# RADIOPROTECTIVE EFFECT OF PALLADIUM α-LIPOIC ACID COMPLEX ON THE DIELECTRIC RELAXATION AND AC CONDUCTIVITY OF RED BLOOD CELLS

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Abstract. The present work studies the effect of palladium  $\alpha$ -lipoic acid complex (PLAC) on the dielectric relaxation and AC conductivity of red blood cells and tests its effect as radioprotector. Blood samples were exposed to gamma radiation at dose level 100 Gy. The samples were divided into four groups: control, control with PLAC, irradiated and irradiated with PLAC. The dielectric properties were measured to determine the relative permittivity, dielectric loss and AC conductivity. The addition of PLAC to the blood samples resulted in increase in the relative permittivity, dielectric loss and AC conductivity of control red blood cells. While the exposure to gamma radiation affected their dielectric properties, the addition of PLAC to the RBCs prior to exposure to 100 Gy gamma radiation was shown to provide a significant protection from radiation-induced damage for the measured parameters in this study.

*Key words*: Palladium lipoic acid complex, red blood cells, dielectric properties, gamma radiation, liquid crystals.

### INTRODUCTION

Palladium lipoic acid complex (PLAC) was formulated to act as a non-toxic chemotherapeutic agent [16]. It is composed of the transition element palladium covalently bound to the antioxidant alpha-lipoic acid, in a unique arrangement that allows the molecule to be both water and lipid soluble, as well as it exists as a liquid crystal. This liquid crystalline structure allows it to store a great deal of energy and thus serves as a semiconductor [17]. It exists in a prescription version called DNA reductase and a dietary supplement called Poly MVA [2]. PLAC was shown to give protection against radiation illness. During oral administration of this material in the emergency treatment of certain brain tumors, it was found that patients receiving concurrent radiation, and depression [19].

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It was examined for its efficacy as a radioprotector; it significantly reduced the  $\gamma$ -radiation-induced mortality in mice and aided recovery from the radiationinduced loss of body weight after 8 Gy exposures. Also, the radiation-induced DNA damage in these cells was reduced when PLAC was administered to animals exposed to a lethal dose of 8 Gy whole-body  $\gamma$ -radiation [45]. Administration of PLAC for seven days prior to whole body exposure to different doses of gamma radiation (2, 4, and 8 Gy) significantly reduced the damage to cellular DNA in bone marrow and blood leukocytes, as well as preventing the radiation-induced lowering of tissue antioxidant levels [32].

PLAC was designed to inhibit anaerobic cells without damaging healthy ones. Research indicates that this compound can influence the electrochemical process occurring in the body, consequently "electrocuting" cancer cells while leaving healthy cells alive. Cancer cells operate in anaerobic conditions (without oxygen) and PLAC appears to target and kill these anaerobic cells in part through its ability to change cells' electrochemical circuitry [18]. A charge transfer from membrane phospholipid to DNA is the presumptive mechanism whereby certain tumors, protozoa, and yeasts are inhibited by this complex. The subcellular site of destruction has been shown to be the membrane [16]. However, not all normal body cells are aerobic. Red blood cells (RBCs) are the main organelle that supplies all the body's tissues and cells with oxygen. They do not have mitochondria and other organelles that may otherwise be present in a normal cell. Therefore, RBCs depend on anaerobic respiration, instead of aerobic respiration. Only glycolysis supplies energy to this cell. What will be the effects of PLAC on these anaerobic normal cells? And can this complex provide protection from radiation induced effects? This work is a part of a study conducted to investigate the effects of PLAC on RBCs properties in vitro, and to test its radio-protective effects.

### MATERIALS AND METHODS

#### PREPARATION OF RED BLOOD CELLS SAMPLES

Blood samples were obtained from adult male Swiss Albino rats, weighing 120–150 g, after dissection using heparnized needles to prevent coagulation. To retain cell viability 5% glucose isotonic solution (commercially available, pyrogen free, prepared for intravenous infusion) was added to blood samples to reach concentration of 20 mM. Each sample was divided into four groups to provide self control comparison: control, control with PLAC (positive control), irradiated and irradiated with PLAC. Each group consisted of 6 samples of volume 8 mL. They were incubated in water bath at 37°C for 30 min before irradiation. Whole blood samples were irradiated in glass bottles tightly closed at room temperature. The short duration of irradiation (about 2 minutes) avoids heating or oxidation of the samples during irradiation. After irradiation, the samples were centrifuged at

3000 rpm for 10 minutes to remove plasma and buffy coats. RBCs were washed by phosphate-buffer saline (PBS, pH 7.4) and centrifuged at 3000 rpm for another 10 minutes at 4 °C, and then the supernatant was removed to obtain the packed cells ready for the experimental measurements.

