INTRODUCTION

Transfusion-Associated Graft-Versus - Host Diseases (TA-GVHD) is well recognized as an uncommon, but frequently fatal, adverse effect of blood component therapy. In this disorder, viable donor lymphocytes transfused to a vulnerable patient organize a disturbing attack on the recipient's tissues. Ionizing radiation has been shown to limit the capacity of lymphocytes to proliferate. Irradiation of cellular blood products using a γ source inactivates T cells, the causative cells of GVHD. The selection of an appropriate dose of γ irradiation that eliminates the risk of TA-GVHD while preserving the quality of the transfused product remains an issue. Based on data showing the elimination of T-cell-mediated allogeneic reactivity in the mixed lymphocyte culture (MLC), many blood banks have been irradiating blood with 25 G**y (Lu and Negrin, 1994).** Gamma irradiation of blood product is considered the support of TA-GVHD prevention. However the lowest

irradiation dose able to completely inhibit the lymphocyte activation in blood components. Transfusion-associated graft-versus-host disease (TA-GVHD) is rare but usually fatal complication of transfusion of any blood component containing viable T lymphocytes.

Ionizing radiations have been found to produce deleterious effect on the biological system. Most cell damage caused by ionizing radiation is mediated by the reactive oxygen species (ROS) generated from the interaction between radiation and water molecules in cells. Under normal conditions, there is a delicate balance between the generation of (ROS) and the cellular antioxidant system. The overproduction of ROS in both intra- and extra cellular species upon exposure of cells or individuals to radiation, results in oxidative stress defined as the imbalance between the proxidants and antioxidants. Once this imbalance takes place, cellular molecules may be damaged by the predominant free radicals. This

leads to oxidative modifications of the cellular molecules **(Yilmaz and Yilmaz, 2006).** Erythrocytes are susceptible to oxidative stress as result of the high polyunsaturated fatty acid content of their membrane and the high cellular concentration of oxygen and hemoglobin, which is a source of oxygen reactive species **(Chicha *et al.*, 1999).**

Also radiation induced degradation of membrane proteins mainly due to aggregation **(Soszynski and Schuessler, 1991).**

Gamma radiation of erythrocytes induces alterations at three different functional units of the membrane: lipid bilayer, protein components and cytoskeleton at the membrane surface **(Bonincontro *et al.*, 1989).** In addition, radiation induces shortening in the lipid fatty acid chain by lipid peroxidation **(Schön *et al.*, 1994).** The production of hydroperoxides and cross-linkages in the membrane lipids can disorder the upper

region of the bilayer favoring penetration of water (**Parasassi *et al*., 1991).**

In order to overcome the potential harmful effect of free radicals and to reduce damage by oxidants, a variety of pharmacological antioxidants such as ceruloplasmin, transferrin, edaravone, and melatonin have been examined **(Gutteridge, 1986; Manda and Bhatia, 2003a; Abzai *et al*., 2004).** In addition, several provitamins and vitamins have been found to be potent radioprotectors (**Konopacka and Rzeszowska-Wolny, 2001; Manda and Bhatia, 2003b).** α-Lipoic acid (LA) is an endogenously produced coenzyme that plays an essential role in α-ketoacid dehydrogenase reactions **(Bilska and Wlodek, 2005**). Its properties as an antioxidant have recently been reviewed **(Bilska and Wlodek, 2005**). LA, or its reduced form, dihydrolipoic acid (DHLA), quenches a number of oxygen free radical species in both lipid and aqueous phases, chelates transition metals, and prevents membrane lipid peroxidation and protein

damage via interactions with vitamin C and glutathione.

