INVESTIGATION OF THE RUTIN IMPACT ON THE DIELECTRIC PROPERTIES OF BLOOD AFTER EXPOSURE TO GAMMA RADIATION

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Abstract. This work is intended to study the radio-mitigation effect of rutin on the dielectric properties of blood. Adult male rats were divided into four groups: control, positive control (treated with rutin for four consecutive weeks), irradiated and irradiated and treated groups. The latter group was treated with rutin for four consecutive weeks after irradiation. The exposure dose was 6 Gy gamma radiation. The electrical properties of blood were measured. The relative permittivity, relaxation time, and area under the loss curve showed significant decrease after exposure to gamma radiation (i.e., in the case of irradiated group) compared to control group. However, the positive control group and the treated group with rutin after irradiation showed a non-significant decrease as compared to the control group. The treatment with rutin resulted in a significant decrease in the effective capacitance (C_{eff}) of erythrocytes membrane compared to control group, while, it did not show significant changes in the irradiated and the treated groups after irradiation. On the other hand, the AC conductivity decreased after exposure to gamma radiation, while no significant changes were observed for the positive control and the treated groups with rutin after irradiation, compared to the control. The treatment with rutin after exposure to radiation was shown to mitigate the damaging effects in the erythrocytes' membrane.

Key words: rutin, gamma radiation, blood, dielectric properties.

INTRODUCTION

Incidents leading to over-exposure of people to ionizing radiation can occur in several places such as industrial, medical or research areas. Radiation accidents can be either nuclear accidents, which involve nuclear facility such as Chernobyl reactor fire, or radiological accidents, which arise when control of a sealed or unsealed source are lost causing exposure to radiation or release of radioactivity. The latter are the more common, and the sources involved may be X-rays sets, or sealed gamma-ray sources or unsealed radio-isotopes. One difficulty in estimating

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exposure doses following a large-scale accidental exposure is the limitation of personnel and resources to carry out rapid sampling and analysis.

There are many differences between controlled body irradiation and accidental exposure, since the determination of pre-irradiation status of the patient and the precise exposure dose allow an appropriate prophylaxis. However, the experience of controlled body irradiation can be therefore only used as a guide. In the cases of radiation accidents, the exposure doses are usually large doses, and the acute radiation syndrome generally starts within few hours of exposure and the severity is dose dependent. The acute radiation syndrome occurs after whole or significant partial body irradiation of over 1 Gy delivered at a relatively high dose rate [81]. The effective medical response at the right time is crucial in reducing radiation harmful effect and mortality. Medical management of radiation injury is a complex operation due to uncertainties in dose, duration, and organs involved in radiation exposure. There are two fundamental approaches to medical intervention that can be achieved following the exposure to radiation:

a) Mitigation, which refers to therapies that could begin immediately after irradiation but before a clear evidence of clinical disease. It is proposed to regulate the downstream patho-physiological events of radiation injury, and thereby to prevent the development of further injury. Examples of radio-mitigators include the use of angiotensin converting enzyme (ACE) inhibitors to prevent the development of radiation-induced renal [49] or central nervous system (CNS) [36] injuries.

b) Treatment, which refers to therapies that could be effective after apparent clinical disease has developed [82]. Ideally, this treatment should be effective against acute and long-term radiation effects, non-toxic, affordable, and chemically stable to permit the ease handling and storage [66].

The potential use of flavonoid compounds as radioprotectors is gaining much interest [9]. They have demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities [20, 30, 32, 39, 63, 45, 50, 76, 69]. Certain flavonoids (*e.g.* luteolin, quercetin, rutin, kaempferol) can have an inhibitory effect on ACE activity [2, 23]. Furthermore, as these compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of free radicals), their anti-peroxidative activity could also be assigned to a concomitant capability of chelating iron ions [1, 47].