# RADIOPROTECTOR

Palladium lipoic acid complex (PLAC), a liquid supplement of concentration 11.65 mg/mL, was obtained as a gift from Garnett Mckeen laboratory, Inc., USA. It was added to the whole blood in concentration of 2%v/v without further purification as described by Menon and Nair 2011 [33].

#### GAMMA IRRADIATION

The irradiation process was carried out in the National Center for Radiation Research and Technology (NCCRT), Atomic Energy Authority, Cairo, Egypt. The irradiation dose was 100 Gy from Cobalt-60 source (dose rate was 3.089 kGy/h) at the beginning of the experimental work. The calibrations of the sources and doses calculation were performed by the Egyptian high-dose reference laboratory. The chosen dose in this study (100 Gy) was based on its significant effects on the measured factors as reported in previous studies [12, 50].

### DIELECTRIC MEASUREMENTS

The dielectric properties were measured using LCR meter (HIOKI 3531) manufactured in Japan, in the frequency range 0.4 to 5 MHz. The measuring cell is a parallel plate conductivity cell with platinum electrodes with area 4  $cm^2$  and separating distance 2 cm.

The red blood cells were re-suspended in buffered saline (pH 7.4 and conductivity 0.65 S/m), and the hematocrit was adjusted at 3%. The samples were incubated in water bath at 37 °C during measurement.

The measured parameters were the capacitance *C* and resistance *R*, from which the permittivity  $\varepsilon'$  and AC conductivity  $\sigma_{AC}$  can be calculated as follows [37, 48]:

$$C = A \varepsilon' \varepsilon_0 / d \tag{1}$$

where A is the area of the electrode and d is the distance between the two electrodes and  $\varepsilon_0$  is the vacuum permittivity (F/m). The permittivity can be expressed in complex quantity as:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \tag{2}$$

The real part represents the permittivity constant and is given by:

$$\varepsilon' = \varepsilon_{\infty} + \frac{\varepsilon_s - \varepsilon_{\infty}}{1 + \omega^2 \tau^2}$$
(3)

where  $\varepsilon_s$  is the limiting low frequency permittivity, and  $\varepsilon_{\infty}$  is the permittivity value at the end of the dispersion,  $\tau$  is the relaxation time and the imaginary part  $\varepsilon''$  (the dielectric loss):

$$\varepsilon'' = \frac{(\varepsilon_{\rm s} - \varepsilon_{\infty})\omega\tau}{1 + \omega^2\tau^2} \tag{4}$$

In order to separate the AC conductivity component from the total conductivity measured (DC and AC) the following relationship was applied [37]:

$$\sigma_{\rm AC} = \omega \, \varepsilon_{\rm o} \, \varepsilon^{\prime \prime} \tag{5}$$

The total area under the  $\varepsilon''$  loss curve is proportional to the total concentration of dipoles in the material and their dipole moment, irrespective of their distribution of relaxation times [40]. It is related to the relaxation strength,  $\Delta \varepsilon (\Delta \varepsilon = \varepsilon_s - \varepsilon_{\infty})$  by:

$$\operatorname{area} = \pi \Delta \varepsilon / 2 \tag{6}$$

#### MEMBRANE PERMEABILITY

The membrane permeability was studied by measuring the effects of triton X-100, a nonionic detergent, with cell membrane. Triton X-100 has two types of effects on red blood cell membrane: the permeability and damage effects, which happen simultaneously, but with two different mechanisms [6]. The damaging effect was measured as the change in the turbidity of cells suspension as a function of detergent concentration. The permeability effect was measured by recording the release of hemoglobin by visible spectroscopy after sedimentation of the detergent-treated RBCs [21]. Turbidity is commonly measured by spectrophotometer using wavelength far from the absorbed range by the sample. Dilute RBCs suspension in PBS (pH 7.4) was added to different dilutions of Triton X-100 solution ranging from 0.034 to 0.272 mM. After incubation of the samples at 37°C for 20 min, the transmittance was recorded at 600 nm, and the turbidity T was calculated from the following equation:

