Evaluation of tryptophan role on some biochemical and histological alterations in irradiated rats.

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The effect of tryptophan daily oral dose (81.7 mg/kg wt) for 21 days on some biochemical and histological parameters in female albino rats exposed to gamma irradiation at dose 7 Gray (Gy) was investigated. The hematological studies revealed that there were significant reduction in red blood cell count, hemoglobin, hematocrit, platelets and leucocytes values in irradiated rats and elevation of thiobarbituric acid reactive substances level. Catalase activity decreased significantly after irradiation. Some histological changes were noticed in both lymph nodes and spleen of irradiated rats. Tryptophan administration dose (81.7 mg/kg wt) revealed ameliorative effects in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and some biochemical and histological changes occurred due to gamma irradiation.

Key words: Tryptophan, gamma irradiation, biochemical parameters, histological parameters.

Exposure to gamma irradiation induces significant alterations in both physiological and metabolic processes as well as disorders in organs functions and blood biochemical level (Roushdy et al., 1997; Koc et al., 2002). Radiation produced highly reactive and dangerous molecular species called free radicals in cells and tissues, which have high energies and can break chemical bonds. Free radicals may be formed within cells as well as in the extra cellular medium and can interact with membrane lipids, nucleic acid, carbohydrate and protein. As a result of interaction with membrane lipids, lipid peroxidation chain reactions may disturb membrane structure, while interactions with protein may cause structural changes, which lead to some biochemical disorder functions and resulted in acute and chronic diseases (Weiss et al., 1990; Felemovicius et al., 1995).

Effect of gamma rays may results in metabolic disturbance as well as the structure and function of body system. These disturbances are invariably dependent on the radiation dose and the ability of the cell to repair the damage (Sener et al., 2004). Gamma irradiation hazards, due to free radicals generation, present an enormous challenge for biological and medical safety (El-Missiry et al., 2007).

Tryptophan is an essential amino acid for the biosynthesis and structure of proteins that are implicated in a variety of metabolic processes. It is also the main precursor of serotonin, melatonin secreted by pineal gland and niacin (Millward, 1998, Beck, 2001; Feoli et al., 2006). Melatonin, a neuro-hormone with a range of functions (Szczepanik, 2007; Kedizora et al., 2008), one of its main abilities is to scavenge and reduce free radicals during the endotoxic shock process (Mizok, 2000; Cardinalli, 2007).

Tryptophan deficient diet leads to a negative tryptophan balance or a state of malnutrition in the organism, as well as a reduction in the final degradation of tryptophan metabolites, like serotonin, picolinic acid, niacin and melatonin (Miller et al., 2008), while the degradation products of melatonin participate in the activation and suppression of immune responses (Saleem et al., 2008).

Melatonin level increased nearly ten folds from low day-time to high nocturnal value.
(Pääkkönen et al., 2006) and has been documented as a direct free radical scavenger and an indirect antioxidant, as well as an important immunomodulatory agent (Reiter et al., 2004). Melatonin, a powerful endogenous antioxidant, plays a role in the reduction of oxidative damage (Taysi et al, 2003).

Mcbride et al., 2010, reported that drugs (tryptophan, pyrrolysine, serine, selenocysteine, threonine, tyrosine and valine) and their compositions useful in preventing and negative side effects associated with radiation exposure or clinical radiotherapy are disclosed. This study aims to investigate the possible ameliorative effects of tryptophan against biochemical changes and tissues injuries in rats after exposed to gamma irradiation.

MATERIALS AND METHODS

Animals: 32 adult female albino rats (~ 140 g) were used and purchased from the National Research Centre, Dokki, Cairo, Egypt, and allowed to acclimate for 7 days. They were housed in controlled environmental conditions (temperature 25 ± 2˚C and 12 hrs. dark/light cycles) with standard diet and water ad libitum.

Experimental design: Animals were divided into the following groups each of eight rats:

First group: normal rats served as control.
Second group: rats received daily oral dose of tryptophan (81.7 mg/kg wt) for three weeks.
Third group: rats exposed to gamma irradiation (7 Gy) for one time (acute dose).
Fourth group: rats were irradiated with 7 Gy of gamma irradiation followed by three weeks daily oral administration of tryptophan (81.7 mg/kg wt).

Gamma irradiation: Whole body gamma irradiation was performed using Co cell 3500 of the Middle Eastern Regional Radioisotope Center for the Arab Countries in Dokki, Giza, Egypt. Animals were irradiated at one dose level of 7 Gy for each rat after adaptation period (7 days).

Blood samples were collected from orbital venous plexus at the end of the experiment (3 weeks). The blood centrifuged at 3000 rpm and the obtained serum was stored at -20 °C until assayed for biochemical analysis.

Biochemical analysis:
Hematology measurements included erythrocyte count (RBCs), hemoglobin (Hb), Hematocrit (Ht), leucocyte count (WBCs), platelets (PLt), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were carried out according to Dacie and Lewis (1984).

