PROPHYLAXIS AND MITIGATION OF RADIATION-INDUCED CHANGES IN THE ELECTRICAL PROPERTIES OF ERYTHROCYTES BY PALLADIUM LIPOIC ACID NANO-COMPLEX (POLY-MVA)

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Abstract. The aim of the present work is to study the prophylactic and mitigation effect of poly-MVA against acute whole body gamma irradiation. Adult male rats were exposed to 6 Gy single dose from Cs-137 source. The electrical properties of erythrocytes suspension were measured in the frequency range 40 kHz to 5 MHz. The lipid peroxidation of liver homogenate, the mean corpuscular volume, hemoglobin concentration and hematocrit were also measured. The animals received daily oral administration of 2 mL/kg body weight of poly-MVA for three different time intervals. The study of the prophylactic effect was applied by two modes of administration: two weeks before irradiation and another group which received continuous administration for two weeks before and two other weeks after irradiation (total time of administration 28 days). The mitigation effect was examined by administration of poly-MVA for two weeks after irradiation. The results showed that exposure to gamma radiation caused a decrease in the AC conductivity, relative permittivity, area under the loss curve and membrane effective capacitance, while caused increase in the lipid peroxidation I day after exposure to radiation. The later change persisted until the 14^{th} day recorded after irradiation. The administration of poly-MVA after irradiation alleviated the radiation-induced damage as appeared from the non-significant change in the measured parameters compared to control. The group which received continuous administration, before and after irradiation, showed better results compared to the group which received administration before irradiation only. The obtained results showed that poly-MVA can be used as prophylactic as well as mitigator agent on planned and accidental radiation exposure.

Key words: erythrocytes, gamma radiation, dielectric properties, lipid peroxidation, poly-MVA, radio-prophylaxis, mitigation effects.

INTRODUCTION

The increase applications of nuclear technologies in power production, medicine and industry enhance the probability of accidental radiation exposure to occupational workers, patients and public. In comparison with planned radiation

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exposure during diagnosis and therapy, the management of accidental radiation exposure is relatively complicated due to uncertainties in dose, duration, organs involved in radiation exposure. Although radiation accidents are not common, human error, failure to follow safety precautions and inadequate control and regulation of radiation sources have led to deaths and significant exposures among workers and members of the public [5]. Treatment of the victim in this case needs information and measurements that may take time in order to determine the suitable therapeutic strategy for its case. However, after the event of the radiation accident, it will be important to provide an agent that would mitigate the effects of exposure to ionizing radiation and can promote or increase the efficiency of the treatment. Ideally this agent would be long-lasting, be easily administered (preferably orally) and possesses low toxicity [35]. Development of agents for prophylaxis, mitigation and treatment of radiation injury is an important task for radiological studies. Radio-prophylactics are agents that must be given before radiation exposure; mitigators are given after radiation exposure, but before the appearance of overt evidence of injury; and treatment refers to those agents that are given after the development of clinical symptoms [48]. Treatment agents include suppressors of the renin-angiotensin system and suppressors of chronic oxidative stress, but also include agents such as pentoxifylline to treat radiation fibrosis and growth factors to facilitate recovery from hematological injury [30]. In recent years, radio-prophylactic agents with a novel mode of action have been investigated. Some types aimed to increase survival rate by stimulating the function and regeneration of the stem cell population that is decreased due to radiation induced damage [22, 52]. They can be naturally occurring compounds that function as antioxidants and immuno-stimulants. Among these categories are: superoxide dismutase (SOD) enzymes that scavenge superoxide anion by catalyzing its conversion to hydrogen peroxide and oxygen. Alpha-tocopherol succinate was found to be the best of all tocopherols tested for induction of cytokines [46] and flavonoids, which exhibit strong antioxidant activities, are a family of polyphenolic compounds found in fruits and vegetables [45]. Other types targeted the DNA directly, such as methylproamine, which belongs to the bis-benzimidazol family. It has two benzimidazole groups and one phenol group, conferring minor DNA groove-binding properties. Its mechanism of radioprotective activity is to donate an electron from the ligand to damaged DNA [9, 27]. DNA-reductase is another agent which has the ability to shunt electron energy from itself to DNA. It is the original family member of palladium lipoic acid complex, available commercially as dietary supplement called poly-MVA [1]. Its active ingredient is the palladium–lipoic acid polymer, which exists as a trimer of palladium-lipoic acid joined to thiamine in an arrangement which allows it to be both water and lipid soluble, and the initials “MVA” stand for minerals, vitamins, and amino acids [16]. Palladium, which is a transition mineral, serves as highly efficient aerobic catalyst [47]. It shares a common resonance frequency with DNA; hence, electron transfer between the molecules is facilitated. Besides the lipoic acid palladium core unit, poly-MVA

contains free lipoic acid minerals (molybdenum, rhodium, and ruthenium), vitamins (B_1, B_2, and B_{12}) and amino acids (N-acetyl cysteine and formyl methionine) [11]. This formulation is designed to provide energy for compromised body systems by changing the electrical potential of human cells and increasing DNA's charge density within the cell. It was applied to target cancer cells, which operate in anaerobic conditions (without oxygen) and poly-MVA appears to target and kill these anaerobic cells in part through its ability to change cells' electrochemical circuitry [17]. Toxicological studies indicated that the LD_{50} of poly-MVA exceeded 5000 mg/kg, and no mutagenic effect of the combination was observed in the Ames test [8].