$$I = I_0 e^{Tl} \tag{7}$$

where  $I_0$  and I are intensities of the incident and transmitted light respectively, and l is the length of the light path through the sample [10]. The permeability was measured as the absorbance at 540 nm of the hemoglobin released after

centrifugation of the RBCs at 3000 rpm for 10 min. The percentage of hemolysis was taken against complete hemolysis [5]:

$$\%H = \frac{A_{\text{sample}}}{A_{100\%\text{lysis}}} \times 100 \tag{8}$$

where  $A_{\text{sample}}$  and  $A_{100\% \text{lysis}}$  are the absorbance of the hemoglobin released from RBCs in normal saline and after complete hemolysis in distilled water respectively.

#### STATISTICAL ANALYSIS

The experimental results were expressed as mean values  $\pm$  standard deviation. The significance of the difference between the values of the treated groups and control was evaluated by the Student t-test and values with p < 0.05 were considered as statistically significant.

# **RESULTS AND DISCUSSION**

The RBC is unique amongst eukaryotic cells in that it is anuclear; it has no cytoplasmic organelles. All the structural properties are linked to the membrane; a fluid assembly of amphiphilic molecules into a two-dimensional liquid crystalline structure [55]. This structure is rigid enough to form a stable container but is fluid to allow lateral transport of the different membrane components and a differential permeability essential for cell homeostasis [14].

Palladium lipoic acid complex (PLAC) possesses a powerful antioxidant effect, which can be partly attributed to its lipoic acid fraction. Since this naturally occurring acid is soluble in both fat and water, PLAC are able to pass across cell membranes and work intracellularly [34]. The active ingredient in PLAC is the palladium-lipoic acid polymer, which allows palladium-lipoic acid to be soluble and exists as liquid crystal. It acts as a liquid electrical transistor that transfers electrical current from the cell membrane to the mitochondria which redistributes the electrical current throughout the cell via the existing electrical pathways [17]. Plasma membrane oxido-reductases (PMORs) are trans-membrane electron transport systems that have been found in the membranes of all cells, and have been extensively characterized in the human RBCs [25]. They transfer reducing equivalents from intracellular reductants to extracellular oxidants, thus their most important role seems to enable cell response to changes in intra- and extracellular redox environments [31]. Glycolytic ATP, the energy supplying process to the cell and a major factor affecting RBCs viability, can be channeled to membrane ion pumps, where the glycolytic enzymes are organized into complexes on the membrane and are regulated by oxygenation and phosphorylation [8].

#### DIELECTRIC PROPERTIES

It was reported that all electrical parameters were significantly correlated with pH and ATP [51]. Membrane permeability governs the acid base status and directly controls the transport of oxygen and carbon dioxide through the blood [13]. Different factors affect the permeability of water, nonelectrolytes and electrolytes. For example, the surface potential at the membrane surface will affect the ability of charged ions to cross. Also, the order of the membrane; generally the more ordered the membrane, the less permeable it is [15]. The dynamical ionic transport through the membrane, and hence its permeability, affects the electrical conductivity of RBCs suspension [41]. The capacitance determines the amount of charge that can be stored across a membrane when a cell is exposed to an electric field, and depends strongly on the level of folding of the cell membrane [20]. In this study, the dielectric properties, which include both conductive and capacitive properties, were tested. This dielectric dispersion mechanism is closely related to ion flow through the membrane pores, which is a microscopically important process in membrane/electrolyte systems and in biological cells and tissues. In biological tissues, such ionic activities are associated with cell viability, cell membrane composition, and tissue microstructure [28]. The addition of PLAC only to RBCs was shown to increase the relative permittivity, dielectric loss and AC conductivity compared to control values (Figs. 1, 2, and 3). Also, it resulted in increase in the number of dipoles in the cell membrane, as appeared from the significant increase in the area under loss peak (Table 1).