Rutin (also called as rutoside, quercetin-3-rutinoside, or sophorin) is a citrus flavonoid glycoside [38]. It is a yellow crystalline flavonol glycoside ($C_{27}H_{30}O_{16}$) that occurs in various plants (rue, tobacco, buckwheat, passion flower, tea, apple, etc.). It is a vital nutritional component of food stuff [24]. The name 'rutin' comes from the plant *Ruta graveolens*. Chemically, it is a glycoside comprising flavonolic-aglycone-quercetin along with disaccharide rutinose.

Many *in vitro* and *in vivo* studies were carried out to investigate the anti-oxidant potential of rutin and other flavanoids [12, 13, 14, 26, 27, 33, 34, 41, 44, 57, 72, 75, 79, 85]. Rutin is considered to be a good antioxidant because of its ability to bind free radicals and metal ions [41]. It is capable of chelating iron ions (with II and III valence), which can initiate oxygen free-radical formation [79]. It can be used as an anti-inflammatory agent because of the binding of free radicals that prevents the induction of inflammatory cytokine transcription factors [26, 27]. Its property to bind free radicals plays an important role in protecting DNA from various types of oxidative damage. Reactive oxygen species (oxygen radicals, superoxide anion, hydroxides, peroxyls, alkoxyls, etc.) can contribute to the development of oncological diseases [12]. It, also, inhibits lipid peroxidation, which is one of the driving factors in the development of various cardiovascular and neuro-degenerative diseases [13, 34, 72].

Rutin has been investigated for potential radio-protective effects. Its oral administration to Swiss albino mice, for five consecutive days before exposure to 10 Gy gamma radiation, resulted in an increase of the radiation tolerance and the dose reduction factor of 1.15. It significantly elevated the levels of reduced glutathione, glutathione-S-transferase, catalase, superoxide dismutase, and decreased lipid peroxidation in the mouse liver homogenate at 24 h after whole body exposure to 10 Gy [54]. It was shown to inhibit various free radicals generated *in vitro*, which may be attributed to the presence of a phenolic group that contributes to scavenging the radiation-induced free radicals and inhibition of oxidative stress [56]. Its anti-genotoxic potential was assessed in terms of chromosomal aberrations, micronucleus test, and alkaline comet assay after exposure to 3 Gy gamma radiation. Its oral administration before irradiation resulted in the decrease in the DNA damage at the post-irradiation time as compared with irradiation group [55].

High doses of radiation cause loss of intestinal crypts and breakdown of the mucosal barrier then provoking diarrhea, nausea, and vomiting. Systemic effects may include malabsorption which renders the idea of oral less efficient mitigator. However, oral feeding is preferred to maintain functioning of the intestinal mucosa and reducing the inherent infection risk of parenteral nutrition [31]. The experiments showed that rutin and its aglycone can interact with various structures at the molecular level (free radicals, protein systems, enzymes). This enables rutin to be used for the prevention and treatment of various diseases [37]. This work is a part of a study dedicated to investigate the radio-mitigation effect of rutin through evaluation of its effect on the dielectric properties of the blood. Blood is a suitable candidate for monitoring the radiation effect as it is a representative sample for the whole body exposure since it circulates all over the body. The erythrocytes can be easily separated to obtain cells with intact membranes. Also, its anucleate cells represent a useful model for measuring the membrane properties without the interference of intracellular membranes [71]. The dielectric properties of cell

suspensions provide suitable parameters characteristic of cell states and dynamics related to different physical, chemical or biological interactions [22]. Also, the measurement of the cell dielectric properties can give important information on the cell's physiology, in particular the properties of the membrane and cell interior. Several studies investigated the sensitivity of the dielectric parameters to the physiological and structural states of erythrocytes. Many functional parameters showed a good correlation to cell capacitance and conductance, this resulting in the introduction of fitted equations to obtain viscosity [60] and ATP, K, Na and Cl ions' concentration [73] from dielectric measurements.

