ABSTRACT

In the present experiments, a study of the radioprotective effects of natural antioxidants, rutin alone, vitamin E alone or each of them combined with synthetic radioprotector, cysteine, have been investigated in feverish irradiated rats. Furthermore, the oxidative stress biomarkers of the feverish whole body irradiated rats were examined. The data revealed deleterious damage of radiation exposure which was manifested as significant increase in lipid peroxidation measured as malondialdehyde (MDA). On the other hand, the activity of plasma superoxide dismutase (SOD) and the blood level of glutathione (GSH) were reduced after irradiation.

Effect of hyperthermia on oxidative stress biomarkers in rats were studied in this work and data showed that the increase in body temperature intensified lipid peroxidation processes where MDA in plasma and liver homogenate was increased, GSH level in blood and liver homogenate was also increased while plasma SOD activity was reduced.

Effect of rutin alone, vitamin E alone or each of them combined with cysteine on oxidative stress biomarkers in hyperthermic irradiated rats was investigated. Results indicated that, pretreatment with vitamin E and rutin alone or combined with cysteine before the onset of hyperthermia significantly attenuated fever–induced increase in free radical formation and lipid peroxidation.

Radiation exposure at acute single dose of 6.5 Gy did not change the body temperature when measured on the 3rd day following exposure.

In order to determine any antipyretic effect of the drugs used, the body temperature of each animal was measured before induction of hyperthermia as well as 18 hours following yeast injection. Rats were treated with the tested drugs before induction of fever then exposed to whole body gamma radiation at acute single dose of 6.5 Gy and body temperature of each animal was measured 3 days after irradiation. Only rutin had an antipyretic effect in yeast-induced hyperthermia in rats.

INTRODUCTION

There is a considerable evidence that indicates that the cytotoxic effect of ionized radiation is mediated by the rapid generation of oxygen free radicals, via a process that involves intracellular water radiolysis to yield superoxide, hydrogen peroxide and hydroxyl radicals (Panes and Granger, 1996).

The indirect effect of radiation in biological systems depends on the effect of irradiation of water and the presence of oxygen in the tissue being irradiated. The end products of radiolysis of water without oxygen are:

\[ \text{H}_2\text{O} \rightarrow \text{OH}^- + \text{H}^- + \text{H}^+ + \text{OH}^- \]

\text{H}^- \text{ and OH}^- \text{ released by ionizing radiation, are the most important free radicals comprising 55 \% of the initial relative yield (Nair et al., 2001).}
Most of the indirect action involves reactive species derived from water molecules. Radiation interacts with water, some of the products formed can then react with other solute molecules and radicals of solute molecules can form final stable products (Mulcahy et al., 1992).

Ionizing radiation manifests its toxicity via free radicals from water radiolysis (Feurgard et al., 1998). However, the basis of the oxygen enhancement of radiation lethality is the generation of hydroxyl radicals, which are responsible for most of the indirect radiation damage that affects cell survival (Kachur et al., 1998).

Factors that determine the biological effects of ionizing radiation include: the type of radiation, the dose received, the rate at which the radiation dose is delivered, nutritional factors, the type of irradiated tissues, the age and sex of the exposed subject, as well as the rate of delivery of the irradiation dose whether in fractions or in a single exposure (Beir, 1990). Thus larger radiation doses used in single treatments tend to cause more injury than the same dose given in fractions or over a prolonged period.

A large number of drugs have been screened for their radioprotective efficacy, however, none of them was accepted clinically because of the inherent toxicity at useful concentrations (Singh and Yadav, 2005).

Radioprotection may include scavenging of free radicals, protection of cellular and subcellular entities against oxidative damage, repair of target molecules like DNA and protein and restoration of cell proliferation. Compounds having such properties can offer protection against radiation damage (Agarwala and Goel, 2002).

Hyperthermia is the condition where body temperature is passively elevated above the thermoregulatory set-point (Kluger et al., 1998). The term hyperthermia is used in two ways: as a sign, reflecting only a rise of body temperature; and as a syndrome, in which an unregulated rise in body temperature is caused by mechanisms other than those producing fever (Lifshitz, 1994).