Catalase (CAT) was assayed according to Johansson and Borg (1988). Thiobarbituric acid reactive substance (TBARs) was measured by the method of Yoshioka et al. (1979).

Histological examination:
Tissue specimens of spleen and lymph nodes were fixed in 10% formalin saline. Trimming was done on the fixed tissue specimens and washed in tap water for 12 hours. Serial alcohols (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3 microns thickness by slide microtome. The obtained tissue sections were collected on the glass slides and stained by hematoxylin and eosin stain for histological examination by the light microscope (Banchroft et al., 1996).

Statistical analysis:
All values were expressed as mean ± SE. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Duncan’s test. P values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Hematological parameters level:
Table (1) shows the effect of irradiation and tryptophan on hematological parameters in female albino rats. Administration of tryptophan resulted in non-significant modification in hematological parameters, compared with the control at the end of the experiment. Irradiation at dose level 7 Gy showed significant decrease in RBC’s count, hemoglobin, hematocrit, platelets and leucocytes values, which reflecting of anemia. The red cell indices (MCV, MCH and MCHC) did not appear to be adversely affected. On the other hand, irradiated group treated with
lipoxygenation appears to be decreased in the irradiated animals compared to the control. In this respect, some investigators reported some alterations in the hematological parameters after exposure to an irradiation, some of which are in harmony with the present investigation. Red blood cell count, hemoglobin and Hematocrit values were depressed (Ashry and Hussein, 2007). The depression observed in RBCs, Hb and Ht could be attributed to the destruction of erythrocyte precursor in bone marrow (Ashry and Hussein, 2007). Irradiation causes retardation in incorporation of iron and decrease in hemoglobin binding to erythrocyte membrane (Derval and Sichevskaia, 2000). The underlying cause of the increased RBCs lyses by irradiation appears to be a decreased production of erythropoietin (Alfrey et al., 1997), however slightly variation of MCV and MCH values from control were noted. These findings indicate that anemia may occur following acute exposure to single radiation dose. Anemia is a common problem associated with irradiation of large body area that includes bone marrow (Henke et al., 1999). Significant depression in bone marrow viable cells in irradiated animals is concomitant with these results. Erythrocyte hemolysis is caused by lipid peroxidation (Sato et al., 1999).

Table (1). Effect of tryptophan and gamma irradiation on hematological parameters in adult female albino rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Tryptophan</th>
<th>Gamma irradiation</th>
<th>Gamma irradiation + tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (x10^6/mm³)</td>
<td>6.09 ± 0.10</td>
<td>6.24 ± 0.21</td>
<td>4.70 ± 0.12</td>
<td>3.80 ± 0.28</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.63 ± 0.38</td>
<td>12.66 ± 0.39</td>
<td>9.40 ± 0.21</td>
<td>8.30 ± 0.48</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.56 ± 1.10</td>
<td>39.00 ± 1.05</td>
<td>30.67 ± 0.66</td>
<td>20.75 ± 1.44</td>
</tr>
<tr>
<td>Platelets (x10^9/mm³)</td>
<td>294.00 ± 94.75</td>
<td>317.00 ± 47.01</td>
<td>45.33 ± 0.88</td>
<td>65.25 ± 8.60</td>
</tr>
<tr>
<td>Leucocytes (x10^9/mm³)</td>
<td>8.30 ± 1.44</td>
<td>9.58 ± 1.17</td>
<td>1.97 ± 0.32</td>
<td>1.74 ± 0.14</td>
</tr>
<tr>
<td>MCV (µm³)</td>
<td>61.67 ± 8.50</td>
<td>62.50 ± 2.72</td>
<td>65.25 ± 1.73</td>
<td>54.60 ± 1.03</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.09 ± 0.58</td>
<td>20.28 ± 0.71</td>
<td>20.00 ± 0.58</td>
<td>21.84 ± 1.20</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>30.96 ± 0.00</td>
<td>32.46 ± 0.07</td>
<td>30.64 ± 0.23</td>
<td>40.00 ± 1.36</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Values with same superscript in the raw are not statistically different.

Table (2). Effect of tryptophan and gamma irradiation on serum thiobarbituric acid reactive substances (TBAR’s) concentration and catalase activity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Tryptophan</th>
<th>Gamma irradiation</th>
<th>Gamma irradiation + tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (U/ml)</td>
<td>0.84 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.73 ± 0.02</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>TBAR’s (µmol/dl)</td>
<td>19.90 ± 0.81</td>
<td>10.70 ± 0.87</td>
<td>26.63 ± 0.90</td>
<td>19.13 ± 1.30</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Values with same superscript in the raw are not statistically different.