In a previous study, the addition of lipoic acid resulted in decrease in the relative permittivity and dielectric loss of RBCs, no induced changes in AC conductivity which was attributed to the interaction of the lipoic acid with red blood cells membrane which decreased the number of dipoles while no significant effect on the membrane permeability was detected [50]. The addition of palladium to lipoic acid in the PLAC complex changes its properties; and resulted in significant increase in the membrane relative permittivity, which is a measure of its polarizability in the electric field. It is also related to the structural arrangement of the lipid bilayer and to the conformation and localization of proteins in the membrane [7]. The increase in the number of dipoles can also be attributed to increase in energy production in the RBCs as a result of addition of PLAC, since the ATP molecule possesses a large dipole moment [29]. The increase in AC conductivity indicates increase membrane permeability which may result from enhanced interchange of intra- and extracellular fluids [38, 39]. This result is in contrast to the non-significant decrease in the conductivity observed in a previous study after addition of lipoic acid. Positive correlation was found between cell membrane permeability, ATPase activation, and ATP in RBCs, which suggests that activation of the ion pump is concomitant with a significant increase in intracellular ATP [4, 51]. Also, the glycolytic ATP can be channeled to membrane

ion pumps allowing its direct consumption without release into the cytoplasm [8]. This result supports that the addition of PLAC enhances the ATP production in the RBCs.

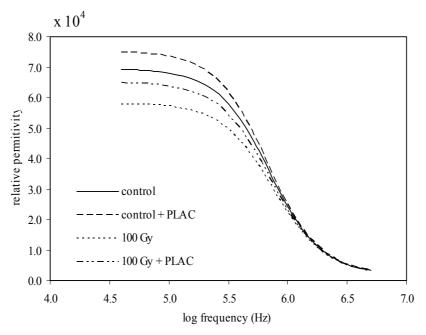


Fig. 1. Relative permittivity for control, treated with PLAC, irradiated and treated irradiated red blood cells.

Gamma irradiation of RBCs resulted in significant decrease in the relative permittivity dielectric loss and AC conductivity. The effect of exposure of RBCs to gamma radiation was extensively studied. It was shown to induce alterations in the functional units of the membrane: lipid bilayer, protein components and cytoskeleton at the membrane surface [7]. It also induces shortening in the lipid fatty acid chains by lipid peroxidation [47], and the produced hydroperoxides and cross-linkages in the membrane lipids can disorder the upper region of the bilayer favoring penetration of water and ending by hemolysis [36]. Free radicals formed during irradiation can cause a variety of membrane changes including lipid peroxidation, hydrolysis of phospholipids head groups, lipid-lipid crosslinks, disulfide bridge formation and amino acid residue damage in membrane proteins and lipid-protein crosslinks [30]. The effects of free radicals on the RBCs membrane and cytoskeleton may contribute to the leak of hemoglobin out of the cells. The hemolysis of the RBCs reflects the loss of integrity of the cells which can lead to the liberation of intracellular hemoglobin [47]. In addition, ionizing radiation was reported to cause oxidation of the sulphydryl groups to the

corresponding dithiols and induce conformational changes of membrane proteins [22]. The decrease in the dielectric permittivity and loss reflects the radiation induced damage in the cell membrane, since they are related to the structural arrangement of the lipid bilayer and also to the conformation and localization of proteins in the membrane [7]. The significant decrease in the conductivity of RBCs as a result of exposure to 100 Gy gamma radiation can be attributed to the radiation induced damage in cell membrane with the subsequent loss of ions, electrolytes and intracellular components [50]. Irradiation up to 100 Gy gamma X-rays produced a significant decrease in the activity of ATP-ase [35], affecting the membrane transport activity.

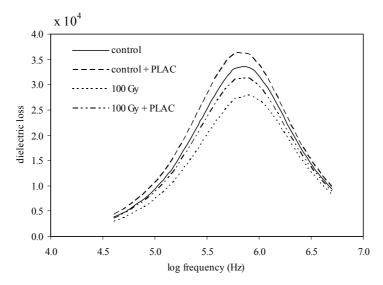


Fig. 2. Dielectric loss for control, treated with PLAC, irradiated and treated irradiated red blood cells.

