THE RADIOPROTECTIVE EFFICIENCY OF COMBINED ADMINISTRATION OF NATURAL ANTIOXIDANTS (RUTIN AND VITAMIN E) AND A SULFHYDRYL COMPOUND IN IRRADIATED RATS

Rasha R. Radwan, Esmat A. Shaban and Sanaa A. Kenawy*

Department of Drug Radiation Research, National Center for Radiation Research and Technology, Atomic Energy Authority
* Department of Pharmacology and Toxicology – Faculty of Pharmacy – Cairo University

ABSTRACT

In the present study, the radioprotective effects of natural antioxidants, rutin alone, vitamin E alone or both of them combined with a synthetic radioprotector, cysteine, have been investigated in irradiated rats. Furthermore, the oxidative stress biomarkers and certain liver function tests of the whole body irradiated rats were examined. The effect of irradiation was evaluated by exposing the whole body of rats to gamma radiation at acute single dose of 6.5 Gy. Rutin (1.064 mmol/kg) was daily administered orally for two weeks before irradiation, vitamin E (50 mg/100g) was injected intraperitoneally daily for seven days before irradiation, while, cysteine (30 mg/kg) was intra-peritoneally administered 30 min. only before irradiation. Blood and liver malondialdehyde (MDA), glutathione (GSH) and plasma superoxide dismutase (SOD) levels were evaluated. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were also measured in various groups. The data revealed deleterious damage of radiation exposure which was manifested as a significant increase in lipid peroxidation measured as MDA. On the other hand, the activity of SOD and the level of GSH were reduced after irradiation. In addition, the activities of serum ALP, AST and ALT were markedly elevated after radiation exposure. Administration of rutin or vitamin E alone or combined with cysteine before radiation provided a protective effect as measured by the tested parameters. It could be concluded that treatment with the natural antioxidants can control radiation-induced oxidative damage in the biological system. This study indicates that the use of combination of agents is a promising approach for maximizing radioprotection with minimal adverse effects. Administration of cysteine increases the radioprotective effects of rutin and vitamin E against the damaging effects of ionizing radiation.

INTRODUCTION

The need for protection against the toxic effects of ionizing radiation comes from different directions: occupational exposure, nuclear accidents, environmental sources and protection of normal tissue during the therapeutic irradiation of cancer (Roberts et al., 1995). One of the possibilities of increasing radioresistance of an organism is the use of chemical protectors that substantially reduce the damaging effect of radiation (Hassan, 1994). It has been considered that, radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissues (Weiss and Landauer, 2003).

Radioprotectors could be identified as chemical compounds capable of ameliorating the biological influences of ionizing radiation when administered before radiation exposure. The efficiency of these radioprotectors is greatly dependent on their chemical properties, period of treatment and the post irradiation time elapsing after radioprotectors application (Monig et al., 1990). However, no ideal, safe synthetic radioprotector is available to date, so the search for alternative sources, including plants, has been going on for several decades (Arora et al., 2005).
A large number of drugs have been screened for their radioprotective efficacy, however, because of the inherent toxicity at useful concentrations, none of them could find clinical acceptance (Singh and Yadav, 2005). Sulphydryl radioprotectors are one of the best radioprotectors known today. Their use encounters two great difficulties; their toxicity and the short period during which they are active (Hassan and El-Kady, 2002).

As many other flavonoids rutin is a good antioxidant. Rutin and its aglycone quercetin have been shown to inhibit lipid peroxidation in vivo and in vitro (Ngère-Salvayre et al., 1991). They were found to be scavengers for superoxide anion (Middleton and Kandaswami, 1992) as well as hydroxyl radicals (Metodiewa et al., 1997). In addition, they effectively bind iron thus limit its ability to catalyze free radicals formation (Afanas’ev et al., 1989).

Vitamin E is classified as a fat-soluble vitamin because of its solubility in solvents of low polarity. The solubility in aqueous media is, therefore, relatively low and vitamin E will tend to either partition into tissue lipids or, in translation through the body, locate in hydrophobic domains of molecular structures like lipoproteins. Within the cell, vitamin E partitions into the hydrophobic core of the various cell membranes. The relative concentration of vitamin E differs from one membrane to another (Wang and Quinn, 1999).

Low to moderate doses of several radioprotective agents acting via different mechanisms markedly improved the degree of protection while maintaining toxicity within acceptable limits (Maisin, 1998).

The aim of the present work was to study the oxidative stress biomarkers and certain liver function tests of the whole body gamma irradiated rats. For this purpose, normal groups of animals were exposed to gamma radiation. Blood and liver malondialdehyde and glutathione levels as well as plasma superoxide dismutase activity were evaluated. Serum AST, ALT and ALP activities were also measured.

The therapeutic potential of natural antioxidants such as vitamin E and rutin in absence or presence of cysteine was examined in the irradiated rats, in order to study their possible effect on the oxidative stress and liver function.