MATERIALS AND METHODS

Adults Wistar male albino rats, 120–150 g, were obtained from the National Centre for Radiation Research and Technology (NCCRT), Cairo, Egypt. The rats were housed in cages and maintained in a 12 h light/dark cycle. They were allowed to acclimatize to the environmental conditions for one week before the experiment starting and were kept on standard food pellets containing all nutritive elements and water *ad libitum*. Rutin was purchased from Sigma-Aldrich (St. Louis, MO, USA). The experimental animals were divided into four groups ($n = 6$):

1. Control healthy rats (received distilled water).
2. Positive control group: animals treated with rutin dissolved in distilled water by oral administration with daily dose of 70 mg/kg body weight [35], for four consecutive weeks.
3. Irradiated group (the animals were exposed to 6 Gy gamma radiation).
4. Irradiated and treated group in which the animals were exposed to gamma radiation then treated with rutin for four consecutive weeks.

The animals were anesthetized by exposure to diethyl ether in a closed container by open-drop method. They were then sacrified 24 h after last injection with rutin. Blood samples were collected by heart puncture. The blood samples were diluted immediately after withdrawal in isotonic buffered saline (pH 7.4 and conductivity 0.63 S/m) to avoid any changes in the cell membrane structures. The hematocrit (Hct) was adjusted at 3%. The samples were incubated in water bath at 37 °C during the measurement.

DIELECTRIC MEASUREMENT

The dielectric measurements were carried out using LCR meter HIOKI 3531, manufactured in Japan, in the frequency range 40 kHz – 5 MHz. The measured parameters were capacitance, C (in F) and conductance, G (in S). The measuring cell is a parallel plate conductivity cell with platinum electrodes with area of 4 cm^2 and separating distance of 2 cm. To reduce the electrode polarization during the

measurements, the electrodes were made from platinum metal, which is known to have the lowest possible impedance [68], and coated with platinum black layer [29]. The wire of the measuring cell was made from coaxial shielded silver wire, to eliminate stray capacitance. The erythrocytes (Hct 3%) were suspended in buffered saline (pH 7.4 and conductivity 0.63 S/m).

The relative permittivity ε' can be obtained from the following equation [70]:

$$\varepsilon' = \frac{C}{\varepsilon_o}\frac{d}{A} \tag{1}$$

where A (m^2) is the area of the electrode, d (m) is the distance between the two electrodes, C (F) is the capacitance and ε_o is the vacuum permittivity.

The permittivity can be expressed in complex quantity as:

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

The real part in Eq. (2) represents the permittivity constant and is given by:

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

where ε_s is the limiting low frequency permittivity, and ε_∞ is the permittivity value at the end of the dispersion, τ (s) is the relaxation time (= $1/2\pi f_c$), where f_c (Hz) is the characteristic frequency.

The imaginary part, ε'', in Eq. (2), that is the dielectric loss, is given by:

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

Equations (3) and (4) are commonly known as the Debye's dispersion formulas. The conductivity σ (S/m) is given by:

$$\sigma = G\frac{d}{A} \tag{5}$$

To separate the AC conductivity component from the total measured conductivity (DC and AC) the following relationship was applied [53]:

$$\sigma_{AC} = \omega\,\varepsilon_0\varepsilon'' \tag{6}$$

where ε" = ε' tan δ, and the dissipation factor which satisfies the following relation**:**

$$\tan\delta = 2\pi f\,(C/G) \tag{7}$$

where f is the frequency (Hz). The dispersion of erythrocytes suspension shows a slight deviation from simple Debye type, and the empirical Cole-Cole equation is commonly applied:

$$\varepsilon^* = \varepsilon_\infty + \frac{(\varepsilon_o - \varepsilon_\infty)}{1 + (j\omega\tau)^{1-\alpha}} \tag{8}$$

where α is an experimental Cole-Cole parameter with values between 0 and 1.