The effects of PLAC as radioprotector can be regarded as a result of both its radical scavenging and inducing energy production. Previous studies reported that PLAC is composed of lipoic acid (free radical scavenger) with palladium (an energy source) which results in the unique electronic and redox properties of palladium  $\alpha$ -lipoic acid [15, 27]. Its oxygen radical absorbance capacity ORAC value is 5.65 [3], i.e. it is approximately five times more potent antioxidant than  $\alpha$ -lipoic acid. This formulation demonstrated that it serves as both a highly active free radical scavenger and alternative energy source for body cells [2]. It also increases the level of GSH and reduced the MDA level, and may facilitate a chain breaking antioxidant effect on the lipid peroxidation process [53]. Due to its antioxidant effect, it acts as a therapy of diseases associated with oxidative stress, either directly as a free radical scavenger or indirectly due to its synergistic action with other antioxidants [9]. Also, it was reported that it enhances the enzymatic

activities for both Krebs cycle and respiratory complexes I–IV, with a concentration of about 13 times less than that of pure  $\alpha$ -lipoic acid [52]. In this study, the addition of PLAC to irradiated group resulted in increase in the dielectric factors from the irradiated group and approach to control, which shows that it provides a significant degree of radio-protection against gamma radiation. These effects can be attributed to its ability to neutralize the free radicals formed by the radiation and the enhancement of the cellular energy production in the form of ATP as previously shown in the positive control group.

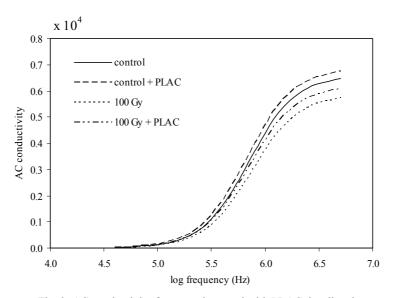


Fig. 3. AC conductivity for control, treated with PLAC, irradiated and treated irradiated red blood cells.

# MEMBRANE PERMEABILITY

Another study of the effect of PLAC on the membrane permeability was performed by the detergent test. The interaction of triton X-100 with RBCs consists of an ensemble of stages: the initial incorporation of detergent molecules into the membrane leads to a decrease in molecular packing which causes an increase in permeability, due to a colloid osmotic imbalance, resulting in hemolysis. Finally, at higher detergent concentrations, full membrane solubilization is achieved, its molecular components being incorporated into detergent micelles [44]. The detergent–membrane interaction concerns the physico-chemical and molecular aspects of this interaction. One interesting aspect is the interplay between bilayer and micellar structure as a function of lipid and detergent concentration, as well as their molar ratios [44]. The detergent attacks the membrane in a systematic manner according to its spatial organization [26].

# Table 1

Relative permittivity, area under loss peak, AC conductivity at 5 MHz and  $\alpha$ ' (relaxation time distribution factor) for control, treated with PLAC, irradiated and treated irradiated groups

Groups	Statistics	ε <sub>s</sub>	Area under loss peak	$\sigma_{5MHz}(S/m)$
control	mean	6.94×10 <sup>4</sup>	1.06×10 <sup>5</sup>	6.46×10 <sup>3</sup>
	<i>S. D</i> .	3.54×10 <sup>3</sup>	5.50×10 <sup>3</sup>	241.55
PLAC	mean	7.51×10 <sup>4</sup> *	1.15×10 <sup>5</sup> *	6.74×10 <sup>3</sup> *
	S. D.	4.62×10 <sup>3</sup>	$1.04 \times 10^{3}$	84.22
100 Gy	mean	5.81×10 <sup>4</sup> *	8.80×10 <sup>4</sup> *	5.73×10 <sup>3</sup> *
	S. D.	1.63×10 <sup>3</sup>	$2.54 \times 10^{3}$	105.49
100 Gy + PLAC	mean	6.50×10 <sup>4</sup>	9.78×10 <sup>4</sup>	6.08×10 <sup>3</sup> *
	S. D.	1.96×10 <sup>3</sup>	$3.07 \times 10^{3}$	105.29