**MATREALS AND METHODS**

**Animals**

Adult male Wistar albino rats, weighing 150-200 g, were obtained from the National Research Center (Giza, Egypt). The animals were kept under suitable laboratory conditions throughout the period of investigation. They were allowed free access to food consisting of standard pellets and water *ad libitum.*

**Drugs**

Rutin (Byron Chemical Company, USA): It was freshly dissolved in distilled water and orally administered daily for two weeks before irradiation in a dose of 1.064 mmol/kg/day.

Vitamin E (Pharco Pharmaceuticals, Egypt): It was dissolved in sunflower oil and injected i.p daily for seven days before irradiation in a dose of 50 mg/100g/day.

Cysteine (Sigma Chemical Company, USA): It was freshly dissolved in distilled water and i.p administered 30 min. before irradiation in a single dose of 30 mg/kg.

**Experimental design**

A pilot experiment was carried out in order to select the suitable time for evaluating the effect of radiation. Four groups of animals (each of 7 rats) were used. The 1st gp included non irradiated animals, while the other 3 gps were examined 1, 3 and 7 days following exposure to radiation, respectively.

Three days following exposure to radiation was selected to be the suitable time for evaluating the toxic effects of radiation.

Ten groups of animals (each of 7 rats) were used in the present study. The animals of the 1st four groups were not irradiated.

1<sup>st</sup> group: received saline and served as normal control.

2<sup>nd</sup> group: received rutin (1.064 mmol/kg, orally) daily for two weeks.

3<sup>rd</sup> group: received vitamin E (50mg/100g, i.p) daily for seven days.

4<sup>th</sup> group: received cysteine (30 mg/kg, i.p) 30 min. before sacrifice.

5<sup>th</sup> group: received saline then irradiated (6.5 Gy) and served as irradiated control.

6<sup>th</sup> group: received rutin (1.064 mmol/kg, orally) daily for two weeks then irradiated (6.5 Gy).

7<sup>th</sup> group: received vitamin E (50mg/100g, i.p) daily for seven days then irradiated (6.5 Gy).

8<sup>th</sup> group: received cysteine (30 mg/kg, i.p) 30 min. before irradiation.

9<sup>th</sup> group: received rutin (1.064 mmol/kg, orally) daily for two weeks and cysteine (30 mg/kg, i.p) 30 min. before irradiation.

10<sup>th</sup> group: received vitamin E (50mg/100g, i.p) daily for seven days and cysteine (30 mg/kg, i.p) 30 min. before irradiation.
The third day following irradiation was selected on which animals were sacrificed, blood was collected and liver was isolated.

**Irradiation of animals**

Rats were exposed to whole body gamma radiation by an acute single dose of 6.5 Gy delivered at a dose rate of 0.48 Gy/min. The radiation source was $^{137}$Cs using Gamma Cell-40 biological irradiator, belonging to the National Center for Radiation Research and Technology, Cairo, Egypt.

**Measured parameters**

Lipid peroxide levels were determined in plasma and liver homogenates as thiobarbituric acid reactive substances (TBARS). It was determined according to the method of Yoshioka et al. (1979).

GSH level in blood was determined according to the method described by Beutler and his colleagues (1963), while GSH level in liver homogenate was determined according to the method described by Ahmed et al. (1991).

SOD activity was determined in blood. The assay was carried out according to the method of Marklund and Marklund (1974).

Alkaline phosphatase activity was determined kinetically in serum using a test reagent kit according to the method of Rec. GSCC. (1972), whereas, AST and ALT were determined in serum. AST and ALT were assayed colorimetrically using a test reagent kit according to the method of Reitman and Frankel (1957).

**Statistical Analysis**

All the values were expressed as means ± S.E. Comparisons between means were carried out using one-way ANOVA followed by Tukey-Kramer multiple comparison test using Instat software, version 2 (Graphpad Software, Inc., San Diego, USA).

**RESULTS**

Effect of whole body $\gamma$ irradiation on oxidative stress biomarkers and the activities of liver enzymes in rats 1, 3 and 7 days following irradiation.

The results are shown in Table (1) and illustrated in Figure (1).

Blood GSH level of normal animals was $51.60 \pm 2.64$ mg%, while that of irradiated rats was significantly decreased in a time-dependent manner by 15, 38 and 45% at 1, 3 and 7 days following exposure to $6.5$ Gy $\gamma$ radiation, respectively, as compared with that of normal animals.

Liver GSH content of normal animals was $22.00 \pm 0.60$ mg/g tissue, while that of irradiated rats was significantly decreased in a time-dependent manner by 20, 31 and 39% at 1, 3 and 7 days after animal exposure to $6.5$ Gy $\gamma$ radiation, respectively.

Plasma SOD activity of normal animals was found to be $1.27 \pm 0.01$ U/ml, while that of irradiated rats was significantly decreased in a time-dependent manner by 12, 41 and 46% at 1, 3 and 7 days after exposure to radiation, respectively.