Tryptophan showed significant decrease in the hematological parameters, while MCH and MCHC significantly increased compared to control. Ionizing radiation is a potent free radicals former that affects the cellular enzyme systems which in turn produce excessive amounts of free radicals (Athar et al., 1993). Catalase activity was significantly increased in tryptophan treated group. After irradiation at dose level 7 Gy, the results show marked decrease in catalase activity of both gamma irradiated and irradiated treated with tryptophan groups comparing with the control. The obtained results are in agreement with Noaman and Ashry (2006) who noticed depressed catalase activity on using a single dose of gamma rays (10 Gy). These depletion may be attributed to “oxidative stress” occur when antioxidant balance is disrupted by excessive production of reactive oxygen species such as superoxide radical, hydroxyl radical, hydrogen peroxide, singlet oxygen and/or inadequate antioxidant defense mechanisms as superoxide dismutase activity and glutathione reductase production (Cuzzocrea et al., 2001 and Nordberg and Arner, 2001). Once this imbalance appears, cellular molecules may be damaged by the predominant free radicals, this leads to oxidative modifications of lipid and lipid peroxidation (Romero et al., 1998). Polyunsaturated bonds of membrane cholesterol and fatty acids can readily react with the generated free radicals and undergo lipid peroxidation through the abstraction of...
hydrogen, then react with molecular oxygen to form intermediates terminated by either scavenging reaction or continued to form lipid peroxidation products (Danato, 1980). However, when the oxidative damage is extreme as a result of irradiation, these reactive oxygen species scavenging enzymes are degraded (Hasegawa et al., 1992).

The results showed significant elevation of thiobarbituric acid reactive substances level of irradiated group, while tryptophan treated group significantly decrease and no significant change in irradiated-tryptophan group compared to the control. This elevation of thiobarbituric acid reactive substances is an index that endogenous lipid peroxidation was observed under gamma irradiation (Turk et al., 2002). Therefore, its measurement is frequently used to determine the level of oxidative stress (Murugan and Pari, 2006). The obtained results demonstrated that oxidative stress increased after exposure to ionizing radiation and in consequence of the systemic inflammatory response syndrome which led to dysfunction of various organs and system (Miesel et al., 1996 and Soliman et al., 2005).

In the group treated with tryptophan after irradiation, TBARs level reach to normal range. This effect of TRP is due to its antioxidant activity. So, TRP can be employed in the treatment of some illnesses such as autoimmune diseases like lupus erythematosus and multiple sclerosis (Bitzer-Quintero et al., 2010).

**Histological studies:**

In the present study sections from lymph nodes and spleen have been examined to delineate structure differences between the studied groups. Lymph nodes sections of control group rats showed normal histological structure, normal lymphoid cells and normal blood vessels (Fig. 1). Rats treated with tryptophan exhibit normal configuration (Fig. 2).

Animals were exposed to whole body gamma irradiation at dose 7 Gy showed distortion in the architecture pattern of lymph nodes, dilatation and congestion of blood vessels associated with lymphoid depletion focal haemorrhage (Fig. 3). Administration of tryptophan after irradiation leads to improvement of injury of lymphoid cells and appeared normal histological structure (Fig. 4). Histological observations in spleen tissue of control rats through light microscope (X400) revealed normal lymphoid follicle from follicular artery surrounded by lymphocytes (Fig. 5). Administration of tryptophan showed normal appearance and no histological changes (Fig. 6). After administration of 7 Gy of gamma irradiation showed fragmented nuclei, lymphocytic depletion hemorrhage, presence of megakaryocytic and lymphocytic necrosis (Fig. 7). For rats treated with tryptophan after irradiation, spleen showed normal appearance (Fig. 8).
In the present study, gamma-irradiation induced injury to lymph nodes and spleen cells. These results agree with those of Mansoub (2011), who investigated the effect of gamma irradiation (5, 7.5 and 12 Gy) on spleen tissue and found necrosis in some parts of the spleen. Also, Abdou and Osman (2008) found the similar observations and reported that spleen of gamma irradiated rats (4 Gy) showed marked dilation and congestion of splenic blood vessels as well as presence of multiple megakaryocytic. Studies from exposed human and animals indicate that radiation from cobalt can affect a wide variety of tissues with greater levels of cellular divisions (Blatt et al., 1994).

Necrosis and inflammation were the key features of high dose radiation injury (Mansoub, 2011). Cohen (2002) explained that tissue injury caused by ionizing radiation is initiated by oxidative injury to deoxyribonucleic acid. It is established that tissue injury elicits acute inflammation whose features among others include swelling of the affected part. Also, Kutsyi (2011) concluded that radiation induced proteolysis of histone H1 and core histones in spleen nuclei leads to chromatin decondensation and DNA degradation by nucleases.

Non-histological changes appeared for rats treated with tryptophan after irradiation. Tryptophan administration prevented most of previously described changes. This finding due to the antioxidant activity of tryptophan
which consider the precursor of the scavenger or immunomodulator molecules melatonin and picolinic acid, can be found in the diet; and could be an alternative nutritional supplement used to regulate the immune response in the generation of free radicals (Bitzer-Quintero et al., 2010). Radio protective effect of tryptophan was concluded by Basu et al., (1992) who noticed that the combination of tryptophan with thiol compound had a radio protective effect in protecting splenic tissue following 8 Gy gamma radiation.

From the present study, it appears that tryptophan could play ameliorative and curative role against irradiation. This role had noticed in histological examination more than biochemical studies.

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