Cytokines released by monocytic cells play a central role in the genesis of fever. The cytokines primarily involved in the development of fever include interleukins (IL–I, IL–6) and tumor necrosis factor (TNF–α) (Klir et al., 1993; Mackowiak, 1998). The interaction between these cytokines is complex, with each being able to up-regulate and down-regulate their own expression as well as that of the other cytokines. These cytokines bind to their own specific receptors located in preoptic region of the anterior hypothalamus (Spacer and Breder, 1994; Mackowiak, 1998).

The cytokine–receptor interaction activates phospholipase A₂, resulting in the liberation of plasma membrane arachidonic acid as substrate for the cyclooxygenase pathway. Some cytokines appear to increase cyclooxygenase expression directly, leading to liberation of prostaglandin E₂ (PGE₂), which diffuses across the blood brain barrier, leading to activation of responses designed to decrease heat loss and increase heat production (Mackowiak, 1998).

Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE₂ biosynthesis (Chattopadhyay et al., 2005). Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects (Cheng et al., 2005). A natural antipyretic agent with reduced or no toxicity is therefore, preferred.

Yeast induced pyrexia in rats was lowered significantly with ethyl acetate extract of Acacia catechu. The antipyretic effect of the test drug may be due to presence of flavonoid compounds such as quercetin, as some flavonoids are predominant inhibitors of cyclooxygenase or lipoxygenase (Rajnarayana et al., 2001).

Fever could induce reactive oxygen species (ROS) generation, cellular hypoxia and metabolic stress (Hall et al., 2000). In vivo hyperthermia–generated ROS have been shown to oxidize carboxyl and thiol groups of amino acid residues (Berlett and Stadtman, 1997; Dean et al., 1997) thus altering protein structure and function.

It was shown that body temperature depends critically on a precisely regulated ratio of reduced/oxidized glutathione (GSH/GSSG) within the brain. This balance is lost after pyrogens enter the circulation and cause oxidative stress (Riedel et al., 2003).

The increase in body temperature was shown to intensify the lipid peroxidation process, which indicates that pyrexia is associated with increased oxidative stress (Mutalik et al., 2003). There is an observation by Brzezińska-Slebodzińska (2001) observed a significant increase in the thiobarbituric acid reactive substances level in serum and tissue homogenates during fever and that antioxidant supplementation decreased the lipid peroxidation processes.

Whole body gamma irradiation was found to exert many alterations in biological systems (Cherdyntseva et al., 2005). The produced changes include oxidative stress imbalance (Azab et al., 2004).

Radiation induced–lipid peroxidation is a highly destructive process which brings about changes in
structure, fluidity and permeability of membranes (Cheeseman and Slater, 1993) leading to a significant increase in lipid peroxides which are toxic to the cells and decrease in the level of the antioxidant glutathione in blood and liver homogenate (El-Shamy et al., 2001). Furthermore, exposure of mammals to gamma radiation was shown to produce reduction in plasma superoxide dismutase enzyme activity which acts against free radicals (Nommura and Yamaoka, 1999).

Since hyperthermia is known to alter certain oxidative stress biomarkers (Hall et al., 2000; Mladenov et al., 2006), it seems that the response of the feverish whole body could be different when exposed to gamma radiation.

The aim of the present study is to investigate the oxidative stress biomarkers of the feverish rat when exposed to whole body gamma radiation and the therapeutic potential of natural antioxidants such as vitamin E and rutin in absence or presence of cysteine.

**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 was also provided *ad libitum.*

**Drugs**

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

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

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

4- Brewer’s yeast (Matroh, Egypt): was freshly suspended in distilled water and administered i.m 18 hours before irradiation in order to induce fever. A single dose of 1ml/100g of 44% w/v aqueous suspension was used.

N.B: The concentration of each drug was adjusted so that the dose of each drug administered was contained in 1 ml suspension or solution per 100 g body weight.

**Experimental design**

Nine groups of animals each of 7 rats were used in the present study.

1st group: received saline and served as normal control.

2nd group: received saline then irradiation (6.5 Gy) and served as irradiated control.

The other groups were feverished 18 hr following administration of Brewer’s yeast (1ml/100g of 44 % yeast suspension, i.m). The feverish animals were further subdivided into 7 groups each consisted of 7 rats.

1st group: received saline and yeast and served as hyperthermic control.

2nd group: received saline then irradiated (6.5 Gy) and received yeast and served as hyperthermic irradiated control.

