PGFRE pretreated irradiated group had maximum protection of DNA content against 8Gy gamma irradiation in comparison to PGFRA, PGSM and PGSC. In Experimental-3 group a non-significant increase in DNA content was observed at all the post irradiation days as compared to Experimental-4 group (Table:7; Figure:37). In only PG fruit rind and seed extracts DNA content remained near to the normal at all the autopsy intervals. Amongst all the experimental groups, experimental group 1 (PGFRE +8Gy) had a significantly (P<0.05) more increase as compared to other experimental groups (2,3 and 4).

RNA content

RNA content decreased significantly in the control animals treated with 8Gy which was maximum on the 7th post irradiation day. Then it increased slightly till the last post irradiation interval. Reduction in RNA content by 9.19%, 10.04%, 13.39%, 14.79% and 13.00% was observed after 3hr, 1st, 3rd, 7th and 14th post irradiation day respectively, as compared to their control group.

Decrease in the amount of RNA was recorded in the experimental animals exposed to 8Gy after 3 hrs. Maximum decrease was observed on the 7th post irradiation day. The RNA content was always higher than the control group (P<0.001) in experimental group. Then it started to increase after 7th day which continued to increase uptill the 28th day. In experimental-1 group it was more by 9.43%, 8.31%, 11.24%, 12.97%, 15.04% and in Experimental -2 group by 9.00%, 7.87%, 10.97%,
Table 8: Variations in the RNA content (mg/g tissue) in the liver of Co$^{60}$ gamma ray irradiated Swiss albino mouse with and without Punica granatum pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
<th>3h</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8Gy)</td>
<td></td>
<td>1.166±0.012 a***</td>
<td>1.155±0.005 a***</td>
<td>1.112±0.008 a***</td>
<td>1.094±0.005 a***</td>
<td>1.117±0.007 a***</td>
<td>ANS</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td></td>
<td>1.276±0.012 b***</td>
<td>1.251±0.006 b***</td>
<td>1.237±0.004 b***</td>
<td>1.236±0.008 b***</td>
<td>1.285±0.008 b***</td>
<td>1.287±0.014 b***</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td></td>
<td>1.282±0.010</td>
<td>1.283±0.008</td>
<td>1.284±0.008</td>
<td>1.284±0.014</td>
<td>1.286±0.009</td>
<td>1.287±0.014</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td></td>
<td>1.271±0.012 c*** fNS</td>
<td>1.246±0.014 c*** fNS</td>
<td>1.234±0.008 c*** fNS</td>
<td>1.237±0.011 c*** fNS</td>
<td>1.241±0.009 c*** f NS</td>
<td>1.241±0.015 f NS</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td></td>
<td>1.281±0.012</td>
<td>1.283±0.008</td>
<td>1.284±0.009</td>
<td>1.283±0.011</td>
<td>1.284±0.018</td>
<td>1.284±0.014</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td></td>
<td>1.263±0.007 d***</td>
<td>1.242±0.007 d***</td>
<td>1.233±0.006 d***</td>
<td>1.234±0.008 d***</td>
<td>1.236±0.012 d***</td>
<td>1.247±0.011</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td></td>
<td>1.281±0.019</td>
<td>1.283±0.009</td>
<td>1.285±0.010</td>
<td>1.286±0.010</td>
<td>1.286±0.018</td>
<td>1.285±0.011</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td></td>
<td>1.242±0.011 e* g NS</td>
<td>1.238±0.007 e* g NS</td>
<td>1.227±0.007 e* g NS</td>
<td>1.234±0.011 e* g NS</td>
<td>1.235±0.012 e* g NS</td>
<td>1.244±0.030 g NS</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td></td>
<td>1.283±0.010</td>
<td>1.284±0.008</td>
<td>1.285±0.016</td>
<td>1.285±0.015</td>
<td>1.284±0.007</td>
<td>1.286±0.010</td>
</tr>
</tbody>
</table>