Serum ALP activity of normal animals was $105.17 \pm 3.08$ U/L, and that of irradiated rats at 3 and 7 days was significantly increased by 66 and 50% after animal exposure to $6.5$ Gy $\gamma$ radiation, respectively.

Serum AST activity of normal animals was $70.28 \pm 0.92$ U/L, and that of irradiated rats at 3 and 7 days was significantly increased by 57 and 30 % after animal exposure to $6.5$ Gy $\gamma$ radiation, respectively, as compared with that of normal animals.

Serum ALT activity of normal animals was $52.28 \pm 0.92$ U/L, and that of irradiated rats at 3 and 7 days was significantly increased by 83 and 61% after irradiation, respectively, as compared with normal values.

The results concerning liver MDA content and activities of serum ALP, AST and ALT revealed that 3 days following exposure to $\gamma$ radiation is the suitable time for the further investigations.

Effect of rutin or vitamin E alone or combined with cysteine on oxidative stress biomarkers in normal and whole body irradiated rats.

The results are shown in Tables (2) and (3) and illustrated in Figures (2), (3) and (4).

Daily oral administration of rutin (1.064 mmol/kg) for two weeks to normal non irradiated animals only elevated SOD activity by 14 % (Table 2, Figure 2). However, when administered two weeks before irradiation, it resulted in a reduction of plasma and liver MDA levels by 20 and 25%, respectively, elevation of blood and liver GSH content by 51 and 29% respectively, and elevation of plasma SOD activity by 45 % (Table 2 and Figure 3).
Table (1): Effect of whole body gamma irradiation on oxidative stress biomarkers and the activities of liver enzymes in rats 1, 3 and 7 days following irradiation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal</th>
<th>Days after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>Plasma MDA (nmol/ml)</td>
<td></td>
<td>6.30 ± 0.13</td>
<td>7.90* ± 0.70</td>
</tr>
<tr>
<td>Liver MDA (nmol/g tissue)</td>
<td></td>
<td>161.00 ± 0.85</td>
<td>173.20 ± 1.13</td>
</tr>
<tr>
<td>Blood GSH (mg %)</td>
<td></td>
<td>51.60 ± 2.64</td>
<td>43.84* ± 1.99</td>
</tr>
<tr>
<td>Liver GSH (mg/g tissue)</td>
<td></td>
<td>22.00 ± 0.60</td>
<td>17.56 ± 0.39</td>
</tr>
<tr>
<td>Plasma SOD (U/ml)</td>
<td></td>
<td>1.27 ± 0.01</td>
<td>1.11* ± 0.03</td>
</tr>
<tr>
<td>Serum ALP (U/L)</td>
<td></td>
<td>105.17 ± 3.08</td>
<td>116.07 ± 3.51</td>
</tr>
<tr>
<td>Serum AST (U/L)</td>
<td></td>
<td>70.28 ± 0.92</td>
<td>76.14 ± 2.23</td>
</tr>
<tr>
<td>Serum ALT (U/L)</td>
<td></td>
<td>52.28 ± 0.92</td>
<td>56.00 ± 1.53</td>
</tr>
</tbody>
</table>

Three groups of animals each consisting of 7 rats were exposed to γ radiation (6.5 Gy). Blood was collected and liver was isolated from the animals of the three irradiated gps 1, 3 and 7 days following γ radiation, respectively. A fourth gp was unexposed to γ radiation and served as normal gp. Each value represents mean ± S.E of the mean. Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. * Significantly different from normal group at p ≤ 0.05.

Figure (1): Effect of whole body gamma irradiation on oxidative stress biomarkers and the activities of liver enzymes in rats 1, 3 and 7 days following irradiation.

Administration of a single i.p dose of cysteine (30 mg/kg) before irradiation resulted in reduction of plasma and liver MDA levels by 16 and 28%, respectively, elevation of blood and liver GSH contents by 40 and 21%, respectively, and elevation of plasma SOD activity by 23% (Tables 2, 3 and Figures 3, 4).

Administration of daily i.p dose of vitamin E (50 mg/100g) for seven days to normal animals only elevated SOD activity to be 1.43 ± 0.03 U/ml (Table 3 and Figure 2), while, when given for seven days before irradiation, it resulted in reduction of plasma and liver MDA levels by 24 and 34 %, respectively, elevation of blood and liver GSH content by 47 and 36 %, respectively, and elevation of plasma SOD activity by 40 % (Table 3 and Figure 4).

Combined therapy of rutin and cysteine before irradiation resulted in a reduction of plasma and liver MDA levels by 27 and 37 %, respectively, elevation of blood and liver GSH contents by 65 and 43% respectively, and elevation of plasma SOD activity by 60 % (Table 2 and Figure 3).