3rd group: received rutin (1.064 mmol/kg, oral) daily for two weeks and received yeast then irradiated (6.5 Gy).

4th group: received vitamin E (50mg/100g, i.p) daily for seven days and received yeast then irradiated (6.5 Gy).

5th group: received yeast and cysteine (30 mg/kg, i.p) 30 min. before irradiation.

6th group: received rutin (1.064 mmol/kg, oral) daily for two weeks and received yeast and cysteine (30 mg/kg, i.p) 30 min. before irradiation.

7th group: received vitamin E (50mg/100g, i.p) daily for seven days and received yeast and cysteine (30 mg/kg, i.p) 30 min. before irradiation.

The third day following irradiation was selected at which the animals were sacrificed, blood collected and livers isolated for further studies.

**Irradiation of animals**

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

**Measured parameters**

Lipid peroxides 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 in blood was determined according to the method described by Beutler and his colleagues (1963), while GSH 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 kinetically according to the method of Marklund and Marklund (1974).

Rectal body temperature was measured in intact animals using a medical thermometer.

Statistical Analysis

All the values are expressed as means ± S.E. Comparisons between means were carried out using different statistical tests according to the determined parameter.

1- The concentration of GSH and MDA and the activities of SOD were evaluated and statistical analysis was carried out by means of one-way ANOVA followed by Tukey-Kramer multiple comparison test using Instat software, version 2 (Graphpad Software, Inc., San Diego, USA).

2- Data of febrile responses of animal groups with different treatments and the time course study of irradiation were analysed by repeated measures two-way ANOVA followed by Duncan’s multiple range test using Statistica software, version 5 (Statsoft, Inc., USA).

RESULTS

Effect of rutin or vitamin E alone or combined with cysteine on oxidative stress biomarkers in hyperthermic irradiated rats.

The results are shown in Tables (1) and (2) and illustrated in Figures (1), (2) and (3).

Plasma MDA level of normal animals was 6.30±0.13 nmol/ml, whereas that of hyperthermic, irradiated and hyperthermic irradiated groups were markedly elevated by 22, 65 and 93 %, respectively, relative to the normal group (Table 1 and Figure 1).

Liver MDA content of normal animals was 161.00 ± 0.85 nmol/g tissue, whereas that of hyperthermic, irradiated and hyperthermic irradiated groups were markedly elevated by 31, 93 and 118 %, respectively, relative to the normal group (Table 1 and Figure 1).

Blood GSH level of normal animals was 51.60 ± 2.64 mg %, while that of irradiated rats was significantly decreased by 38 % relative to that of normal group. On the other hand, blood GSH level of hyperthermic rats was significantly increased by 20 % relative to that of normal group. Blood GSH level of hyperthermic irradiated group did not change as compared to the normal value (Table 1 and Figure 1).

Liver GSH content of normal animals was 22.00 ± 0.60 mg/g tissue, while that of irradiated rats was significantly decreased by 31 %. On the other hand, liver GSH content of hyperthermic rats was significantly increased by 17 % relative to that of normal group (Table 1 and Figure 1).

Plasma SOD activity of normal animals was 1.27 ± 0.01 U/ml, while that of hyperthermic, irradiated and hyperthermic irradiated groups were markedly decreased by 19, 37 and 45 %, respectively, relative to that of normal rats (Table 1 and Figure 1).

Oral daily administration of rutin (1.064 mmol/kg) for two weeks before hyperthermia and irradiation resulted in reduction of plasma and liver MDA by 23 and 27 %, respectively, elevation of blood and liver glutathione by 17 and 12 %, respectively, and elevation of plasma SOD by 54 % relative to hyperthermic irradiated animals (Table 1 and Figure 2).

Administration of a single i.p dose of cysteine (30 mg/kg) before hyperthermia and irradiation resulted in reduction of plasma and liver MDA by 19 and 31 %, respectively, and elevation of plasma SOD by 30 % relative to hyperthermic irradiated animals (Table 1, 2 and Figure 2, 3).

Daily administration of i.p dose of vitamin E (50 mg/100g) for seven days before hyperthermia and irradiation, resulted in reduction in plasma and liver MDA by 32 and 34 %, respectively, elevation of blood and liver GSH by 14 and 19 %, respectively, and elevation of plasma SOD by 40 % relative to hyperthermic irradiated animals (Table 2 and Figure 3).