The healthy normal Swiss albino mouse without any treatment is = 1.284 ±0.008
Significance level = *P<0.1; ** P <0.05; ***P<0.001 ; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co$^{60}$ gamma radiation.
Control = Irradiated only to 8Gy of Co$^{60}$ gamma radiation
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1=b; Control V/s Experimental 2= c; Control V/s Experimental 3= d; Control V/s Experimental 4= e; Experimental 1 V/s Experimental 2 =f; Experimental 3 V/s Experimental 4 = g
PGFRE = Punica granatum fruit rind Ethanol extract. PGFRA = Punica granatum fruit rind Acetone extract. PGSM = Punica granatum seed Methanol extract. PGSC = Punica granatum seed Chloroform extract
Figure 38: Variations in the RNA content (mg/g tissue) in the liver of Co\textsuperscript{60} gamma ray irradiated \textit{Swiss albino mouse} with and without \textit{Punica granatum} fruit rind pretreatment

![Graph showing RNA content variations](image1)

Figure 39: Variations in the RNA content (mg/g tissue) in the liver of Co\textsuperscript{60} gamma ray irradiated \textit{Swiss albino mouse} with and without \textit{Punica granatum} seed pretreatment

![Graph showing RNA content variations](image2)
Observations

13.07%, 11.10% was observed after 3hr, 1st, 3rd, 7th and 14th day post irradiation respectively, in comparison to their respective control. In Experimental-1 group, a non significant increase in RNA content was observed at all the post irradiation days as compared to Experimental-2 group(Table:8; Figure:38).

In experimental-3 group it was more by 8.31%, 7.53%, 10.88%, 12.79%, 10.65% and in Experimental -4 group by 6.51%, 7.18%, 10.34%, 12.79%, 10.56% was observed after 3hr, 1st, 3rd, 7th and 14th day post irradiation respectively, in comparison to their respective control. In the plant extract treated animals RNA content remained near to the normal. In Experimental-3 group, a small but nonsignificant increase in RNA content was observed at all the post irradiation days as compared to Experimental-4 group (Table:8; Figure:39). Amongst all the experimental groups, experimental-1group had more RNA as compared to other experimental groups.

The DNA content is correlated to the RNA content in the irradiated animals. The co-efficient of correlation calculated between DNA and RNA contents of the same groups was 0.992 for 8Gy control, 0.997 for PGFRE, 0.994 for PGFRA and PGSM, 0.989 for PGSC pretreated irradiated animals separately. In the only plant extract (PGFRE, PGFRA, PGSM and PGSC) treated group it is 1.

Total Protein content

Total Proteins in mice liver decreased significantly after 3hrs of radiation exposure. In 8Gy control group total protein content significantly decreased (P<0.001) as compared to the sham irradiated group (173.00±4.04) after 24 hrs. Reduction in protein content was 15.04%, 13.72%, 10.34%, 12.40% and 14.28% after 3hr, 1,3,7
Observations

and 14\textsuperscript{th} post irradiation days respectively, in control group in comparison to the sham irradiated group.

The protein content decreased in all the experimental groups after 3h of post irradiation as compared to normal but it remained significantly higher (P<0.001) than the control group till 3\textsuperscript{rd} day post irradiation. Then it started to decrease uptill the last interval. In experimental -1 group decrease in total protein content was 14.83\%, 15.03\%, 11.95\%, 10.94\%, 11.84\% and in experimental-2 group 12.38\%, 13.07\%, 9.64\%, 10.51\%,11.18\% on 3hr,1,3,7 and 14\textsuperscript{th} post irradiation days respectively, as compared to their control group. In Experimental-1 group, it was a little bit higher at all the post irradiation days as compared to Experimental-2 group (Table:9; Figure:40).

Total protein content of experimental-3 group was 14.82\%, 13.72\%, 11.11\%, 10.94\%, 11.86\% higher and in experimental-4 group 11.95\%, 11.76\%, 10.27\%, 11.79\%, 12.50\% higher on 3hr,1,3,7 and 14\textsuperscript{th} post irradiation days respectively, as compared to their control group. In only PG fruit rind and seed extract treated groups total protein content was slightly higher than the normal (Table:9; Figure:41). Amongst all the experimental groups, in the experimental-1 group it was highest as compared to other experimental groups.