Improved animal models are required for testing of therapeutics for treatment and prophylaxis of radiation injury [48]. In addition, animal experimentation is necessary to accurately predict the medical consequences of radiation exposure in humans and to develop new mechanistically targeted interventions [35]. This work is a part of a study conducted to test the efficiency of poly-MVA as mitigator after exposure to a single sublethal dose of gamma radiation (6 Gy), in comparison to its effect as radio-prophylactic agent, on the biophysical properties of rats' erythrocytes.

MATERIALS AND METHODS

CHEMICALS

Poly-MVA is a liquid dietary supplement that contains 23.5 mg/mL of lipoic acid–palladium complexes as well as minerals (molybdenum, rhodium, ruthenium), vitamins (B_1, B_2 and B_{12}), and amino acids (N-acetyl cysteine and formyl methionine). The palladium lipoic acid formulation, in this study, was obtained as a gift from Garnett McKeen Laboratory, Inc., USA.

ANIMALS

Adult male Swiss Albino rats weighing 200g were used. They were divided into 4 groups of 18 animals each: control group, treated group with poly-MVA, irradiated group and treated-irradiated group. Rats were kept under standard conditions along the experimental period. Food and water were supplied daily *ad libitum.* All animals were housed according to the ethical rules in compliance with institutional guidelines. The conditions were the same for all animals throughout the study. The animals were housed in standard cages (26 × 42 × 15 cm) with sawdust, at constant room temperature (25±1 °C) and relative humidity (45–55%) with a 12 hours light/dark cycle. They were dissected at three times intervals: 1, 7 days and 14 days after exposure to radiation.

IRRADIATION

Animals were placed in a specially designed well-ventilated acrylic pie cage which holds up to 20 rats. The whole body of the animals was exposed to 6 Gy gamma radiation from the biological irradiator gamma cell-40, cesium-137 source with dose rate: 0.769 cGy/s (manufactured at the atomic energy agency, Canada) at the National Center for Radiation Research and Technology, Cairo. The activity of the irradiation source allows the animals to receive the recommended dose in this study in 13 min.

TREATMENT

Poly-MVA was taken orally with daily dose of 2 mL/kg body weight. The effect of poly-MVA was tested after three modes of administration:

Group A consisted of two subgroups: subgroup treated with poly-MVA (positive control) and subgroup treated after irradiation for two consecutive weeks. The measurements were performed 1 day after first administration and/or irradiation, and continued to the 7^{th} and 14^{th} days.

Group B consisted of two subgroups treated with poly-MVA for two consecutive weeks; one of the subgroups was exposed to radiation at the end of administration period, and the other was kept without irradiation as positive control of this group. The measurements were performed 1 day after administration and/or irradiation, and continued to the 7^{th} and 14^{th} days.

Group C consisted of two subgroups: one subgroup was treated with poly-MVA for two consecutive weeks before irradiation and the administration continued for two other weeks, resulting in total administration time of 4 weeks. The measurements started 1 day after irradiation, and continued at the 7^{th} and 14^{th} days during poly-MVA administration. The positive control subgroup received administration of poly-MVA for four consecutive weeks (total time of administration 4 weeks). The measurements started after two weeks of administration at three time intervals: at the end of 2, 3 and 4 weeks.

PREPARATION OF ERYTHROCYTES SAMPLES

Animals were anesthetized by exposure to diethyl ether in a closed container by open-drop method. The blood samples were withdrawn from the left ventricle of the heart using heparinized needles. The blood samples were centrifuged at 3000 rpm for 5 min. The plasma and buffy coat were removed by aspiration. The erythrocytes were washed twice in buffered saline (pH 7.4) and separated by centrifugation at 3000 rpm for 10 min.

DIELECTRIC MEASUREMENTS

The dielectric measurements were performed using LCR meter HIOKI 3531, manufactured in Japan, in the frequency range 40 kHz to 5 MHz. The capacitance (*C*), conductance (*G*) and impedance (*Z*) were calculated using the appropriate relations by means of the LCR meter software. The erythrocytes suspension (hematocrit 3%) was suspended in buffered saline (pH 7.4 and conductivity 0.627 S/m). The samples were incubated in water bath at 37 ^{o}C during the measurement. The relative permittivity ε', dielectric loss ε", area under loss peak, AC conductivity and membrane effective capacitance C_{eff} were calculated as previously discussed [42–44].

ESTIMATION OF LIPID PEROXIDATION IN THE LIVER

Lipid peroxidation product in the liver homogenate, malondialdehyde (MDA), was measured by thiobarbituric acid assay according to the method of Ohkawa *et al*. [33]. The method is based on MDA reaction with thiobarbituric acid to give reactive substances, a red species that can be detected spectrophotometrically at 535 nm.