Combined therapy of rutin and cysteine before hyperthermia and irradiation resulted in reduction in plasma and liver MDA by 36 and 39 %, respectively, elevation in blood and liver GSH by 26 and 22 %, respectively, and elevation in plasma SOD by 65 % relative to hyperthermic irradiated animals (Table 1 and Figure 2).

Combined therapy with vitamin E and cysteine before hyperthermia and irradiation resulted in reduction in plasma and liver MDA by 38 and 42 % respectively, elevation in blood and liver GSH by 20 and 27 %, respectively, and elevation in plasma SOD by 59 % relative to hyperthermic irradiated animals (Table 2 and Figure 3).
Fever was induced by i.m. administration of Brewer's yeast (1 ml/100g of 44% yeast suspension) in five gps of animals each of 7 rats. Eighteen hours following induction of fever, the animals of 4 gps were irradiated (6.5 Gy), while the fifth one was unexposed to $\gamma$ radiation and served as hyperthermic control. The four hyperthermic irradiated gps received saline (10 ml/kg), rutin (1.064 mmol/kg, oral/day for 2 weeks), cysteine (30 min. before irradiation) and rutin+cysteine as mentioned above. Blood was collected and liver was isolated for further investigation.

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>MDA (nmol/ml)</th>
<th>GSH (mg%)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Liver</td>
<td>Blood</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>Hyperthermic control (1 ml/100g yeast)</td>
<td>7.70*±0.41</td>
<td>211.40*±2.40</td>
<td>61.84*±0.49</td>
<td>25.73*±0.58</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>Hyperthermic (1 ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td>12.16*a±0.16</td>
<td>351.56*a±5.05</td>
<td>48.53*a±0.39</td>
<td>20.01*a±0.29</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg, oral/day for 2 weeks) +Hyperthermic (1ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td>9.32*b±0.31</td>
<td>257.64*b±3.05</td>
<td>56.57*b±0.50</td>
<td>22.37*b±0.47</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before irradiation) +Hyperthermic (1 ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td>9.86*c±0.41</td>
<td>243.66*c±2.13</td>
<td>54.21*c±0.59</td>
<td>21.57*c±0.49</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg) +Cysteine (30ng/kg) +Hyperthermic (1 ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td>7.81*c±0.26</td>
<td>213.33*c±2.16</td>
<td>60.9*c±0.72</td>
<td>24.41*c±0.46</td>
</tr>
</tbody>
</table>

Table (1): Effect of rutin alone or combined with cysteine on oxidative stress biomarkers in hyperthermic irradiated rats.
Table (2): Effect of vitamin E alone or combined with cysteine on oxidative stress biomarkers in hyperthermic irradiated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/ml)</th>
<th>GSH (mg%)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Liver</td>
<td>Blood</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>
</tr>
<tr>
<td>Hyperthermic control (1 ml/100g yeast)</td>
<td>7.70²b±0.41 (22 %)†</td>
<td>211.40²a±2.40 (31 %)†</td>
<td>61.84²a±0.49 (20 %)†</td>
</tr>
<tr>
<td>Irradiated (6.5 Gy) (Saline 10 ml/kg)</td>
<td>10.37³b±0.27 (65 %)†</td>
<td>311.41³a±6.23 (93 %)†</td>
<td>31.83³a±1.11 (-38 %)†</td>
</tr>
<tr>
<td>Hyperthermic (1 ml/100g yeast) + Irradiated (6.5 Gy)</td>
<td>12.16⁴ab±0.16 (93 %)†</td>
<td>351.56⁴ab±5.05 (118 %)†</td>
<td>48.53⁴ab±0.39</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g, i.p./day for 1 week) + Hyperthermic (1ml/100g yeast) + Irradiated (6.5Gy)</td>
<td>8.29⁵ac±0.31 (-32 %)†</td>
<td>231.47⁵ac±2.56 (-34 %)†</td>
<td>55.21⁵ac±0.56 (14 %)†</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before irradiation) + Hyperthermic (1 ml/100g yeast) + Irradiated (6.5Gy)</td>
<td>9.86⁶bc±0.41 (-19 %)†</td>
<td>243.66⁶abc±2.13 (-31 %)†</td>
<td>54.21⁶abc±0.59</td>
</tr>
<tr>
<td>Vitamin E (50mg/100g) + Cysteine (30mg/kg) + Hyperthermic (1 ml/100g yeast) + Irradiated (6.5Gy)</td>
<td>7.52⁷bc±0.29 (-38 %)†</td>
<td>204.66⁷bc±2.56 (-42 %)†</td>
<td>58.38⁷bc±0.38 (20 %)†</td>
</tr>
</tbody>
</table>