**Lipid peroxidation**

At all the post irradiation intervals lipid peroxidation remained significantly higher in the control group than the normal. The maximum lipid peroxidation level was observed at 7\textsuperscript{th} day post irradiation. Then it decreased till the last interval. LPO
Table 9: Variations in the Total Protein content (mg/g tissue) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
<th>3h</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8Gy)</td>
<td></td>
<td>146.98±1.41 a***</td>
<td>149.26±1.125 a***</td>
<td>155.11±0.562 a**</td>
<td>151.54±0.650 a***</td>
<td>148.28±0.562 a***</td>
<td>ANS</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td></td>
<td>168.78±0.557 b***</td>
<td>171.70±0.976 b***</td>
<td>173.65±1.48 b***</td>
<td>166.12±1.17 b***</td>
<td>165.85±1.95 b***</td>
<td>163.24±1.41</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td></td>
<td>173.00 ±0.560</td>
<td>174.94±0.565</td>
<td>176.90±1.13</td>
<td>176.58±0.98</td>
<td>174.62±0.97</td>
<td>173.97±1.56</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td></td>
<td>165.19±1.41 c*** f\textsuperscript{NS}</td>
<td>167.72±2.25 c*** f\textsuperscript{NS}</td>
<td>170.07±1.80 c*** f\textsuperscript{NS}</td>
<td>167.47±1.30 c*** f\textsuperscript{NS}</td>
<td>164.87±1.48 c*** f*</td>
<td>162.95±1.025 f\textsuperscript{NS}</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td></td>
<td>173.32±1.487</td>
<td>173.97±0.565</td>
<td>175.99±1.60</td>
<td>174.30±1.125</td>
<td>173.98±1.125</td>
<td>172.67±1.68</td>
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<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td></td>
<td>168.77±1.68 d***</td>
<td>169.75±1.49 d***</td>
<td>172.35±1.71 d***</td>
<td>168.12±1.97 d***</td>
<td>165.87±2.91 d***</td>
<td>160.64±1.81</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td></td>
<td>172.65±1.71</td>
<td>173.64±1.68</td>
<td>175.28±1.125</td>
<td>173.97±2.03</td>
<td>172.67±1.68</td>
<td>171.70±1.68</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td></td>
<td>164.55±2.13 e*** g\textsuperscript{NS}</td>
<td>166.82±2.98 e*** g\textsuperscript{NS}</td>
<td>171.05±1.81 e*** g\textsuperscript{NS}</td>
<td>169.42±1.41 e*** g\textsuperscript{NS}</td>
<td>166.82±1.49 e*** g\textsuperscript{NS}</td>
<td>164.23±1.81 g\textsuperscript{NS}</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td></td>
<td>171.12±1.00</td>
<td>173.22±2.24</td>
<td>174.62±0.975</td>
<td>172.35±2.03</td>
<td>172.03±2.25</td>
<td>171.44±1.94</td>
</tr>
</tbody>
</table>

The healthy normal Swiss albino mouse without any treatment is = 173.00±4.04
Significance level = *P<0.1; ** P <0.05; ***P<0.001 ; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
Control = Irradiated only to 8Gy of Co\textsuperscript{60} gamma radiation
ANSA= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1=b; Control V/s Experimental 2= c; Control V/s Experimental 3= d; Experimental 1 V/s Experimental 2 =f; Experimental 3 V/s Experimental 4 = g
PGFRE = Punica granatum fruit rind Ethanol extract.
PGFRA = Punica granatum fruit rind Acetone extract.
PGSM = Punica granatum seed Methanol extract.
PGSC = Punica granatum seed Chloroform extract.
Figure 40: Variations in the total protein content (mg/g tissue) in the liver of Co$_{60}$ gamma ray irradiated *Swiss albino mouse* with and without *Punica granatum* fruit rind pretreatment.