Combined treatment of vitamin E with cysteine before irradiation resulted in reduction of plasma and liver MDA levels by 34 and 42 %, respectively, elevation of blood and liver GSH contents by 59 and 50 %, respectively, and elevation of plasma SOD activity by 48 % (Table 3 and Figure 4).
Table (2): Effect of rutin alone or combined with cysteine on oxidative stress biomarkers in normal and whole body irradiated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>SOD</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma (U/ml)</td>
<td>Liver (mg/ml)</td>
<td>Blood (mg%)</td>
</tr>
<tr>
<td>Normal (Saline 10 ml/kg)</td>
<td>6.30±0.13</td>
<td>161.00±0.85</td>
<td>51.60±2.64</td>
<td>22.00±0.60</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg, oral / day for 2 weeks)</td>
<td>5.83±0.19</td>
<td>154.74±1.61</td>
<td>57.83±0.49</td>
<td>23.83±0.84</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before sacrifice)</td>
<td>6.04±0.20</td>
<td>144.63±2.49</td>
<td>53.63±1.17</td>
<td>23.23±0.65</td>
</tr>
<tr>
<td>Irradiated (6.5 Gy) (Saline 10 ml/kg)</td>
<td>10.37±0.27</td>
<td>311.41±6.23</td>
<td>31.83±1.11</td>
<td>15.10±0.59</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg) +Irradiated (6.5 Gy)</td>
<td>8.24±0.27</td>
<td>233.24±9.37</td>
<td>47.96±1.22</td>
<td>19.57±0.62</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before sacrifice)</td>
<td>8.74±0.19</td>
<td>226.61±5.34</td>
<td>44.61±1.25</td>
<td>18.26±0.29</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg) +Cysteine (30 mg/kg) +Irradiated (6.5 Gy)</td>
<td>7.54±0.24</td>
<td>196.47±3.56</td>
<td>52.67±1.22</td>
<td>21.64±0.97</td>
</tr>
</tbody>
</table>

Three groups of animals each consisting of 7 normal rats were used in all experiments. Blood was collected and liver was isolated post treatment in the 1st three gps and 3 days following γ irradiation in the last four gps. Each value represents the mean ± S.E of the mean. Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. * Significant difference from normal group at p ≤ 0.05 @ Significant difference from irradiated group at p ≤ 0.05

Table (3): Effect of vitamin E alone or combined with cysteine on oxidative stress biomarkers in normal and whole body irradiated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>SOD</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma (U/ml)</td>
<td>Liver (nmol/ml)</td>
<td>Blood (mg%)</td>
</tr>
<tr>
<td>Normal (Saline 10 ml/kg)</td>
<td>6.30±0.13</td>
<td>161.00±0.85</td>
<td>51.60±2.64</td>
<td>22.00±0.60</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g, i.p./day for 1 week)</td>
<td>5.76±0.15</td>
<td>151.53±2.29</td>
<td>56.20±0.85</td>
<td>24.61±0.85</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before sacrifice)</td>
<td>6.04±0.20</td>
<td>144.63±2.49</td>
<td>53.63±1.17</td>
<td>23.23±0.65</td>
</tr>
<tr>
<td>Irradiated (6.5 Gy) (Saline 10 ml/kg)</td>
<td>10.37±0.27</td>
<td>311.41±6.23</td>
<td>31.83±1.11</td>
<td>15.10±0.59</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g) +Irradiated (6.5 Gy)</td>
<td>7.90±0.36</td>
<td>205.14±3.87</td>
<td>46.63±1.71</td>
<td>20.61±0.92</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before irradiation) +Irradiated (6.5 Gy)</td>
<td>8.74±0.19</td>
<td>226.61±5.34</td>
<td>44.61±1.25</td>
<td>18.26±0.29</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g) +Cysteine (30 mg/kg) +Irradiated (6.5 Gy)</td>
<td>6.84±0.39</td>
<td>181.70±2.24</td>
<td>50.67±1.37</td>
<td>22.70±0.82</td>
</tr>
</tbody>
</table>

Three groups of animals each consisting of 7 normal rats were used in all experiments. Blood was collected and liver was isolated post treatment in the 1st three gps and 3 days following γ irradiation in the last four gps. Each value represents the mean ± S.E of the mean. Statistics carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. * Significant difference from normal group at p ≤ 0.05 @ Significant difference from irradiated group at p ≤ 0.05
Figure (2): Effect of rutin, cysteine and vitamin E on blood level and liver contents of malondialdehyde and glutathione and the activity of plasma superoxide dismutase in normal rats.

Four groups of animals each consisting of 7 normal rats were used. They received saline (10 ml/kg control gp), rutin (1.064 mmol/kg, oral/day for 2 weeks), cysteine (30 mg/kg, i.p., 30 min. before sacrifice) and vitamin E (50 mg/100g, i.p./day for 1 week), respectively. The 1st gp served as normal gp. Blood was collected and liver was isolated for further investigation. Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

* Significantly different from normal control group at $p \leq 0.05$.