Fever was induced by i.m. administration of Brewer’s yeast (1 ml/100g of 44% yeast suspension) in five gps of animals each of 7 rats. Eighteen hours following induction of fever, the animals of 4 gps were irradiated (6.5 Gy), while the fifth one was unexposed to γ radiation and served as hyperthermic control. The four hyperthermic irradiated gps received saline (10 ml/kg), vitamin E (50 mg/100g, i.p./day for 1 week), cysteine (30 min. before irradiation) and vitamin E/cysteine as mentioned above. Blood was collected and liver was isolated for further investigation. 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.

† Compared to the normal group.

* Significant difference from normal group at p ≤ 0.05

† Significant difference from irradiated group at p ≤ 0.05

‡ Significant difference from hyperthermic group at p ≤ 0.05

§ Significant difference from hyperthermic + irradiated group at p ≤ 0.05
Figure (1): Blood level and liver contents of MDA and GSH and the activity of plasma SOD in hyperthermic, irradiated and hyperthermic irradiated rats.

Four groups of animals each consisting of 7 rats were used, the 1st received saline (10 ml/kg) and served as normal gp., while the other received saline and was exposed to γ radiation (6.5 Gy). Fever was induced by administration of Brewer's yeast in two groups, only one of them was exposed to gamma radiation. 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 group at p ≤ 0.05.

Figure (2): Effect of rutin alone or combined with cysteine on oxidative stress biomarkers in hyperthermic irradiated rats.

Four groups of animals each consisting of 7 rats in which fever was induced by administration of Brewer's yeast. 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 as mentioned above then the animals were exposed to γ radiation (6.5 Gy). The 1st gp served as hyperthermic irradiated control. Blood was collected and liver was isolated for investigation of MDA, GSH and the activity of plasma SOD.

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

* Significantly different from hyperthermic irradiated group at p ≤ 0.05.
Figure (3): Effect of vitamin E alone or combined with cysteine on oxidative stress biomarkers in hyperthermic irradiated rats.

Four groups of animals each consisting of 7 rats in which fever was induced by administration of Brewer's yeast. They received saline (10 ml/kg), vitamin E (50 mg/100g, i.p./day for 1 week), cysteine (30 mg/kg, i.p., 30 min. before irradiation) and vitamin E+cysteine as mentioned above then the animals were exposed to γ radiation (6.5 Gy). The 1st gp served as hyperthermic irradiated control. Blood was collected and liver was isolated for investigation of MDA, GSH and the activity of plasma SOD. Statistical analysis was carried out by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

* Significantly different from hyperthermic irradiated group at p ≤ 0.05.

Effect of exposure to radiation on body temperature in normal and feverish rats.

The results are shown in Table (3).

The body temperature of normal animals at zero time was 37.37 ± 0.05 °C.

Induction of fever by i.m injection of Brewer's yeast (1 ml/100g of 44% yeast suspension) caused significant elevation of body temperature after 18 hours of yeast injection to be 39.10±0.20 °C.

On the other hand, rectal temperature of irradiated animals did not show any change as compared to normal rats.

Hyperthermic irradiated rats showed elevation of body temperature after 18 hours of yeast injection to be 38.84 ±0.10 °C. The value was still different than that of the normal gp but did not alter significantly relative to the value of the hyperthermic gp.

Effect of rutin alone, vitamin E alone or combined with cysteine on body temperature in hyperthermic irradiated rats.

The results are shown in Table (4).

The body temperature of normal animals was 37.31 ± 0.05 °C.

Daily oral administration of rutin (1.064 mmol/kg) for two weeks alone or combined with a single dose of cysteine (30 mg/kg, i.p.) 30 min. before irradiation showed significant reduction in Brewer's yeast– induced hyperthermia in rats to be 37.30 ± 0.04 and 37.41 ± 0.05, respectively.
single dose of cysteine (30 mg/kg, i.p.) 30 min. before irradiation showed significant reduction in Brewer's yeast–induced hyperthermia in rats to be 37.30 ± 0.04 and 37.41 ± 0.05, respectively.