S.D.: standard deviation

\*: statistically significant

The analysis of membrane solubilization can be presented by both percentage of turbidity and percentage hemolysis versus detergent concentration. In the turbidity curve (Fig. 4a) the concentration of detergent necessary to start up and to terminate the solubilization process was obtained by y-axis intercepts of the straight lines: the  $D_{sat}$  and  $D_{sol}$  respectively [44]. Both factors depend on the behavior of the detergent with structure and composition properties of the RBCs membrane. It was shown that the detergent resistance increases monotonously with acyl chain order/rigidity in the hydrophobic region [46]. Differentiation of the sigmoidal curve of turbidity yields a Gaussian distribution curve (Fig. 5) from which one can calculate the area under the curve (A) which is a function of the mass of the RBCs membrane digested by the detergent [11]. The fitting equations of the experimental data were previously discussed [49]. Addition of PLAC was shown to increase this factor significantly, which can be explained by the interaction of the complex with the membrane resulted in increasing the mass digested by the detergent. Exposure to 100 Gy showed significant decrease in the membrane mass as a result of the damaging effect to the membrane structure. Addition of PLAC prior to irradiation was shown to protect the membrane as seen from the non-significant change of the cell membrane mass (Table 2).

At low detergent concentration, it causes the breakdown of the hydrophobic barrier of the membrane and increases its permeabilization to solutes while keeping larger anionic proteins in the cytoplasm [21]. Small ions and associated water molecules enter the cell; creating a positive osmotic pressure, result in swelling and subsequent bursting of the cell, a process referred to as colloid-osmotic lysis [42]. In the hemolysis curve (Fig. 6)  $C_{\text{sat}}$  and  $C_{\text{sol}}$  are considered as the detergent concentration required for inducing onset of hemolysis, and total lysis respectively [43].

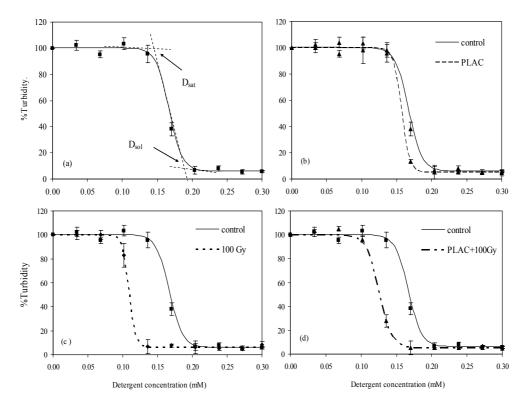


Fig. 4. Membrane solubilization curve of Triton X-100 on control red blood cells showing: graphical calculation of  $D_{\text{sat}}$  and  $D_{\text{sol}}$  (a), control and PLAC group (b), control and irradiated group and control and treated irradiated group (d).

The hemolysis in the presence of detergent depends intrinsically on the osmotic fragility of RBCs [23], and the permeability of the cell membrane to water and to the permeating solute [24].  $D_{sat}$  and  $D_{sol}$  of the group treated with PLAC alone or before irradiation did not show significant changes from the control group (Table 2). However, both parameters showed significant decrease for the irradiated group. The interaction of lipoic acid with cell membrane was reported to enter the non-polar part of the membrane, weakens the van der Waals interaction between the acyl chains, and consequently leads to a disordering in the system [1].

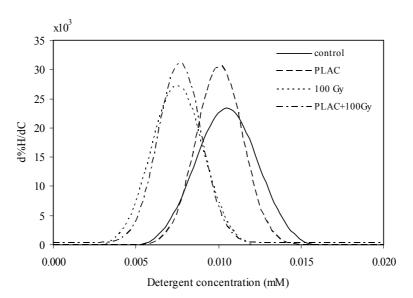


Fig. 5. Differentiation of the membrane solubilization curve of control red blood cells, PLAC, irradiated and treated irradiated groups.

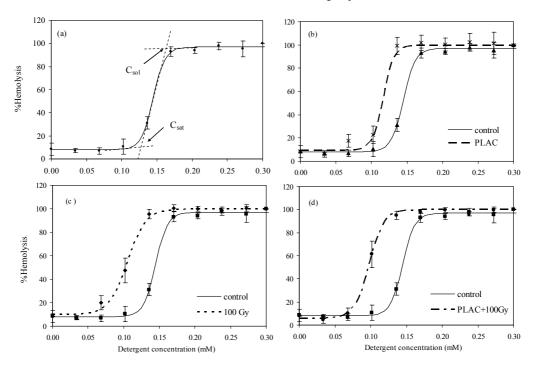


Fig. 6. Hemolysis curves of control red blood cells showing: graphical calculation of  $C_{sol}$  and  $C_{sat}$  (a), control and PLAC group (b), control and irradiated group and control and treated irradiated group (d).