Figure (3): Effect of rutin alone or combined with cysteine on blood and liver contents of malondialdehyde and glutathione and the activity of plasma superoxide dismutase in whole body $\gamma$ irradiated rats.

Four groups of animals each consisting of 7 rats were used. They received saline (10 ml/kg), rutin (1.064 mmol/kg, oral/day for 2 weeks), cysteine (30 mg/kg, i.p., 30 min. before irradiation) and rutin+cysteine then the animals were exposed to gamma radiation. The 1st gp served as irradiated control. Blood was collected and liver was isolated 3 days following $\gamma$ irradiation for further investigation. Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

* Significantly different from irradiated group at $p \leq 0.05$. 
The pilot experiment carried out in the current study in order to select the suitable time for evaluating the effect of radiation exposure showed that the plasma level of MDA was increased on the 1st, 3rd, and 7th day following exposure, while the increase of liver MDA content was only significant on the 3rd and 7th day following exposure.

The increased plasma and liver MDA contents found in the present study due to exposure to gamma radiation is in agreement with those of previous studies (Saada and Azab, 2001; Azab et al., 2004) which revealed that exposure to ionizing radiation induced lipid peroxidation. Similar results were produced in rat liver microsomes (Varshney and Kale, 1990), rat spleen lymphocytes (Kucherenko et al., 1991) and plasma of inflamed rats (El-Ghazaly and Khayyal, 1995).

The present results are also in accordance with the results of Ramadan and El-Ghazaly (1997), who found that whole body gamma irradiation (6.5 Gy) induced a significant increase in MDA concentration of plasma and liver homogenate of rats on the 1st, 2nd, 7th and 14th day post-irradiation. Whole body exposure of rats to lower dose of y-radiation at 3.5 Gy (Sridharan and Shyamaladevi, 2002) as well as at 4.5 Gy (Abbady et al., 1999) also caused increases in lipid peroxidation. Another study of Hassan et al. (1996) demonstrated that MDA contents in liver, spleen, intestine, kidney, lung and brain of rats 3 days post exposure with sublethal dose of 4 Gy were markedly increased as compared to normal values.

**DISCUSSION**

The pilot experiment carried out in the current study in order to select the suitable time for evaluating the effect of radiation exposure showed that the plasma level of MDA was increased on the 1st, 3rd, and 7th day following exposure, while the increase of liver MDA content was only significant on the 3rd and 7th day following exposure.

The increased plasma and liver MDA contents found in the present study due to exposure to gamma radiation is in agreement with those of previous studies (Saada and Azab, 2001; Azab et al., 2004) which revealed that exposure to ionizing radiation induced lipid peroxidation. Similar results were produced in rat liver microsomes (Varshney and Kale, 1990), rat spleen lymphocytes (Kucherenko et al., 1991) and plasma of inflamed rats (El-Ghazaly and Khayyal, 1995).

The present results are also in accordance with the results of Ramadan and El-Ghazaly (1997), who found that whole body gamma irradiation (6.5 Gy) induced a significant increase in MDA concentration of plasma and liver homogenate of rats on the 1st, 2nd, 7th and 14th day post-irradiation. Whole body exposure of rats to lower dose of y-radiation at 3.5 Gy (Sridharan and Shyamaladevi, 2002) as well as at 4.5 Gy (Abbady et al., 1999) also caused increases in lipid peroxidation. Another study of Hassan et al. (1996) demonstrated that MDA contents in liver, spleen, intestine, kidney, lung and brain of rats 3 days post exposure with sublethal dose of 4 Gy were markedly increased as compared to normal values.
Table (4): Effect of rutin or vitamin E alone or combined with cysteine on serum ALP, AST and ALT activities in whole body γ irradiated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>ALP (Serum (U/L))</th>
<th>AST (Serum (U/L))</th>
<th>ALT (Serum (U/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Saline 10 ml/kg)</td>
<td></td>
<td>105.17±3.08</td>
<td>70.28±0.92</td>
<td>52.28±0.92</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg, oral/day for 2 weeks)</td>
<td></td>
<td>98.17±2.38</td>
<td>68.14±1.29</td>
<td>49.57±0.78</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before sacrifice)</td>
<td></td>
<td>96.63±3.08</td>
<td>66.43±1.66</td>
<td>48.43±0.97</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g, i.p./day for 1 week)</td>
<td></td>
<td>94.28±2.29</td>
<td>63.43±2.36</td>
<td>46.43±1.09</td>
</tr>
<tr>
<td>Irradiated (6.5 Gy, Saline 10 ml/kg)</td>
<td></td>
<td>174.53±3.37</td>
<td>110.71±3.21</td>
<td>95.50±2.30</td>
</tr>
<tr>
<td>Rutin (1.064mmol/kg) + Irradiated (6.5 Gy)</td>
<td></td>
<td>142.54±2.51</td>
<td>92.21±2.21</td>
<td>79.71±3.36</td>
</tr>
<tr>
<td>Cysteine (30mg/kg) +Irradiated (6.5 Gy)</td>
<td></td>
<td>133.98±3.11</td>
<td>87.07±2.01</td>
<td>76.00±2.19</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g) + Irradiated (6.5 Gy)</td>
<td></td>
<td>127.76±3.11</td>
<td>83.50±2.75</td>
<td>72.14±2.18</td>
</tr>
<tr>
<td>Rutin (1.064mmol/kg) + Cysteine (30mg/kg) + Irradiated (6.5 Gy)</td>
<td></td>
<td>125.43±2.91</td>
<td>79.14±0.79</td>
<td>64.43±1.19</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g) + Cysteine (30mg/kg) + Irradiated (6.5 Gy)</td>
<td></td>
<td>114.53±3.36</td>
<td>75.86±1.75</td>
<td>58.43±1.76</td>
</tr>
</tbody>
</table>