Several methods have been proposed for the evaluation of the Cole-Cole α parameter. In this study, the geometrical method applied by Ülgen and Sezdi [78] was used. In the Cole-Cole plot (Fig. 1) the imaginary part is maximum at the characteristic frequency, $\omega_c = 2\pi f_c$, which can be determined as follows:

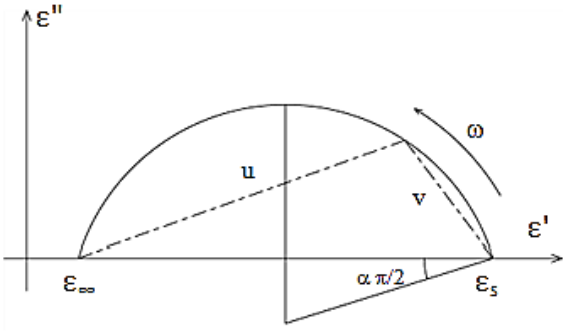

Fig. 1. The depressed Cole-Cole plot.

$$\frac{|v|}{|u|} = (\omega\tau)^{1-\alpha} \tag{9}$$

By plotting $\ln\left\{\frac{|v|}{|u|}\right\}$ against ln ω yields a straight line; its intersection with ln ω axis gives the characteristic frequency, ω_c, and the slope, (1–α), where:

$$|v| = \sqrt{(\varepsilon'')^2 + (\varepsilon_o - \varepsilon')^2} \tag{10}$$

$$|u| = \sqrt{(\varepsilon'')^2 + (\varepsilon' - \varepsilon_\infty)^2}. \tag{11}$$

The Cole-Cole dispersion can be represented in circuit terms as a parallel combination of a resistor and a constant phase element (CPE) with complex-valued impedance (Z_{CPE}) given by:

$$Z_{CPE} = K\,(j\omega)^{-n} \tag{12}$$

where K is a constant and n = α. This CPE impedance reduces to a simple resistance for n = 0 and to a capacitive reactance for n = 1 [46]. The equivalent circuit for a biological cell is shown in Fig. 2.

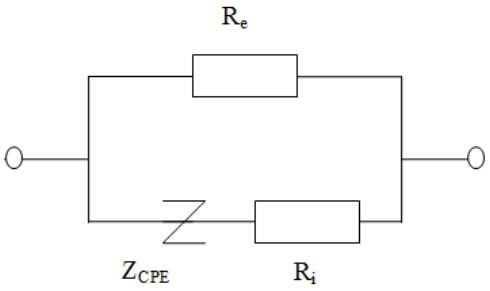

Fig. 2. Electrical equivalent circuit for a biological cell [78].

where R_i and R_e are the resistances of intracellular and extracellular fluids. The Cole-Cole parameters: R_i, R_e, f_c, and α are related to the membrane complex impedance (Z^*) by the following equation [78]:

$$Z^* = R_\infty + \frac{R_0 - R_\infty}{1 + \frac{R_i + R_e}{K}(j\omega)^\alpha} \tag{13}$$

where $\alpha \leq 1$, $R_\infty = \frac{R_e.R_i}{R_e + R_i}$, $R_0 = R_e$. Here, R_0 and R_∞ are the resistances at $f = 0$ and $f = \infty$, respectively, and they are related to the effective membrane capacitance (C_{eff}) by [73]:

$$C_{eff} = \frac{1}{2\pi f_c (R_i + R_e)^{1/(1-\alpha)}}. \tag{14}$$

STATISTICAL ANALYSIS

All the data were analyzed with one-way analysis of variance (ANOVA) followed by a *post hoc* test (LSD alpha) for multiple comparisons using the SPSS (version 20). The data were expressed as mean ± standard error (SD). P values smaller than 0.05 were considered to be statistically significant.

RESULTS

The dielectric characteristics of living cells include both conductive and capacitive properties. The conductivity depends on the dynamical ionic transport through the membrane, so it is a measure of the permeability of the cell membrane, although movement of ions over the cell surface (surface conductance) also plays a role [59].

Measuring the dielectric properties of erythrocytes after exposure to gamma radiation, in the frequency range 40 kHz – 5 MHz, can reflect the induced damage in the cell membrane polar groups. The relative permittivity or dielectric constant is a measure of membrane polarizability in the electric field. It is related with the structural arrangement of the lipid bilayer and with the conformation and localization of proteins in the membrane and, consequently, with the spatial distribution of charged and dipolar groups at the hydrophobic interface [7].