DETERMINATION OF HEMOGLOBIN CONCENTRATION

The hemoglobin concentration was measured spectrophotometrically at 540 nm using Drabkin's reagent and hemoglobin standard, obtained from EAGLE Diagnostics, USA.

DETERMINATION OF HEMATOCRIT

The determination of the hematocrit (*Hct*) was performed as follows: blood samples, in the hematocrit microcapillary tube (75 mm / 75 μL), were centrifuged for 5 minutes at 11,500 rpm. Then the hematocrit values were determined by means of the microcapillary tube reader [23].

MEAN CORPUSCULAR VOLUME (MCV)

The mean corpuscular volume (*MCV*) in femto liter expresses the average size of erythrocytes. It is related to the hematocrit (*Hct*) by the following relations [23]:

$$MCV = \frac{Hct}{erythrocytes\ count\ (\text{million per liter})} \tag{1}$$

STATISTICAL ANALYSIS

In this study, the values are expressed as mean values and 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

EFFECT OF poly-MVA

Liver is the primary organ responsible for drug metabolism [6]. MDA was measured to evaluate the effect of poly-MVA on lipid peroxidation in the liver and the radiation-induced oxidative stress at the considered dose (6 Gy). The administration of poly-MVA did not result in significant changes in MDA concentration for all groups. Also, the mean corpuscular volume, hemoglobin concentration and hematocrit showed normal values (Tables 1 and 2). The dielectric parameters (relative permittivity, dielectric loss and AC conductivity) showed non-significant changes for all treated groups, except the 28th days of group C which increased significantly from the control group. The effective capacitance did not show significant changes from control (Table 3).

EFFECT OF GAMMA RADIATION

Table 1 shows the significant increase (75%) in the MDA concentration after 1 day of exposure to radiation which persisted until the 14th days recorded in this experiment. The hemoglobin concentration and hematocrit decreased significantly after exposure to radiation, until they reached 50% of the control value at the 14th day (Table 2). The mean corpuscular volume showed a significant increase after 1 day and persisted until the 7th day, and decreased on the 14th day (Table 1). Fig. 1 shows the dielectric dispersion (the corresponding frequency dependence of permittivity), dielectric loss and AC conductivity of control erythrocytes. The dielectric loss curve can be evaluated by calculating its total area, which is proportional to the total concentration of dipoles in the material and their dipole moment, irrespective of the distribution of their relaxation times [36]. Whole body exposure to gamma radiation at dose level 6 Gy was shown to decrease the relative permittivity, dielectric loss, conductivity and effective capacitance of the cell membrane 1 day after exposure, and continued to decrease until the 14th day (Fig. 2, 3 and Table 3).

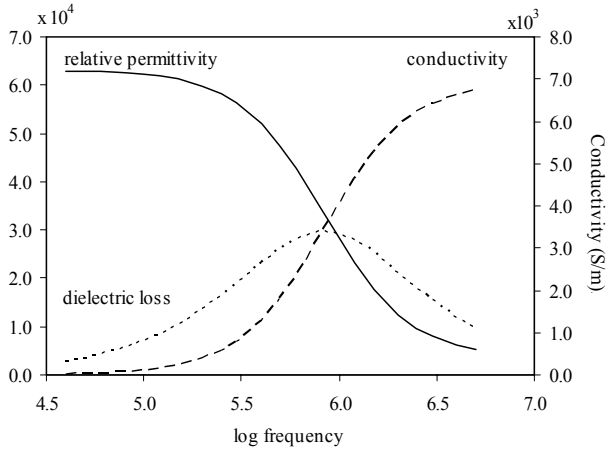

Fig. 1. The relative permittivity, dielectric loss and conductivity of control erythrocytes.

RADIO-MITIGATION EFFECT OF poly-MVA

The mitigation effect of poly-MVA was evaluated by its daily administration immediately after irradiation for two weeks (group A). The MDA concentration showed a non-significant change from control (Table 1). The hemoglobin concentration and hematocrit showed a significant decrease from control, however their values were higher than the irradiated values (10.31 g/dL compared to 7.42 g/dL at the 14th days and 0.39 compared to 0.25 for the hematocrit) (Table 2). The MCV and effective capacitance showed no significant changes from the control (Tables 1 and 3).The relative permittivity did not show significant change from control up to the 14th day (Fig. 2). The area under loss peak decreased significantly 1 day after irradiation and increased to reach control value on the 7th and 14th days (Fig. 2). The AC conductivity showed non-significant change from control which increased significantly at the 14th day (Fig. 3).