Groups of animals each consisting of 7 normal rats were used in all experiments. Blood was collected and liver was isolated post treatment in the 1st four gps and 3 days following γ irradiation in the last six gps for further investigation.

Each value represents the mean ± S.E of the mean.

Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

* Significant difference from normal group at p ≤ 0.05

@ Significant difference from irradiated group at p ≤ 0.05

Figure (5): Effect of rutin, vitamin E and cysteine on serum ALP, AST and ALT activities in normal animals.

Four groups of animals each consisting of 7 normal rats were used. They received saline (10 ml/kg), rutin (1.064 mmol/kg, oral/day for 2 weeks), cysteine (30 mg/kg, i.p., 30 min. before sacrifice) and vitamin E (50 mg/100g, i.p./day for 1 week) respectively. The 1st gp served as normal. Blood was collected post treatment for further investigation.
In the current study, whole body γ-irradiation (6.5 Gy) induced a significant decrease in GSH level in blood and liver homogenate of rats 1st, 3rd and 7th day post-irradiation. The present results were in agreement with those of Yamaoka et al. (2000) and Baliga et al. (2004).

Gamma irradiation was shown to generate free radicals as a secondary event following the ionization of biological molecules (Kiefer, 1990). In this respect, the fixed relation was proved between oxidative stress and decrease in GSH level (Alhar and Iqbal, 1998; Osman, 2003). This leads to the suggestion that the reduction in GSH content might be attributed to its consumption by free radicals.

On the contrary, the experiments carried out by Sridharan and Shyamaladevi (2002) showed that, whole body exposure of rats to γ rays (3.5 Gy) caused increases in the concentrations of GSH and total sulphydryl groups (TSH), probably to counteract the damages produced by the lipid peroxides.

In the current study, plasma SOD activity of normal as well as whole body γ irradiated animals were measured. Results showed a significant reduction in blood SOD activity 1st, 3rd, and 7th day following radiation exposure which was in conformity with the findings of El-Shamy et al. (2001), who found a decrease in blood and liver activity of SOD at 24 and 72 hr post irradiation with a dose of 6.5 Gy.

The present decrease in blood SOD activity after whole body gamma irradiation was in agreement with the results of the studies carried out by Nommura and Yamaoka (1999); Yamaoka et al. (2000) and El-shamy et al. (2001).

It is more likely that the decrease in SOD enzyme activity could be attributed to its inactivation by the increase in reactive oxygen species or lipid peroxides (Hasegawa et al., 1992). There is support for this concept in recent studies by Raja et al. (2007), who reported that during hepatic injury, superoxide radicals generate at the site of damage and modulate SOD, resulting in the loss of activity and accumulation of superoxide radicals, which cause a damage for liver cells.

It seems that serum AST and ALT are the most sensitive markers employed in the diagnosis of hepatic damage due to their location in the cytoplasm and hence released into the circulation after cellular damage (Pradeep et al., 2007). Data of the present investigation showed that whole body γ irradiation (6.5 Gy) induced a significant elevation in activities of serum ALP, AST as well as ALT. The increase in the enzyme activities was only on the 3rd and 7th day following exposure to γ rays compared to normal values. This was in agreement with previous reports (Todorov and Damianov, 1985; Khamis et al., 1989; Kafafy, 2000; Ramadan et al., 2002).

Since the increase in serum liver enzymes was prominent only on the 3rd and 7th days following exposure, this leads to the suggestion that the period, 1 day following exposure might not be enough to influence MDA content in liver homogenate and serum ALP, AST and ALT activities.