![Figure 40](image)

Figure 41: Variations in the total protein content (mg/g tissue) in the liver of Co$_{60}$ gamma ray irradiated *Swiss albino mouse* with and without *Punica granatum* seed pretreatment.

![Figure 41](image)
level increased by 13.35%, 18.53%, 21.69%, 24.94% and 23.24% on 3h, 1, 3, 7 and 14 days post irradiation respectively, in control group when compared to normal level.

Lipid peroxidation level remained significantly lower in *Punica granatum* extract pretreated irradiated groups from their respective controls at all the intervals. In experimental-1 LPO level was reduced by 8.03%, 11.53%, 10.02%, 9.70%, 10.27% and in experimental-2 LPO level was reduced by 7.49%, 10.03%, 8.69%, 6.79%, 9.27% on 3h, 1, 3, 7 and 14 days post irradiation respectively, in comparison to their respective control group. In Experimental-1 group, more decrease in LPO was observed at all the post irradiation days as compared to Experimental-2 group (Table:10; Figure:42).

In experiment-3 LPO level was reduced by 8.71%, 11.33%, 10.53%, 8.83%, 9.89% and in experiment-4 by 6.94%, 9.18%, 9.20%, 6.61%, 9.08% on 3h, 1, 3, 7 and 14 days post irradiation days, in comparison to their respective control groups. In Experimental-3 group, a small decrease in LPO content was observed at all the post irradiation days as compared to Experimental-4 group (Table:10; Figure:43). PGFRE pretreated irradiated group had lower LPO as compared to other PG fruit rind and seed extract pretreated and then irradiated groups.

In only plant extract treated group LPO remained lower than their corresponding control and experimental group. In PG fruit rind and seed extract treated group LPO content remained near to the normal. Amongst all the experimental groups, experimental-1 had lowest LPO levels as compared to other experimental groups.
Table 10: Variations in the Lipid peroxidation (nmol MDA/mg of protein) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td>1.468±0.021 a***</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>1.350±0.018 b**</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>1.296±0.017</td>
</tr>
<tr>
<td></td>
<td>1.305±0.021</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>1.358±0.016 c** f\textsuperscript{NS}</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>1.305±0.012</td>
</tr>
<tr>
<td></td>
<td>1.315±0.027</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>1.340±0.023 d**</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>1.299±0.028</td>
</tr>
<tr>
<td></td>
<td>1.304±0.020</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>1.366±0.033 e* g\textsuperscript{NS}</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>1.309±0.015</td>
</tr>
</tbody>
</table>

The healthy normal Swiss albino mouse without any treatment is = 1.295±0.021
Significance level = *P<0.1; ** P <0.05; ***P<0.001; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
Control = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
ANS= Animal not survived

PGFRE = Punica granatum fruit rind Ethanol extract.  
PGFRA = Punica granatum fruit rind Acetone extract.  
PGSM = Punica granatum seed Methanol extract.  
PGSC = Punica granatum seed Chloroform extract
Figure 42: Variations in the Lipid peroxidation content (n mol MDA/mg of protein) in the liver of Co$_{60}$ gamma ray irradiated *Swiss albino* mouse with and without *Punica granatum* fruit rind pretreatment.

![Graph showing variations in Lipid peroxidation content with and without Punica granatum pretreatment.](image)

Figure 43: Variations in the Lipid peroxidation content (n mol MDA/mg of protein) in the liver of Co$_{60}$ gamma ray irradiated *Swiss albino* mouse with and without *Punica granatum* seed pretreatment.

![Graph showing variations in Lipid peroxidation content with and without Punica granatum seed pretreatment.](image)
Reduced Glutathione

Glutathione (GSH) content decreased after radiation exposure in the liver of irradiated mice. It decreased up till 7th day post irradiation in control group in comparison to normal. Decrease in GSH content by 32.12%, 36.97%, 42.03%, 44.76% and 39.69% was seen on 3h, 1, 3, 7 and 14 day post irradiation, respectively, in control group in comparison to the normal.