Table 1

The mean corpuscular volume of erythrocytes and MDA of control, treated, irradiated and treated-irradiated groups at different time intervals (1, 7 and 14 days)

Treatment with poly MVA	Groups	Statistics	MCV (fl)			MDA Content (nM/g tissue)		
			1 day	7 days	14 days	1 day	7 days	14 days
No treatment	control	mean	109.78	109.78	109.78	95.07	95.07	95.07
		S. D.	6.07	6.07	6.07	9.020	9.020	9.020
	6Gy	mean	117.61	117.69	113.50	166.92	165.72	164.98
		S. D.	14.65	10.360	10.100	22.080	18.760	30.280
		p	0.050	0.018	0.151	0.001	0.0002	0.008

Table 1 (continued)

After irradiation (group A)	Poly-MVA	mean	108.92	103.70	106.59	87.84	97.83	99.55
		S. D.	5.420	7.290	1.170	11.190	13.080	11.900
		p	0.373	0.220	0.022	0.156	0.347	0.247
	6Gy + Poly-MVA	mean	105.66	106.87	104.04	109.32	107.19	103.61
		S. D.	5.270	3.560	8.120	12.223	12.850	14.949
		p	0.039	0.080	0.098	0.204	0.056	0.229
Before irradiation (group B)	Poly-MVA	mean	107.33	104.75	109.39	101.62	102.28	96.48
		S. D.	4.470	4.850	4.530	4.300	12.190	7.920
		p	0.228	0.102	0.451	0.108	0.211	0.425
	6Gy + Poly-MVA	mean	96.10	103.37	97.22	135.43	128.92	120.99
		S. D.	1.240	4.900	4.180	11.200	27.690	16.500
		p	0.0002	0.035	0.008	0.024	0.025	0.125
Continuous treatment (group C)	Poly-MVA	mean	106.59	107.22	107.90	99.55	94.88	93.32
		S. D.	1.170	5.740	11.250	11.900	20.750	16.000
		p	0.022	0.265	0.367	0.247	0.494	0.415
	6Gy + Poly-MVA	mean	106.91	107.64	107.40	99.66	79.77	107.77
		S. D.	6.540	3.330	3.330	9.898	12.333	13.665
		p	0.267	0.204	0.181	0.427	0.075	0.155

S. D.: standard deviation *p* : probability value of the Student t-test.

RADIO-PROPHYLACTIC EFFECT OF poly-MVA

The radio-prophylactic effect of poly-MVA was studied by its daily administration for two weeks before exposure to gamma radiation for two different groups. In the first group the administration of poly MVA was stopped after irradiation (group B) and in the second group the administration continued after irradiation for two more weeks (group C).

Table 2

The hemoglobin concentration and hematocrit of control, treated, irradiated and treated-irradiated groups at different time intervals (1, 7 and 14 days)

Treatment with poly MVA	Groups	Statistics	Hb Conc (g/dL)			*Hct*		
			1 day	7 days	14 days	1 day	7 days	14 days
No treatment	control	mean	13.36	13.36	13.36	0.50	0.50	0.50
		S. D.	1.530	1.530	1.530	0.03	0.03	0.03
	6Gy	mean	12.81	11.72	7.42	0.46	0.42	0.25
		S. D.	1.020	2.240	1.24	0.040	0.060	0.060
		p	0.057	0.018	0.0002	0.011	0.0003	0.0003

Table 2 (continued)

After irradiation (group A)	Poly-MVA	mean	13.45	13.19	12.79	0.49	0.49	0.45
		S. D.	1.230	0.700	0.390	0.030	0.080	0.020
		p	0.472	0.334	0.094	0.198	0.440	0.003
	6Gy + Poly-MVA	mean	12.84	11.94	10.31	0.452	0.42	0.39
		S. D.	0.730	1.310	3.880	0.030	0.040	0.070
		p	0.061	0.020	0.030	0.0002	0.001	0.002
Before irradiation (group B)	Poly-MVA	mean	13.42	13.27	13.47	0.48	0.45	0.48
		S. D.	0.320	0.860	0.570	0.020	0.030	0.030
		p	0.488	0.860	0.570	0.020	0.030	0.031
	6Gy + Poly-MVA	mean	14.54	12.64	7.78	0.45	0.44	0.25
		S. D.	1.430	0.740	2.960	0.040	0.010	0.090
		p	0.079	0.050	0.039	0.023	0.0008	0.018
Continuous treatment (group C)	Poly-MVA	mean	12.79	12.92	13.28	0.45	0.46	0.48
		S. D.	0.390	0.050	1.290	0.020	0.030	0.010
		p	0.094	0.037	0.425	0.003	0.069	0.023
	6Gy + Poly-MVA	mean	12.11	10.83	9.41	0.43	0.39	0.34
		S. D.	1.030	0.410	0.810	0.010	0.003	0.030
		p	0.071	0.0002	0.002	0.0001	0.0005	0.002

S. D.: standard deviation *p* : probability value of the Student t-test.

In group B the MDA concentration was significantly higher than in the control after the 1st and 7th days of irradiation and decreased on the 14th day. The hemoglobin concentration and hematocrit decreased non-significantly after the 1st and 7th days and then showed sharp decrease on the 14th day to show the same results (7.5 g/dL and *Hct* = 0.25) as the irradiated group without poly-MVA (Table 2). The mean corpuscular volume was within the control value for the test period (Table 1). The relative permittivity and the area under loss peak decreased significantly after the 1st day of irradiation and then increased to reach the control value on the 7th and 14th days (Fig. 2). The AC conductivity showed significant decrease from control until the 14th day (Fig. 3).