Administration of vitamin E (50 mg/100g, i.p.) daily for seven days before induction of fever, a single dose of cysteine (30 mg/kg, i.p.) 30 min. before irradiation or their combined administration did not show any significant protective effect against yeast–induced elevation in body temperature after 18 hours from yeast injection.

Table (4): Effect of rutin or vitamin E alone or combined with cysteine on body temperature in hyperthermic irradiated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Body temperature (ºC) (18 hr Post-yeast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthermic irradiated (1ml/100g yeast + 6.5 Gy)</td>
<td></td>
<td>38.84 ± 0.10</td>
</tr>
<tr>
<td>Rutin (1.064 mmol/kg, oral/day for 2 weeks) +Hyperthermic (1ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td></td>
<td>37.30 ± 0.04</td>
</tr>
<tr>
<td>Cysteine (30 mg/kg, i.p., 30 min. before irradiation) +Hyperthermic (1ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td></td>
<td>38.91 ± 0.04</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g, i.p./day for 1 week) +Hyperthermic (1ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td></td>
<td>38.88 ± 0.04</td>
</tr>
<tr>
<td>Rutin (1.064mmol/kg) +Cysteine (30mg/kg) +Hyperthermic (1ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td></td>
<td>37.41 ± 0.05</td>
</tr>
<tr>
<td>Vitamin E (50 mg/100g) +Cysteine (30mg/kg) +Hyperthermic (1ml/100g yeast) +Irradiated (6.5 Gy)</td>
<td></td>
<td>38.88 ± 0.07</td>
</tr>
</tbody>
</table>

Fever was induced in six groups each consisting of 7 rats. The 1st group was exposed to γ radiation 18 hr following induction of fever and served as hyperthermic irradiated gp. The 2nd gp was treated daily for two weeks by rutin (1.064 mmol/kg) before induction of fever then exposed to γ radiation (6.5 Gy), while, 3rd feversh gp received cysteine (30 mg/kg) 30 min. before irradiation. 4th gp was treated daily for one week by vitamin E (50 mg/100g) before induction of fever then exposed to γ radiation (6.5 Gy). The other two gps received rutin+cysteine and vitamin E+cysteine as mentioned before.

Statistical analysis was carried out by two-way ANOVA followed by Duncan’s multiple range test.

* Significantly different from the hyperthermic irradiated group at the respective time at p ≤ 0.05.

DISCUSSION

The increased plasma and liver MDA contents due to exposure to gamma radiation as recorded in this study is in agreement with those of previous studies (Saada and Azab, 2001; Azab et al., 2004) that 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).

Furthermore, in the study of Osman (2003), exposure of female rats to whole body gamma radiation (single dose, 6.5 Gy) created serious oxidative stress in the biological systems. The effect was early detected, one day post exposure and was continued till the 14th day after irradiation. In addition, 24 hr. post irradiation of rats at 6.5 Gy resulted in increase in blood and liver MDA (El-Shamy et al., 2001). In addition, the present results are in agreement with reports previously published by Hasegawa et al. (1992), Osman (1996) and Saada et al. (1999), who found an increase in lipid peroxides together with a decrease in the antioxidant systems after whole body irradiation of rats.

The acceleration in lipid peroxidation was shown to be attributed to peroxidation of the membrane unsaturated fatty acids due to free radicals propagation (Zheng et al., 1996). The extensive lipid peroxidation results in membrane disorganization by peroxidizing the highly unsaturated fatty acids, which in turn alters the ratio of polyunsaturated to other fatty acids leading to a decrease in the membrane fluidity, which may be sufficient to cause cell death (Rotruck, et al. 1979).

In the current study, whole body γ-irradiation (6.5 Gy) induced significant decrease in GSH level in blood and liver homogenate of rats. The present results were in agreement with those of Yamaoka et al. (2000), Neal et al. (2003) as well as Baliga et al. (2004). Exposure of rats to a fractionated dose of γ radiation up to 9 Gy also induced decrease in GSH content in blood, liver, spleen and intestine on the 3rd, 7th and 15th day post–radiation exposure (Abu-Ghadeer et al., 1999).