In experimental-1 group (PGFRE+8Gy) the difference was 23.80%, 28.40%, 31.54%, 23.25%, 25.79% and in experimental-2 (PGFRA+8Gy) 15.21%, 19.14%, 24.16%, 16.92%, 11.60% on 3h, 1, 3, 7 and 14 day post irradiation, respectively. In Experimental-1 group, significantly (P<0.05) lesser decrease in glutathione content was observed at all the post irradiation days as compared to Experimental-2 group (Table:11; Figure:44). PGSM pre-treatment reduced it by 15.21%, 21.60%, 25.52%, 21.14%, 17.41% and PGSC provides by 13.48%, 18.53%, 26.84%, 20.44%, 12.24% on 3h, 1, 3, 7 and 14 day post irradiation, respectively. In Experimental-3 group, lesser decrease in glutathione content was recorded at all the post irradiation days as compared to Experimental-4 group(Table:11; Figure:45). Glutathione content of the experimental group was found higher than the corresponding control group at all the intervals. GSH content was higher in PGFRE extract pretreated group than PGFRA, PGSM and PGSC extract treated group.

In only Pomegranate extract treated groups glutathione content increased but remained near to the normal. In all the experimental groups, experimental-1 group had highest GSH level but the difference was statistically non significant.
Table 11: Variations in the reduced glutathione content (GSH) (µmol/g) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td>50.74±1.996 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>47.11±2.19 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>43.33±3.23 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>41.29±2.86 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>45.08±2.03 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>ANS</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>62.82±1.98 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>60.49±2.03 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>57.00±2.27 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>50.89±1.53 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>56.71±1.50 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>58.33±1.26 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>70.09±2.19 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>71.25±1.33 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>72.71±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>73.58±2.19 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>74.16±1.51 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>73.87±2.51 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>58.46±1.81 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>56.13±1.76 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>53.80±1.53 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>48.28±2.03 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>50.31±1.61 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>52.05±1.16 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>68.34±1.81 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>69.80±2.30 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>70.96±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>73.00±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>73.29±1.51 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>72.13±2.19 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>58.46±2.62 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>57.29±1.90 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>54.39±1.25 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>50.02±1.45 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>52.93±2.09 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>54.57±1.25 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>69.40±2.81 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>70.66±1.49 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>72.13±2.66 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>72.71±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>74.08±2.88 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>73.29±3.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>57.58±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>55.84±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>54.96±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>49.73±2.52 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>50.60±1.33 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>51.86±2.76 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>68.93±2.62 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>69.22±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>70.57±2.48 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>72.42±3.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>74.74±2.01 <strong>P&lt;0.001</strong></td>
</tr>
<tr>
<td></td>
<td>73.87±1.81 <strong>P&lt;0.001</strong></td>
</tr>
</tbody>
</table>

The healthy normal Swiss albino mouse without any treatment is = 74.75±1.84
Significance level = *P<0.1; ** P <0.05; ***P<0.001 ; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
Control = Irradiated only to 8Gy of Co\textsuperscript{60} gamma radiation
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1=b; Control V/s Experimental 2=c; Control V/s Experimental 3=d; Experimental 1 V/s Experimental 2=f; Experimental 3 V/s Experimental 4=g
PGFRE = Punica granatum fruit rind Ethanol extract. PGFRA = Punica granatum fruit rind Acetone extract.
PGSM = Punica granatum seed Methanol extract. PGSC = Punica granatum seed Chloroform extract
Figure 44: Variations in the reduced glutathione content (µmol/g) in the liver of Co\textsuperscript{60} gamma ray irradiated \textit{Swiss albino mouse} with and without \textit{Punica granatum} fruit rind pretreatment.