Concerning ALP activity, it seems that there is a strong correlation between the liver enzyme activity and that of the serum, i.e. the changes occurred in the serum levels were related to the changes that
occurred in the liver (Roushdy et al., 1984). Elevated serum activity of ALP appears to reflect cholestatic injury (Plaa and Hewitt, 1982; Stacey et al., 1993; Martin and Friedman, 1998). The increase in its activity due to irradiation could be attributed to liver disturbances particularly to cell membrane permeability and release of this enzyme from the tissues to the blood stream (Roushdy et al., 1984).

The observation by Mahdy and El-Kashef (1988) showed that whole body gamma irradiation of albino rats at the dose of 7 Gy seriously increased the activity of both serum AST and ALT on the 3rd up to the 10th days post irradiation. In addition, some investigators have reported that the increase in the activities of serum transaminases after radiation exposure was concordant with the radiation dose received and could be attributed to cellular destruction in several extrahepatic tissues (Manciulea et al., 1978; El-Naggar et al., 1980).

Oxidative stress is a common mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders. Increasing evidence indicates the role of oxidative stress in liver injury, cirrhosis development and carcinogenesis (Stal and Olsson, 2000). Hence, there is a great demand for the development of agents with potent antioxidant effect (Pradeep et al., 2007).

The results of the present investigation showed that administration of rutin, vitamin E and cysteine did not alter plasma level or liver MDA content in normal animals. Similar results were obtained concerning GSH. However, administration of rutin and vitamin E resulted in a significant increase only in plasma SOD activity compared to those of normal animals.

In the current experiments, administration of rutin before radiation exposure ameliorated, to a great extent the damaging effects of radiation on lipid peroxidation in plasma and liver homogenate of rats. This was in agreement with the results of Park et al. (2002) and Osman (2003). This suggested a possible radioprotective effect of rutin against radiation-induced lipid peroxidation indicating its beneficial role in scavenging free radicals and reactive oxygen species as a promising antioxidant (Grinberg et al., 1994; Saija et al., 1995; Haenen et al., 1997). These results revealed the usefulness of flavonoids and polyphenolics as natural antioxidants.

There is a support for this concept in other studies carried out by La casa et al. (2000), who established the use of rutin against lipid peroxidation.

The data of the present study revealed that, oral administration of rutin tended to normalize the depletion of both blood and liver GSH content induced by radiation exposure, results which are in line with previous studies (Schmitt et al., 1995; Rohman and Mac, 2000; Osman, 2003). Rutin also provided protection against radiation-induced reduction in SOD activity which is in accordance with the results of Russo et al. (2000) and Kahraman et al. (2003).

Moreover, the study of Russo et al. (2000) to investigate the superoxide anion scavenging capacity of rutin, revealed that rutin inhibited the superoxide anion radicals formation in a dose-dependent manner by inhibiting xanthine oxidase enzyme activity involved in their formation.

Rutin administered prophylactically for two weeks, protected against irradiation-induced liver dysfunction, as evidenced by prevention of elevation in serum ALP, AST and ALT activities. Results are in accordance with previous reports (Rajnarayana et al., 2001; Ray et al., 2006; Raja et al., 2007).

Cysteine, a synthetic radioprotector used in the present study, did not alter the blood or liver MDA content as compared with normal animals. the results are in line with previous studies done by El-Shamy et al. (2001), Neal et al. (2003) and Azab et al. (2004). Furthermore, pretreatment with cysteine before exposure to radiation in the current work, decreased the elevation in both blood and liver MDA levels triggered by irradiation, but the level did not reach that of normal values. The present findings are in agreement with the results of El-Shamy et al. (2001) as well as Azab et al. (2004).

Several mechanisms have been proposed to explain the radioprotective effects of sulphhydryl compounds including free radical scavenging, hydrogen atom donation by –SH groups and –COOH groups (Upadhyay and Kumar, 2004), repair of free radicals in target molecules, induction of hypoxia, target stabilization by binding to DNA, mixed disulfide formation and general enhanced protection from oxidative stress (Muray, 1998; Agrawal and Kale, 2001). Among these possibilities, the first three deserve the most serious consideration (Hassan and El-Kady, 2002).

The present findings revealed that pretreatment with cysteine minimized the reduction in both blood and liver GSH content induced by irradiation, but the level was less than the normal values. These findings are in conformity with those of Holdiness (1991), Meyer et al. (1994), El-Shamy et al. (2001) as well as Neal et al. (2003). More evidences were provided by Neal et al. (2003) who recorded that N-acetyl cysteine protected the blood cells from GSH depletion following radiation exposure.

Pretreatment with cysteine before exposure to radiation decreased the reduction in blood SOD, but the level did not reach that of the normal values. The current results are in agreement with the data of El-Shamy et al. (2001).
The results of the present experiment revealed that pretreatment with cysteine afforded protection against elevation in serum ALP, AST and ALT activities induced by irradiation, but the level was higher than that of the normal values. It is important to notify that, there was no significant difference in MDA concentration in plasma as well as in liver homogenate between the normal group and the non-irradiated vitamin E treated group. These results are in agreement with the study done by Ramadan and El-Ghazaly (1997).