Electron paramagnetic resonance (EPR) or more often named electron spin resonance (ESR) is used for the investigation of paramagnetic substances. A paramagnetic state of a chemical compound is given, when it has an (unpaired) free electron (**Rohn and Kroh, 2005**). These ‘‘unpaired electrons’’ are found in free radicals (highly reactive molecules) and transition metals ions (e.g. iron, copper, cobalt). Therefore, EPR can be used to identify biological molecules that contain free radicals or transition metal ions in their structure. Even more usefully EPR is a quantitative technique, i.e. we can determine the concentration of unpaired electrons present in a sample even if one does not know the exact nature of the free radical being observed. So Electron Paramagnetic Resonance (EPR) has been proven to be an excellent tool for measuring radiation-induced radicals in various materials and

some human-body tissues **(Trompier *et al.*, 2008).**

FT-IR spectroscopy is used for qualitative and quantitative analysis of organic compounds, and also for determining the chemical structure of many inorganic compounds. Recently FT-IR spectrometry was an analytical method able to determine both changes in erythrocyte and plasma during exercise **(Cyril and Ge´rard, 2004**). One important point is that the whole biochemical contents of any cell may be identified at the sub-molecular level by FT-IR spectrometry (**Moore *et al.*, 1995; Sills *et al.*, 1994).** FT-IR spectrometry was proved to be useful in determining cell susceptibility to oxidative stress (**Sockalingum *et al.*, 1998**). Main cellular changes due to free radical attacks were observed on amide I and II components, nucleotide bases, phosphodiester backbone, and sugar rings **(Melin *et al.*, 1999 and 2001**). For erythrocytes, phospholipids bilayer has been extensively studied by IR

spectrometry **(Chen *et al.*, 2000; Mendelsohn and Moore, 1998; Moore *et al.*, 1999),** notably regarding fatty acid saturation degree **(Inoue *et al.*, 2001**). Since the erythrocyte cell is usually the gold example to test the sensitivity of all new analytical methods. Main erythrocyte IR absorptivities should be easily interpreted: (1) fatty acyl moieties of erythrocytes stem exclusively from phospholipids; (2) hemoglobin accounts for about 80 % of erythrocyte proteins, spectrin for about 10 %, and there is no nucleus within this cell; (3) over membrane carbohydrate residues, sugars present within the cell are only lactate and possibly glucose for diabetics.

Differential Scanning Calorimetry (DSC) is a powerful technique for studying the thermodynamics of transitions in biological macromolecules. The heat flow into the cells is monitored as the temperature is increased at a constant rate. The heat absorbed by the sample due

to temperature increase and any conformational transitions is monitored. From the differential heat flow, the specific heat can be determined. Cells are a complex, interacting mixture of proteins, nucleic acids, and membrane lipids, each of which can undergo order-disorder, endothermic transitions detectable by DSC, and numerous small molecules and ions. A DSC scan should be the sum of the transitions of all components. In addition, other processes such as aggregation and metabolism, both exothermic events, can be detected by calorimetry and must be considered in interpreting a DSC profile. The human erythrocyte is a fairly simple cell which lacks a nucleus and consists primarily of hemoglobin. In addition, it has a low level of metabolism, and thus metabolic heat released during a DSC scan does not represent a problem. DSC technique is sensitive to protein denaturation in intact cells including erythrocytes, bacteria, yeast **(Obuchi *et al*., 2000),**

mammalian cells, and tissue (**Ritchie *et al*., 1993**).

The aim of this work is to study the interaction of electromagnetic radiation (*in vitro*) with erythrocyte cells. Such an interaction was evaluated using Electron Paramagnetic Resonance (EPR) spectroscopy, Infra-red (IR) spectroscopy, Differential Scanning Calorimetry (DSC), AC conductivity and Scanning Electron Microscope (SEM). In addition, study the influence of α-lipoic acid in protecting the red blood cells against reactive oxygen species (ROS) which resulting from the exposure to gamma radiation.