Figure 45: Variations in the reduced glutathione content (µmol/g) in the liver of Co\textsuperscript{60} gamma ray irradiated \textit{Swiss albino mouse} with and without \textit{Punica granatum} seed pre-treatment.
Catalase activity

Catalase activity significantly decreased up till 7th day post irradiation (15.78±0.474 to 12.48±0.272) in only 8Gy irradiated group than the normal (18.19±1.04). After 8Gy irradiation Catalase activity decreased by 13.24%, 20.56%, 28.14%, 31.39% and 28.69% on 3h, 1,3,7 and 14 post irradiation day respectively.

Catalase activity in PG extract pretreated irradiated groups were found higher than the corresponding control group at all the intervals. In Experimental-1 Catalase activity increased by 7.98%, 17.02%, 20.58%, 18.91%, 15.80% & in Experimental-2, by 6.90%, 12.17%, 17.52%, 14.98%, 14.72% on 3h, 1,3,7 and 14 days post irradiation, respectively. In Experimental-1 group, more increase in Catalase activity was recorded at all the post irradiation days as compared to Experimental-2 group (Table:12; Figure:46). In Experimental -3 group, Catalase activity increased by 7.54%, 16.53%, 19.81%, 18.66%, 16.11% and in Experimental-4 group, Catalase activity increased by 5.83%, 10.51%, 12.70%, 11.61%, 10.56% on 3h, 1,3,7 and 14 day post irradiation, respectively in comparison to their corresponding control group. In Experimental-3 group, significant (P<0.1) increase in catalase activity was observed at all the post irradiation days as compared to Experimental-4 group (Table:12; Figure:47). *Punica granatum* fruit rind Ethanol extract pretreated irradiated group had significantly higher catalase activity than the other extract pretreated groups but remained lower than the normal at all the intervals.

In only *Punica granatum* fruit rind and seed extracts treated groups, Catalase activity was near to the normal. Amongst all the experimental groups, experimental-1 had a little more catalase activity as compared to other experimental groups.
Table 12: Variations in the Catalase activity (µ/mg protein) in the liver of Co\textsuperscript{60} gamma ray irradiated *Swiss albino mouse* with and without *Punica granatum* pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td>15.78±0.474 \textsuperscript{aNS}</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>17.04±0.116 \textsuperscript{b*}</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>17.67±0.619</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>16.87±0.222 \textsuperscript{cNS} f\textsuperscript{NS}</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>17.82±0.405</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>16.97±0.150 \textsuperscript{d*}</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>17.61±0.441</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>16.70±0.124 \textsuperscript{eNS gNS}</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>17.55±0.375</td>
</tr>
</tbody>
</table>

The healthy normal *Swiss albino mouse* without any treatment is = 18.19±1.04
Significance level = *P<0.1; ** P <0.05; ***P<0.001 ; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
Control = Irradiated only to 8Gy of Co\textsuperscript{60} gamma radiation
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1=b; Control V/s Experimental 2= c; Control V/s Experimental 3= d; Experimental 1 V/s Experimental 2 =f; Experimental 3 V/s Experimental 4 = g

PGFRE = *Punica granatum* fruit rind Ethanol extract.
PGFRA = *Punica granatum* fruit rind Acetone extract.
PGSM = *Punica granatum* seed Methanol extract.
PGSC = *Punica granatum* seed Chloroform extract.
Figure 46: Variations in the Catalase activity (µ/mg protein) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum fruit rind pretreatment

![Graph showing Catalase activity over time for different pretreatments.]

Figure 47: Variations in the Catalase activity (µ/mg protein) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum seed pretreatment

![Graph showing Catalase activity over time for different pretreatments.]

Superoxide dismutase activity

Superoxide dismutase activity (SOD) significantly (P<0.001) decreased up till 7th day post irradiation in control group as compared to the normal (7.21±0.080). Then it started to recover. Irradiation (8Gy) decreased liver SOD activity by 9.43%, 24.54%, 42.99%, 52.28% and 48.95% after 3h, 1, 3, 7 and 14th post irradiation days respectively, in comparison to normal.