In group C, the MDA showed normal level after irradiation and continued within the normal value until the 14th day (Table 1). The hemoglobin concentration and hematocrit showed non-significant change from control after the 1st day, and started to decrease significantly on the 7th and 14th days; however, they showed higher values (9.41g/dL and *Hct* = 0.34) compared to the irradiated group (7.42 g/dL and 0.25) as shown in Table 2. The relative permittivity, area under loss peak and AC conductivity showed significant decrease after the 1st day of irradiation, which increased to reach the control value on the 7th and 14th days (Fig. 2, 3). The membrane effective capacitance showed non-significant decrease for both prophylactic treated groups (Table 3).

Table 3

The effective capacitance of control, treated, irradiated and treated-irradiated groups at different time intervals (1, 7 and 14 days)

Treatment with poly MVA	Groups	Statistics	C_{eff} (F)		
			1 day	7 days	14 days
No treatment	control	mean	9.65×10^{-11}	9.65×10^{-11}	9.65×10^{-11}
		S. D.	1.17×10^{-11}	1.17×10^{-11}	1.17×10^{-11}
	6Gy	mean	7.57×10^{-11}	7.90×10^{-11}	7.89×10^{-11}
		S. D.	7.12×10^{-12}	7.03×10^{-12}	6.25×10^{-12}
		p	0.018	0.048	0.012
After irradiation (group A)	Poly-MVA	mean	8.89×10^{-11}	8.83×10^{-11}	9.25×10^{-11}
		S. D.	5.56×10^{-12}	9.80×10^{-12}	9.87×10^{-12}
		p	0.234	0.153	0.095
	6Gy + Poly-MVA	mean	8.66×10^{-11}	1.01×10^{-10}	9.85×10^{-11}
		S. D.	3.59×10^{-12}	8.21×10^{-12}	7.50×10^{-12}
		p	0.399	0.152	0.160
Before irradiation (group B)	Poly-MVA	mean	9.11×10^{-11}	9.85×10^{-11}	9.26×10^{-11}
		S. D.	6.85×10^{-12}	4.12×10^{-12}	8.62×10^{-12}
		p	0.406	0.193	0.443
	6Gy + Poly-MVA	mean	7.96×10^{-11}	8.40×10^{-11}	8.62×10^{-11}
		S. D.	7.67×10^{-12}	5.14×10^{-12}	1.02×10^{-11}
		p	0.193	0.273	0.136
Continuous treatment (group C)	Poly-MVA	mean	9.25×10^{-11}	9.13×10^{-11}	1.04×10^{-10}
		S. D.	3.25×10^{-12}	1.26×10^{-11}	7.95×10^{-12}
		p	0.069	0.130	0.245
	6Gy + Poly-MVA	mean	8.12×10^{-11}	8.82×10^{-11}	1.02×10^{-10}
		S. D.	9.48×10^{-12}	1.58×10^{-12}	7.66×10^{-12}
		p	0.290	0.474	0.087

S.D.: standard deviation *p*: probability value of the Student t-test.