1-1-Effect of gamma radiation on erythrocytes

The cell membrane is one of the targets of radiation induced events leading to cellular damage. The reactive oxygen species (ROS) produced by water radiolysis react with numerous macromolecular cellular constituents, phospholipids and proteins, the vital membrane components, are susceptible to radiation damage. Studies on model membranes have shown that polyunsaturated hydrocarbon moieties are easily oxidized by radiation induced free radicals. These reactions produce lipid peroxides and their breakdown products which can alter the membrane structure and can cause damage to the membrane bound enzymes and other molecules **(Verma and Rastogi, 1990).** Ionizing radiations was reported to cause an oxidation of -SH groups to corresponding dithiols and induce conformational changes of membrane proteins. Also the processes of the membrane cholesterol conversion to its

oxy-derivatives are increased under the effect of radiation **(Palmarchuk, 1990).** So both oxidation of the membrane protein thiol groups and lipid peroxidation may result from irradiation of erythrocytes membrane **(Gwozdzinski, 1991).**

Vicker *et al*. (1991) provided an evidence for radiation action. It was mentioned that doses of several Grays can modify or destroy sufficient biological molecules of all classes to be immediately life-threatening to humans, while a few cGy may significantly affect only DNA.

Brugnara and Churchil (1992) reported that irradiation of erythrocytes with a 20 Gy radiation dose caused a significant increase in the external sodium ion concentration. They attributed the change to increased permeability to sodium and potassium ions on the part of the erythrocyte membrane lipid bilayer. These changes could be reversed by incubating the cells at 37°C.

Jian *et al*. (1993) have suggested that the membrane abnormalities that occurred as a result of oxidation attack may increase the rigidity of the lipid bilayer and aggregation of membrane proteins. They also proposed that lipid peroxidation may result in formation of cross linkages between red cells or protein molecules and lead to erythrocytes aggregation.

When the rate of generation of free radicals exceeds the ability of the defense system to detoxify free radicals, this cause tissue damage **(Halliwell and Chirico, 1993**).

Pribush *et al*. (1994) reported that in vitro irradiation up to 200 Gy causes no significant changes in the constituents of the red blood cells but increases the rate of potassium leakage indicating that the major site of radiation damage is the cell membrane.

Gamma-irradiation of erythrocytes induces alterations at the three different functional units of the membrane:

1- Lipid bilayer.

2- Protein components.

3- Cytoskeleton at the membrane surfaces.

Lee and Ducoff (1994) postulated that free radicals formed during radiolysis of water can cause a variety of membrane changes including lipid peroxidation, hemolysis of phospholipids head groups, lipid-lipid cross links, disulphide bridge formation and amino acid residue damages in membrane proteins, and lipid-protein cross links. The combined effect of free radicals on the erythrocytes membrane and cytoskeleton may contribute to the eventual leak of hemoglobin out of the cells.

Riley (1994) demonstrated that the hydroxyl radicals are the most reactive radiolytic species, and are the predominant damaging species oxdatively modifying biological molecules.

Anand *et al*. (1997) hypothesized that in vitro (15, 25 , 50 Gy) gamma-radiation damages erythrocytes membrane by producing reacting reactive oxygen species (ROS), which leads to peroxidation of membrane lipids and oxidation of membrane-bound proteins. He also reported that the gamma-irradiation of blood result in hemoglobin oxidation.

Also, **Saran and Bors (1997)** suggested that the secondary chloride-derived radicals generated during irradiation in PBS could contribute to the damage to erythrocytes. These radicals could cause a greater damage to the plasma membrane than $^{\cdot}OH$ radicals that could lead to a faster post-radiation hemolysis. There are also some other parameters related to membrane structure or other membrane components while could influence the lipid peroxidation reactions. One such parameter which evaluated is the lipid- protein association; the proteins which probably bind with lipids can appreciably inhibit the formation of lipid peroxides. In these

way proteins, the most sensitive components of membranes, can protect membranes against the radiation induced peroxidation during the subsequent dose by interacting with the lipids during the first dose radiation and thus protect erythrocytes before hemolysis (**Zaborowski and Szweda-lewandowska, 1997).**

Racek *et al.* (1997) reported that blood is exposed to oxidation stress and therefore has a high antioxidant capacity (AOC). Consequently, damage to erythrocytes by free radicals may occur. Potassium release from erythrocytes into plasma, followed by hemoglobin release, can be considered a very good indicator of the oxidative damage of erythrocytes membranes.

Also, according to **Soszynski and Schuessler (1998**), spectrin (band 1, 2) and band 3 are the most affected due to radiation exposure.

Exposure to ionizing radiation leads to structural changes in erythrocytes membrane proteins and decrease in its