Pretreatment with pomegranate extract significantly reduced decrease in SOD activity in comparison to their corresponding control group at all the post irradiation intervals. In experimental-1 group SOD activity was more by 7.35%, 14.88%, 22.14%, 27.32%, 30.97% and in Experimental-2 group by 3.52%, 10.11%, 17.76%, 12.79%, 44.02% on 3h, 1, 3, 7 and 14 day post irradiation, respectively. In Experimental-1 group, significantly (P<0.1) lesser decrease in superoxide dismutase activity was observed at all the post irradiation days as compared to Experimental-2 group (Table:13; Figure:48). In Experimental-3 group SOD activity was higher by 6.89%, 20.40%, 46.22%, 43.02%, 44.29% in and Experimental-4 group by 7.04%, 20.59%, 46.71%, 43.02%, 44.02% on 3h, 1, 3, 7 and 14 day post irradiation, respectively. In Experimental-3 group, a little more superoxide dismutase activity was observed at all the post irradiation days as compared to Experimental-4 group (Table:13; Figure:49).

In only plant extract treated groups, SOD activity remained higher than the control but near to the normal. Amongst all the experimental groups, experimental-1 group had significantly higher SOD activity as compared to other experimental groups.
Table 13: Variations in the Superoxide dismutase (SOD)(u/mg protein) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum pretreatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>Control (8Gy)</td>
<td>6.53±0.133</td>
</tr>
<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>7.01±0.044</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>7.19±0.075</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>6.76±0.130</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>7.20±0.050</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>6.98±0.181</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>7.22±0.061</td>
</tr>
<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>6.99±0.144</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>7.21±0.068</td>
</tr>
</tbody>
</table>

The healthy normal Swiss albino mouse without any treatment is = 7.21±0.080
Significance level = *P<0.1; ** P <0.05; ***P<0.001; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co\textsuperscript{60} gamma radiation.
Control = Irradiated only to 8Gy of Co\textsuperscript{60} gamma radiation ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1=b; Control V/s Experimental 2=c; Control V/s Experimental 3=d; Control V/s Experimental 4=e; Experimental 1 V/s Experimental 2=f; Experimental 3 V/s Experimental 4=g
PGFRE = Punica granatum fruit rind Ethanol extract. PGFRA = Punica granatum fruit rind Acetone extract.
PGSM = Punica granatum seed Methanol extract. PGSC = Punica granatum seed Chloroform extract
Figure 48: Variations in the Superoxide dismutase (SOD) (u/mg protein) in the liver of Co$^{60}$ gamma ray irradiated Swiss albino mouse with and without Punica granatum fruit rind pretreatment.

![Bar graph showing variations in SOD levels with and without PGF pretreatment.]

Figure 49: Variations in the Superoxide dismutase (SOD) (u/mg protein) in the liver of Co$^{60}$ gamma ray irradiated Swiss albino mouse with and without Punica granatum seed pretreatment.

![Bar graph showing variations in SOD levels with and without PGSM pretreatment.]

Post irradiation time in days: 3h, 1, 3, 7, 14, 28.

SOD levels: Normal, 8Gy, PGFRE +8Gy, PGFRE only, PGFRA +8Gy, PGFRA only.

Post irradiation time in days: 3h, 1, 3, 7, 14, 28.

SOD levels: Normal, 8Gy, PGSM +8Gy, PGSM only, PGSC +8Gy, PGSC only.
Lactate dehydrogenase activity

Lactate dehydrogenase activity (LDH) of liver significantly increased till 7th day post irradiation in comparison to normal (231.37±2.35). Gamma irradiation increased the liver LDH activity by 6.89%, 9.92%, 19.92%, 25.09% and 21.58% in comparison to normal.

LDH activity in different PG extract treated groups was significantly lesser in comparison to their corresponding controls at all the intervals. LDH activity in experimental-1 group (PGFRE+8Gy) was 240.11±1.87, 244.07±2.60, 259.00±2.57, 279.48±1.89, 265.16±1.23 and 256.51±1.68 on 3h, 1, 3, 7, 14 and 28th day respectively. In Experimental-2 group PGFRA+8Gy pretreatment brought down the levels of LDH which were 237.05±1.17, 240.56±1.89, 248.67±0.973, 253.80±1.87, 248.40±1.77 and 241.37±1.08 on 3h 1, 3, 7, 14 and 28th day respectively in comparison to corresponding control. In Experimental-1 group, significantly (P<0.001) lesser LDH activity was observed at all the post irradiation days as compared to Experimental-2 group (Table:14; Figure:50).