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.

In the current study, plasma SOD activity of normal as well as whole body γ irradiated animals was measured. Results showed a significant reduction
in blood SOD activity following radiation exposure, which was in conformity with the findings of El-Shamy et al. (2001), who found a decrease in blood and liver SOD at 24 and 72 hr post irradiation with a dose 6.5 Gy. The present decrease in blood SOD after whole body gamma irradiation was in agreement with the results of the studies carried out by Hasegawa et al. (1992), 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 a 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 radical, which damages the liver cells.

However, Ramadan and El-Ghazaly (1997) reported that no change was observed in plasma SOD activity on the 1st and 2nd days after irradiation (6.5 Gy), while the activity of SOD decreased significantly on the 7th and 14th day post-irradiation. On the contrary, the results of Shaheen and Hassan (1991) showed a significant increase in SOD activity following exposure to radiation.

Yeast acts as an exogenous pyrogen that can evoke cytokine and non-cytokine mediators capable of activating various phospholipases and ultimately promoting the synthesis of prostaglandins (Dinarello, 1991) causing elevation of body temperature.

Many investigators suggested that fever could be induced as a result of the invasion of endogenous pyrogens released from leukocytes into the central nervous system, especially the hypothalamus, whereas the pyrogens act to release prostaglandins (Coccainiti et al., 1988).

The present results showed that fever caused increase in thiobarbituric acid reactive substances (TBARS) in the blood as well as the liver homogenate. The current data are in agreement with previous studies (Brzezińska-Ślebodzińska, 2001; Niu et al., 2003; Mladenov et al., 2006).

In other studies, it has been shown that the increase in body temperature intensified lipid peroxidation processes where MDA increased in serum, kidney and liver homogenate (Brzezińska-Ślebodzińska, 2001). The increase in lipid peroxidation could be attributed to the production of large amounts of superoxide anion (O$_2^-$) and other oxygen intermediates by macrophages (Teshima et al., 1995). Furthermore, acute heat exposure markedly stimulated the process of lipid peroxidation (Mladenov et al., 2006). In the study of Niu et al. (2003), lipid peroxidation was greater in rats exposed to heat stress (ambient temperature 42 °C to induce heat stroke), compared with control rats.

Fever in the current study, increased GSH level in blood and in liver homogenate. This is in agreement with the results of Mitchell and Russo (1983) and Freeman et al. (1990) who reported that GSH increased rapidly in cells exposed to elevated temperatures. This might be a compensatory mechanism against fever-generated reactive oxygen species (ROS). Tissue GSH levels have been shown to increase in response to experimentally induced oxidative stress (Forman et al., 1995). However, Osorio et al. (2003) suggested that hyperthermia does not produce a great stimulus for development of antioxidant mechanism related to GSH. The enhanced plasma GSH level is a reflection of combined tissue efforts in maintaining the redox state and to cope with the oxidative stress during fever exposure.

In the present study, it has been shown that, fever reduced blood SOD activity. This finding is in harmony with that of Yang and Lin (2002) who reported a decrease in brain homogenate SOD activity in rats exposed to heat stress. The onset of fever was found to be correlated with the production of O$_2^-$ formed by the macrophages (Grisham et al., 1988). The reduction in SOD activity might be attributed to its consumption by free radicals.

Based on the fact that SOD represents one of the endogenous antioxidant defense mechanisms that get rid of the harmful species O$_2^-$, it seems conceivable that the reduction of SOD activity observed in the present study may be due to exhaustion of the enzyme as a consequence of O$_2^-$ overproduction.

Pretreatment with rutin and vitamin E alone or combined with cysteine before the onset of hyperthermia ameliorated, to a great extent the effects induced by hyperthermia including changes in blood and liver MDA, GSH contents and plasma SOD activity.

It was postulated that flavonoids can effectively protect cells and tissues against the deleterious effects of reactive oxygen species (Metodiewa et al., 1997). The antioxidant activity of flavonoids could be attributed to the scavenging of free radicals and other oxidizing intermediates (Russo et al., 2000), hydrogen donors (Korkina and Afanas'ev, 1997), chelation of iron or copper ions (Morel et al., 1994; Sugihara et al., 1999), inhibition of oxidases, scavenging of lipid and protein-derived radicals as well as hypochlorous acid (HOCl) which is effectively trapped by flavonoids (Groot and Rauen, 1998).