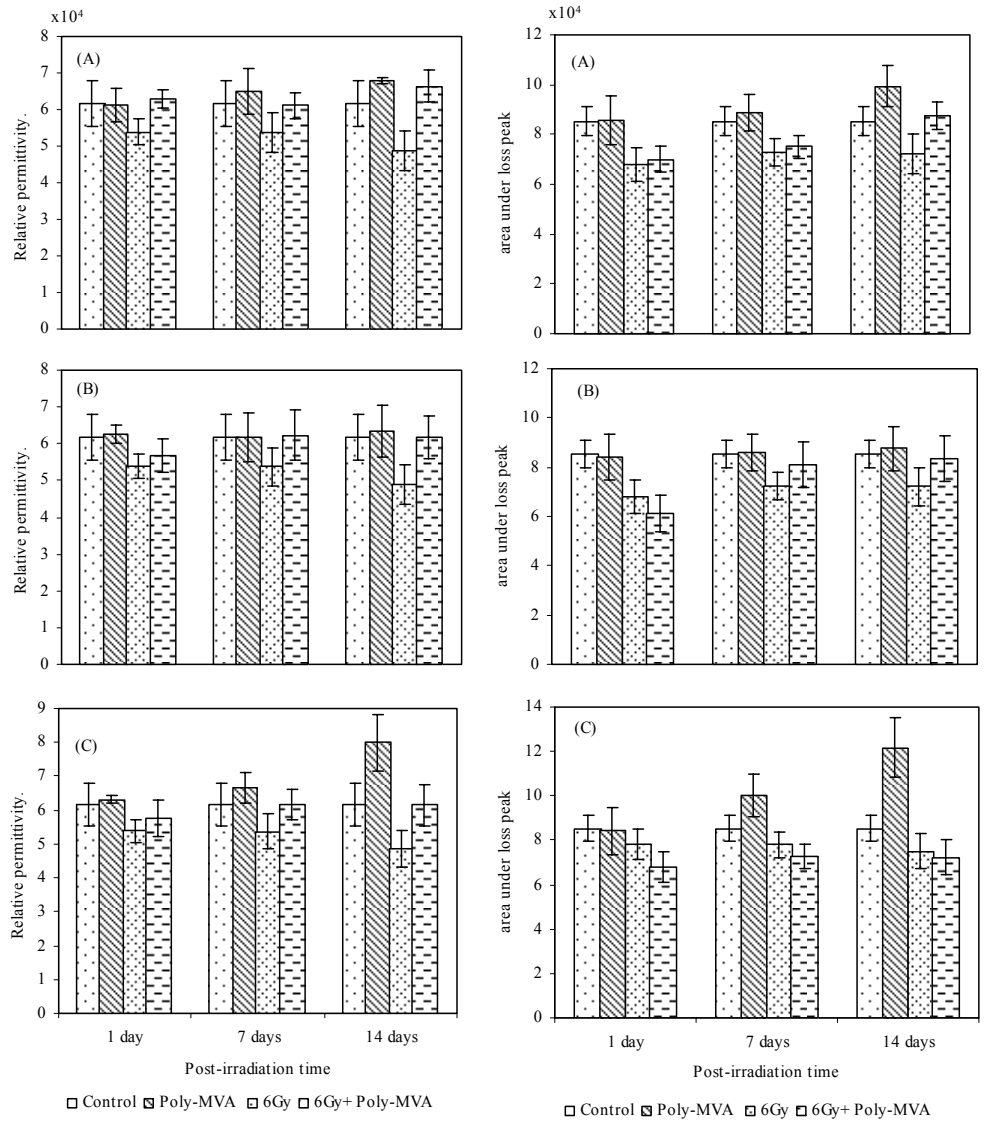

Fig. 2. The relative permittivity and area under loss peaks of control, irradiated and the three modes of administration of poly-MVA, (A) group A (which received poly-MVA administration after irradiation), (B) group B (which received poly-MVA administration before irradiation), and (C) group C (which received poly-MVA administration before and after irradiation), at different time intervals (1, 7 and 14 days).

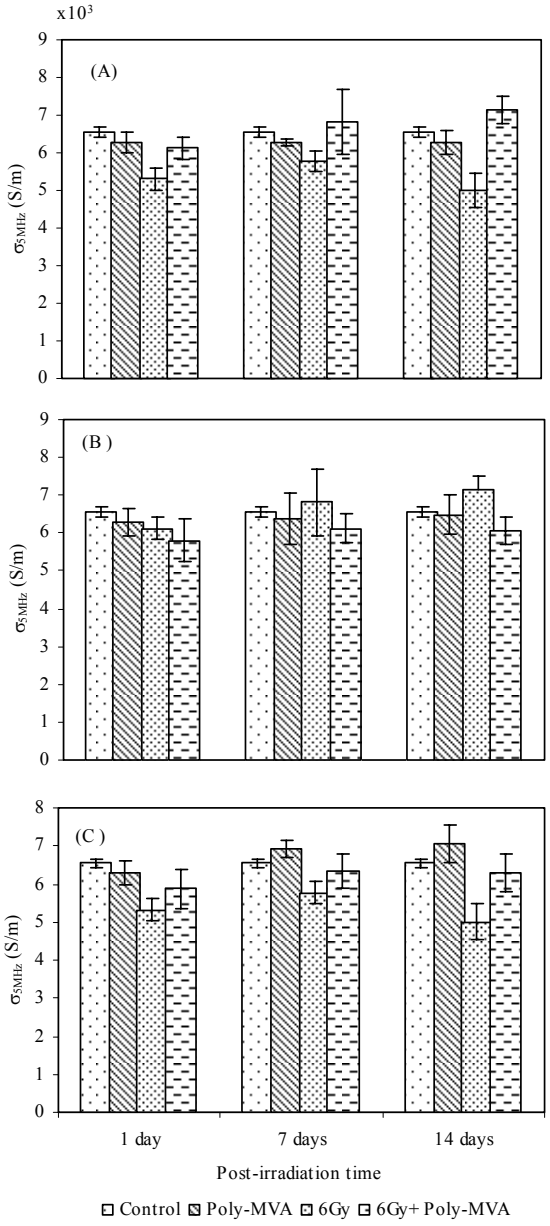

Fig. 3. The conductivity of control, irradiated and the three modes of administration of poly-MVA, (A) group A (which received poly-MVA administration after irradiation), (B) group B (which received poly-MVA administration before irradiation), and (C) group C (which received poly-MVA administration before and after irradiation), at different time intervals (1, 7 and 14 days).

DISCUSSION

At 37 °C, cell membranes are in a liquid-crystalline state [53]. The liquid crystal structure possesses unique electric, magnetic, and thermo-optic properties. These properties are due to the weak intermolecular interaction of the structural elements of liquid-crystal media [20]. Liquid crystals are dielectric materials, which can be defined by their capacitive and conductive properties. Dielectric spectroscopy provides one of the few techniques for probing the nature of the molecular reorientation and there have been several studies on liquid crystals [21]. The dielectric properties of biomembranes provide an insight into the morphological complexities and membrane barrier functions. It is possible, with this technique, to derive the conductivity, a measure of the overall ionic transport across the cell membrane, and the membrane permittivity, which takes into account the static distribution of charges and polar groups across the cell membrane. Also, they have been shown to respond to cell physiologic and pathologic changes [51]. Each subunit in the membrane acts as capacitor, their effective capacitance (C_{eff}) is determined by their relative positions. Hence, it can be considered as the electrical parameter that reflects the resultant effect of the considered treatment. The approach that considers the dielectric parameters of the cell membrane (the permittivity and the electrical conductivity) as an index of possible alterations due to cells interactions with foreign material [4], induced by different drugs or by various physico-chemical agents [3, 4, 13], changes in physiological parameters [34, 43, 44, 50] have been previously applied.