In Experimental-3 (PGSM+8Gy) group LDH activity was 239.75±1.89, 241.64±2.47, 253.80±0.938, 271.91±3.78, 262.72±1.62 and 251.64±1.17 on 3h, 1,3,7,14 and 28th day respectively. The administration of PGSC prior to irradiation brought down the levels of LDH which were 235.69±1.08, 242.45±1.68, 250.56±1.23, 265.87±1.71, 251.91±1.17 and 242.45±1.68 on 3h 1, 3, 7, 14 and 28th day respectively as compared to control. In Experimental-3 group, significantly (P<0.001) lesser LDH activity was observed at all the post irradiation days as compared to Experimental-4 group (Table:14; Figure:51).
Table 14: Variations in the Lactate dehydrogenase activity (LDH)(µmg/hr) in the liver of Co$^{60}$ gamma ray irradiated Swiss albino mouse with and without *Punica granatum* pretreatment

<table>
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<th>Treatment</th>
<th>Post irradiation time in days</th>
</tr>
</thead>
<tbody>
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<td>3h</td>
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<tr>
<td>Control (8Gy)</td>
<td>247.32±2.33</td>
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<tr>
<td>Experimental -1 (PGFRE + 8Gy)</td>
<td>240.11±1.87</td>
</tr>
<tr>
<td>PG Rind Ethanol extract only</td>
<td>234.07±2.60</td>
</tr>
<tr>
<td>Experimental -2 (PGFRA + 8Gy)</td>
<td>237.05±1.17</td>
</tr>
<tr>
<td>PG Rind Acetone extract only</td>
<td>231.37±3.27</td>
</tr>
<tr>
<td>Experimental -3 (PGSM +8Gy)</td>
<td>239.75±1.89</td>
</tr>
<tr>
<td>PG seed Methanol extract only</td>
<td>233.00±4.00</td>
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<tr>
<td>Experimental -4 (PGSC+8Gy)</td>
<td>235.69±1.08</td>
</tr>
<tr>
<td>PG seed Chloroform extract only</td>
<td>230.29±4.21</td>
</tr>
</tbody>
</table>

The healthy normal *Swiss albino mouse* without any treatment is = 231.37±2.35
Significance level = *P<0.1; ** P <0.05; ***P<0.001 ; NS- Not significant
Experimental = The plant extract was given 1hr before irradiation to 8Gy of Co$^{60}$ gamma radiation.
Control = Irradiated only to 8Gy of Co$^{60}$ gamma radiation
ANS= Animal not survived
Statistical Comparison = Control V/s sham irradiated = a; Control V/s Experimental 1 = b; Control V/s Experimental 2 = c; Control V/s Experimental 3 = d; Control V/s Experimental 4 = e; Experimental 1 V/s Experimental 2 = f; Experimental 3 V/s Experimental 4 = g

PGFRE = *Punica granatum* fruit rind Ethanol extract.  
PGFRA = *Punica granatum* fruit rind Acetone extract.  
PGSM = *Punica granatum* seed Methanol extract.  
PGSC = *Punica granatum* seed Chloroform extract.
Figure 50: Variations in the Lactate dehydrogenase activity (LDH) (µmg/hr) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum fruit rind pretreatment.

Figure 51: Variations in the Lactate dehydrogenase activity (LDH) (µmg/hr) in the liver of Co\textsuperscript{60} gamma ray irradiated Swiss albino mouse with and without Punica granatum seed pretreatment.
In only plant extract treated groups LDH activity remained lower than the normal at all the intervals. Amongst all the experimental groups, experimental-1 group had highly significant decrease in LDH activity as compared to other experimental groups.