The potential role of vitamin E to prevent radiation-induced damage has been investigated in the present study. The results demonstrated that administration of α-tocopherol as pretreatment led to a highly significant decrease of MDA levels, as has been reported by several studies (Carpenter, 1991; Schmitt et al., 1995; Ramadan and El-Ghazaly, 1997; Kotzampassi et al., 2003). Administration of α-tocopherol into rats before exposure to radiation seems to exert a beneficial prophylactic effect, since it decreased lipid peroxidation. Therefore, the administration of α-tocopherol before the irradiation of rats could offer a considerable protection against radiation-induced liver injury (Kotzampassi et al., 2003).

It has been observed by Ramadan and El-Ghazaly (1997) that administration of vitamin E before exposure to radiation caused a reduction of MDA concentrations in liver and spleen homogenates as well as in plasma of irradiated rats 1st, 2nd, 7th and 14th day post-irradiation. This protective effect of vitamin E could be attributed to its role in terminating the peroxidative reactions of unsaturated fatty acids, because of its antioxidant capacity and lipophilic character (Halliwell, 1989). Vitamin E has a strong physical interaction with polyunsaturated fatty acids in the cell membrane (Lucy, 1972). It can effectively protect the cell membranes through its protection of polyunsaturated fatty acids against radiation-induced peroxidation (Konings and Drijver, 1979).

The principal role of α-tocopherol as an antioxidant is to scavenge the lipid peroxyl radical before its ability to attack the target lipid substrate (Wang and Quinn, 1999). Pretreatment of rats with α-tocopherol before exposure to γ radiation in the current study, inhibited glutathione depletion induced by radiation, the results are in accordance with the findings of previous studies (Zeji et al., 1994; Schmitt et al., 1995; Kotzampassi et al., 2003). More evidences were provided by Kotzampassi et al. (2003) who showed that α-tocopherol helps to maintain high blood levels of reduced glutathione after irradiation.

In the current experiments, it is important to notify that there was a slight increase in blood SOD activity in the vitamin E treated group when compared to normal rats. On the other hand, pretreatment with vitamin E minimized the reduction in blood SOD activity induced by irradiation, but the level was, however, less than the normal values.

The present results revealed that pretreatment with vitamin E afforded protection against elevation in serum ALP, AST as well as ALT activities induced by irradiation, but the activity was, however, less than that of the normal rats. These results are in agreement with the study done by Zaidi et al. (2005).

The present data indicated that the combined administration of vitamin E or rutin with cysteine exerted a favourable recovery effect in most of the measured parameters. The results were in line with those of previous studies (Shaheen and Hassan, 1991; Kafafy, 2000; Hassan and El-Kady, 2002).

The radioprotective effect of cysteine, vitamin E and their combination on adrenocortical function was estimated by Shaheen et al. (1990) in male rats 24 and 48 hrs after whole body γ irradiation at a dose level of 7.5 Gy. The results showed that, combination of cysteine and vitamin E, effectively enhanced the survival of the irradiated rats than those treated with either agent alone (Hassan, 1994).

Hassan and El-Kady (2002) evaluated the radioprotective effect of cysteine, vitamin E and their combination on gamma irradiation-induced alteration in some haematological parameters such as blood GSH content, RBCs counts and haemoglobin level. The combination of both agents afforded a better protection, so that most of the measured parameters were restored to the pre-irradiated values.

Again, the improvement in the radioprotective action of cysteine by combined administration with vitamin E could be explained in the light of the antioxidant property of vitamin E (Hassan and El-Kady, 2002). The role of vitamin E as a natural antioxidant in various biological systems has been well established (Green, 1969). Being a powerful peroxide inhibitor, vitamin E has been proposed as having a stabilizing effect on membrane structure through interaction with the fatty acyl chains of polyunsaturated phospholipids (Liebler et al., 1986).

The radioprotection provided by combined administration of vitamin E and cysteine is feasible and perhaps, even more efficient against radiation injury to RBCs (Hassan and El-Kady, 2002). This will appreciate the usage of such combination in protecting patients during radiotherapy.

It has been documented that combined administration of cysteine and vitamin E to 3 Gy irradiated rats effectively controlled the radiation induced elevation in AST and ALT activities (Kafafy, 2000).
Protective action of combined administration of cysteine and vitamin E may be explained on the basis of its free radical scavenging ability, peroxide decomposition and catalyzing sulfhydryl disulfide exchange (Jacobs et al., 1983; Ketterer et al., 1983; Kafafy, 2000).

It can be concluded that prophylactic treatment with the natural antioxidants rutin or vitamin E alone or in combination with cysteine produced a protective effect against radiation-induced damage in the biological systems. Thus, such treatment aids in counteracting many of the risks associated with oxidative stress imbalance and liver toxicity, and hence modulate severity of the injury.

REFERENCES