The study of Afanas'ev and Coworkers (1989) added that rutin can inhibit iron ion-dependent lipid peroxidation by chelating iron ions. Rutin has been shown to protect against tert-butyl hydroperoxide–induced oxidative damage to DNA by acting as a metal ion chelator (Aherne and O'Brien, 2000).
It seems that, the overall potential of the antioxidant system was significantly enhanced by the rutin supplement as the plasma and hepatic TBARS levels were lowered while the hepatic SOD and glutathione peroxidase (GPx) activities were increased in the high-cholesterol fed rats. Furthermore, it was suggested that the supplementation with rutin decreased absorption of dietary cholesterol as well as lowered plasma and hepatic cholesterol (Park et al., 2002).

The antioxidant scavenging activity of rutin is clearly pronounced in the amelioration of GSH level. It was proved that γ glutamylcysteine synthetase, the rate limiting enzyme in GSH synthesis, is regulated by phenolic antioxidants (Rohman and Mac, 2000).

Cysteine was found to be protective against radiation illness, excellent free radicals scavenger, peroxide decomposer, catalyst of sulfhydryl disulfide exchange and can possibly implement repair of damaged sites (Kafafy, 2000). It was found to be effective in ameliorating various side effects of radiotherapy (Monig et al., 1990).

Several mechanisms have been proposed to explain the radioprotective effects of sulfhydryl compounds including free radical scavenging, hydrogen atom donation by –SH groups and –COOH groups (Upadhya 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).

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). More evidences were provided by Schmitt et al. (1995) who showed that effective concentrations of α-tocopherol inhibited cellular lipid peroxidation induced by oxidized LDL in cultured endothelial cells. In addition, vitamin E is the most effective and is by far the most important lipid–soluble chain breaking antioxidant in vivo in humans. Thus the content of vitamin E in circulating low density lipoprotein helps to determine their resistance to lipid peroxidation and thus may affect the development of tissue damage (Esterbauer et al., 1989).

One of the ways in which α-tocopherol is believed to stabilize membranes is to form complexes with membrane lipid components that have a tendency to destabilize the bilayer structure thereby countering their effects and rendering the membrane more stable (Wang and Quinn, 1999).

Pretreatment with α-tocopherol before the onset of heat exposure significantly attenuated heat stroke-induced increased free radical formation, lipid peroxidation as well as prolongation of survival time of rats (Niu et al., 2003).

It seems that, exposure of feverish rats to γ radiation resulted in damage of all the measured parameters except blood and liver GSH, since GSH was increased by hyperthermia and irradiation could normalize it.

Data of the present study showed that, the rectal temperature of irradiated group did not change as compared to the normal value which is in conformity with the findings of Abou-Safi et al. (2004). Also, rutin administration before yeast injection in normal rats effectively reduced the elevated body temperature which is in agreement with previous studies (Gunasegaran et al., 2001; Mutalik et al., 2003; Ray et al., 2006). The flavonoids are reported to have antioxidant activity (Sudheesh et al., 1999). Hence, antioxidant activity of rutin may be one of the possible mechanisms by which it reduced the elevated body temperature.

Increased body temperature and pain are among the main reactions of the body against inflammation. Hence, a drug possessing antiinflammatory activity may also exhibit antipyretic properties (Perianayagam et al., 2004). In earlier studies, rutin showed potent antiinflammatory activity (Lindahl and Tagesson, 1997). The antipyretic effect of rutin was studied on hyperthermia in rat model. Rutin showed a significant decrease in rectal temperature. This result suggested that rutin has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature (Milton, 1982). This effect could be due to inhibition of prostaglandin synthesis by its anti-inflammatory activity.

On the other hand, cysteine and vitamin E seem to be devoid of antipyretic activity. This suggests that their antioxidant effectiveness is via their influence on the oxidative stress imbalance (Monig et al., 1990; Zaidi et al., 2005).

One can conclude that prophylactic treatment with the natural antioxidants rutin alone, vitamin E alone or in combination with cysteine produced a potential effect against radiation and hyperthermia–induced damage in the biological systems. Thus, such treatment aids in counteracting many of the risks associated with oxidative stress, and hence modulate severity of the injury.

REFERENCES