EFFECT OF poly-MVA

Oral administration of poly-MVA with 0.38 mg/kg body weight was found to enhance the activities of Krebs cycle dehydrogenases and respiratory complexes in the heart of aged rat. It also enhanced the activity of catalase, manganese-superoxide dismutase, glutathione peroxidase, and the level of lipid peroxidation was decreased significantly compared to the aged control [49]. In this study, the lipid peroxidation test showed non-significant changes from control values for all the treated groups with different modes of administration. Also, the hemoglobin concentration and hematocrit and mean corpuscular volume did not show any significant changes.

In a previous study, the addition of palladium lipoic acid complex to the erythrocytes suspension *in vitro* resulted in increase of the relative permittivity, dielectric loss and AC conductivity compared to control values. 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 [41]. In the present study, the membrane surface charge, total dipole moments and membrane permeability showed normal values in the first days of administration and increased to a

significant value after 28 days of administration. The observed discrepancies between the *in vitro* and *in vivo* studies could be attributed to the difference between the effect of direct interaction of poly-MVA with erythrocytes and its metabolic effects after the oral administration. The increase in the membrane surface charge may be beneficial in improving the flow properties of erythrocytes as it increases the repulsive forces between the cells and vessels. The interaction of polymer nanoparticles with biological membranes is a complex process due to the heterogeneity of both the nanoparticles and the cell membranes [24]. In the case of erythrocytes, it may occur by unspecific means, including diffusion, trans-membrane channels, and electrostatic, van der Waals, hydration forces, or adhesive interactions [39]. An important property of liquid crystals is their orienting ability: they can orient particles suspended in them and will act as molecular matrices, and due to the interaction between the matrix and the particles suspended in it, the latter become sensitive to the external field, with the result that the orientation of the liquid-crystal matrix also changes [20]. In this study, although there were some significant changes on the relative permittivity and the total dipole moment after the administration of poly-MVA, there were no significant changes in the effective capacitance of the erythrocyte membrane, which imply that it did not affect the membrane normal physiological function.

EFFECT OF GAMMA RADIATION

As cell membrane is an important target for radical damage, and blood can reflect the liability of the whole animal to oxidative condition, erythrocytes can be used for determining the effect of radiation with the subsequent involvement of free radicals. Exposure to acute doses of ionizing radiation causes a series of physiological changes which can be direct or late effects. It has been proposed recently that the radiation-induced late effects are caused, in part, by chronic oxidative stress and inflammation. Increased production of reactive oxygen species, which leads to lipid peroxidation, oxidation of DNA and proteins, as well as activation of pro-inflammatory factors has been observed *in vitro* and *in vivo* injury [54]. Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of ROS. It leads to the creation of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes and their physiological functions [14, 18]. The most common of these intermediates are malondialdehyde (MDA); a highly toxic molecule and highly mutagenic product. Its longevity and high reactivity allow it to act inside and outside the cells, interacting with biomolecules such as nucleic acids and proteins, often irreversibly, damaging the mechanisms involved in cell function. MDA is the principal and most studied product of polyunsaturated fatty acid peroxidation, and a current and

scientifically accepted marker of oxidative stress with worldwide recognition [12]. In this study, MDA was shown to increase significantly by about 75% after one day of exposure to radiation which persists until 14 days recorded in this experiment.

Whole-body irradiation leads to a decreased concentration of all cellular elements in the blood. This can be due to direct destruction of mature circulating cells, loss of cells from the circulation by haemorrhage, or leakage through capillary walls and reduced cell production [32]. Following irradiation, acute or chronic, all cells may be affected with the stem cells and early progenitor cells being amongst the most radiosensitive of all [15]. These effects could result in the significant decrease in the hemoglobin concentration and hematocrit decreased after exposure to radiation, until they reached 50% of the control value on the 14^{th} day observed in this study. The exposure to even low doses, such as 0.25 Gy to 1 Gy, was shown to induce transformation of erythrocytes into echinocytes that are susceptible to destruction and sequestration in small sinuses, thus resulting in low circulating numbers [55]. In this study, the mean corpuscular volume showed significant increase suggesting the deformation of erythrocytes from discoid to echinocytes.