In this study, the non-significant change the membrane resistance to detergent suggests that interaction of PLAC with cell membrane didn't affect the molecular packing of its structure. The significant decrease in solubilization parameters for the irradiated group reflects the radiation-induced damage and disorder of the membrane structure. Addition of PLAC before irradiation was shown to provide certain degree of protection against the radiation damage. No change in membrane permeability was found at concentrations up to 0.13 mM ( $C_{sat}$  value) for the control RBCs during the present experiment. This value decreased to 0.103 mM after the addition of PLAC. For the control group, the obtained  $D_{sat}$  and  $D_{sol}$  value is nearly equal  $C_{sat}$  and  $C_{sol}$  (Table 2). However, for the group treated with PLAC showed the solubilization factors are higher than hemolysis factors (0.149 and 0.175 mM compared to 0.103 and 0.14 mM respectively). These results support the previous obtained result in this study, that PLAC increase the membrane permeability. Exposure to gamma radiation also, decreases  $C_{sat}$  and  $C_{sol}$  value significantly (Table 2).

#### Table 2

The membrane solubilization parameters (concentration of detergent necessary to start up and to terminate the solubilization process  $D_{sat}$  and  $D_{sol}$ ) and hemolysis parameters (the detergent concentration required for inducing onset of hemolysis, and total lysis  $C_{sat}$  and  $C_{sol}$ ), the area under the Gaussian distribution curve turbidity (A) for control, treated group with PLAC, irradiated and treated irradiated groups

Groups	Statistics	A	D <sub>sat</sub> (mM)	D <sub>sol</sub> (mM)	C <sub>sat</sub> (mM)	C <sub>sol</sub> (mM)
control	mean	1595.28	0.143	0.176	0.13	0.173
	<i>S. D</i> .	139.74	0.026	0.014	0.019	0.022
PLAC	mean	1706.80*	0.149	0.175	0.103*	0.14*
	S. D.	103.36	0.018	0.018	0.029	0.015
100 Gy	mean	1498.72*	0.104*	0.148*	0.081	0.114*
	S. D.	59.67	0.021	0.012	0.009	0.019
100 Gy +	mean	1629.62	0.13	0.161	0.078	0.122*
PLAC	S. D.	89.42	0.016	0.015	0.014	0.009
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S.D.: standard deviation

\*: statistically significant

It was shown that the by-products of lipid peroxidation, resulted from radiation exposure, cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids [54]. In this study, the decrease in the hemolysis factors can be related to the radiation induced impairment of the membrane permeability. Regarding the group treated by PLAC before exposure to 100 Gy, it showed

decreased hemolysis factor significantly from control, in contrast to the radioprotective effects noticed for the other factors observed in this study. This result may be explained by the effect of the PLAC in increasing the membrane permeability discussed before, which interfered with the radiation-induced impairment in the membrane permeability. This effect cannot be considered as contradiction to the radioprotective effect of the PLAC observed in this study, since the addition of detergent to the RBCs is an experimental condition for testing the membrane properties, not a natural state.

#### CONCLUSION

PLAC formulation is a safe nutritional supplement. Its toxicological studies indicated that the LD<sub>50</sub> of palladium  $\alpha$ -lipoic acid formulation exceeded 5000 mg/kg. Unlike its relative platinum, no evidence of any mutagenic property was demonstrated for palladium [53]. The Oxygen Radical Absorbance Capacity (ORAC) analysis of palladium  $\alpha$ -lipoic acid formulation demonstrates that it is approximately five times more potent antioxidant than DL- $\alpha$ -lipoic acid [3]. The addition of PLAC to the RBCs in vitro was shown to increase its dielectric properties and AC conductivity. These effects were attributed to enhancement of the energy production in the form of ATP, which promote cell functions and increase cell permeability. The study of the effect of triton X-100 on the membrane solubilization and hemolysis of the RBCs support the increase in the membrane permeability induced by PLAC. The addition of PLAC to the RBCs prior to exposure to 100 Gy gamma radiation was shown to provide a significant protection from radiation-induced damage for the measured parameters in this study. The advantage of studying the radioprotective effect of PLAC lies in its structure as a liquid crystal substance, which could represents a novel category of radioprotectors that takes into consideration the liquid crystal properties of the biological systems, rather than restricting the research in the scope of antioxidants or radical scavengers either natural or with minimal side effects.

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