Erythrocytes membrane has a total negative electric charge, which determines the correct course of many processes like transport of metabolic substrates and products through ionic pumps, carriers and membrane channels, for the transfer of information [31] and mainly to prevent aggregation of erythrocytes from each other [19]. The change in both relative permittivity and dielectric loss reflects the change in the protein part of the cell membrane. Whole body exposure to gamma radiation at dose level 6 Gy was shown to decrease the relative permittivity of the cell membrane after one day of exposure, and continued to decrease until the 14^{th} day, which reflects the decrease in membrane surface charge as a result of the oxidation of membrane components. It was reported that the increased oxidative damage on membrane proteins may lead to the decrease of negative surface charge in red blood cells [40]. The decrease in the dielectric 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 effective membrane capacitance indicates that the radiation-induced changes affect the membrane charges as well as their relative positions inside the membrane. Oxidative damage due to reactive oxygen species results in increase of membrane permeability with the subsequent loss of ions, electrolytes and intracellular components [25, 26, 29]. The radiation-induced impairment of membrane permeability could result in the significant decrease in the erythrocytes AC conductivity at 5 MHz.

RADIO-PROPHYLACTIC EFFECT OF poly-MVA

The similarity between chronic tissue injury, chronic inflammation and fibrosis observed in a variety of disease states, including radiation late effects, offers the opportunity to apply antioxidant-based therapies to mitigate and treat late radiation-induced normal tissue injury [38]. Administration of poly-MVA has been shown to alleviate radiation-induced lowering of tissue antioxidant levels in whole-body irradiated mice [28]. In a previous study, poly-MVA was administered to mice for 7 days before exposure to 6 Gy gamma radiation with continuous administration for 5 days post irradiation. On the 12^{th} day post-irradiation, the results showed that poly-MVA enhanced the recovery of irradiated animals from hematopoietic damage as evidenced by the increased number of spleen colonies. The study also showed that poly-MVA protected the cellular DNA of spleenocytes and bone marrow cells of irradiated animals against radiation-induced damages when administered prior to a lethal dose of 8 Gy whole-body gamma radiation, and it suggested that poly-MVA could be safely used as a radioprotector, at least in planned radiation exposure scenarios [37].

The continuous administration of poly-MVA after exposure to gamma radiation showed better results than the administration only before irradiation. As appeared from the data of this study, in group B (which received administration of poly-MVA for 14 days before irradiation and was stopped after irradiation) MDA concentration was significantly higher than in control after the 1^{st} and 7^{th} days of irradiation and decrease on the 14^{th} day. While the MDA in group C (which received oral administration of poly-MVA 14 days before irradiation and continued after irradiation for two more weeks), showed normal level after irradiation and continued within the normal value until the 14^{th} day.

Although the hemoglobin concentration and hematocrit of both groups showed significant decrease on the 14^{th} day, the hemoglobin concentration and hematocrit of group B showed the same results (7.5 g/dL and $Hct = 0.25$) as the irradiated group without poly-MVA (Table 2). However, group C showed higher values (9.41g/dL and $Hct = 0.39$). Also, the mean corpuscular volume for group C was within the control value for the test period. The dielectric parameters of both groups showed similar results. However, group C showed higher values for the effective capacitance which were closer to the control group.

RADIO-MITIGATION EFFECT OF poly-MVA

Mitigators of radiation injury may target the pathways of radiation injuries to prevent or reduce the expression of toxicity [10]. The radiation injuries can be direct as targeting DNA molecules, or indirect by the production of free radicals. Poly-MVA, as a liquid crystal polymer, has a large redox potential, making it a

potent antioxidant agent. It oscillates between an oxidized and reduced form as it accepts unpaired electrons and donates them to its enzymatic site, so it grabs excess electrons, such as free radicals, and shuttles them to the mitochondria, hence, converting them into energy [1]. As a free radicals scavenger, it can spare endogenous antioxidant enzyme system and improve the protection against radiation-induced damage. The administration of poly-MVA after irradiation for two consecutive weeks alleviated the radiation-induced damage in the considered parameters in this study. This appeared in the normal level of MDA, mean corpuscular volume, membrane surface charge and total dipole moments. The hemoglobin concentration, hematocrit, membrane permeability and effective capacitance approached the control values by the 14^{th} day post irradiation. The similarity in the results of the groups A and C (both received poly-MVA after irradiation) shows the efficiency of the physiological effect of poly-MVA in mitigating the radiation damage induced immediately after exposure. Although the 6 Gy dose considered in this study is known to significantly affect the digestive system, the oral uptake of poly-MVA showed positive results in minimizing the radiation-induced damage, reflecting its efficiency in addition to its easy mode of administration.

CONCLUSION

Exposure to acute doses of ionizing radiation causes a series of cellular damages which can be direct or late effects. Whole body exposure to 6 Gy resulted in increase of the lipid peroxidation by 75% after one day of exposure to radiation which persists until 14 days. It also decreased the relative permittivity, dielectric loss, AC conductivity and membrane effective capacitance, which reflects the decrease in membrane surface charge as a result of the oxidation of membrane components. The administration of poly-MVA for 28 days did not affect the electrical properties of the erythrocytes except the increase in the membrane surface charge on the 28^{th} day. The effect of poly-MVA against the radiation-induced damage was tested by three modes of administration: two weeks before irradiation and another group which received continuous administration for two weeks before, two other weeks after irradiation (as prophylactic agent), and two weeks of administration after irradiation (as mitigating agent). The results showed that the best mode of administration was the continuous mode followed by after irradiation and the third was before irradiation, which support the role of poly-MVA as mitigating agent.

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