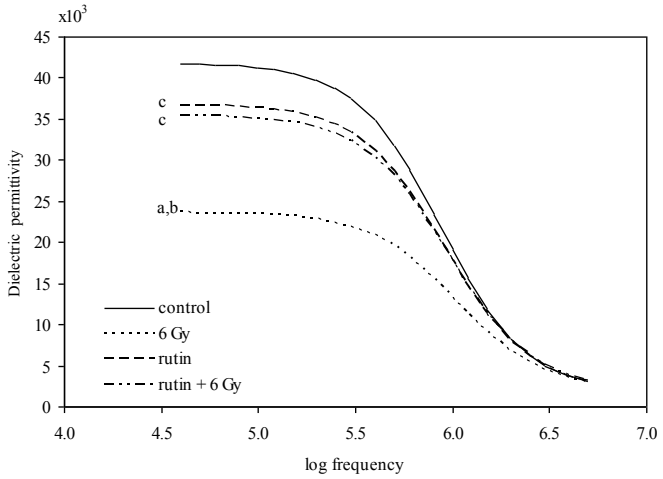

Fig. 3. Relative permittivity for control, treated with rutin, irradiated and treated after irradiation groups (a. significant difference *versus* control group, b. significant difference *versus* positive control group and c. significant difference *versus* irradiated group at $P < 0.05$).

The relative permittivity showed a significant decrease after exposure to gamma radiation compared to control group. The positive control group and the treated group with rutin after irradiation showed a non-significant decrease *versus* control group, while they showed a significant increase when compared to the irradiated group (Fig. 3). 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 [58].

The area under loss curve for the irradiated group showed a significant decrease compared to control group (Fig. 4B). The positive control group and the treated groups with rutin after irradiation showed a non-significant decrease from the control group. However, administration of rutin after irradiation resulted in a significant increase in the area under loss peak when compared to the irradiated group (Figs. 4A, 4B).

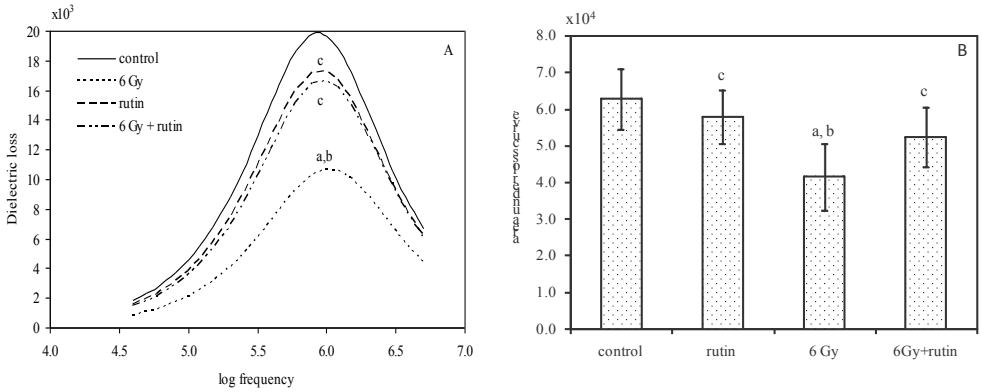

Fig. 4. Loss curves (A) and area under loss curves (B) for control, treated with rutin, irradiated and treated after irradiation groups; a. significant difference *versus* control group, b. significant difference *versus* positive control group and c. significant difference *versus* irradiated group at $P < 0.05$.

Each membrane subunit acts as a capacitor, the effective capacitances (C_{eff}) being determined by the relative positions of the subunits. The treatment with rutin resulted in a significant decrease in the effective capacitance (C_{eff}) of erythrocytes' membrane compared to control group, while, it did not show significant changes in the irradiated and treated groups with rutin after irradiation (Fig. 5).

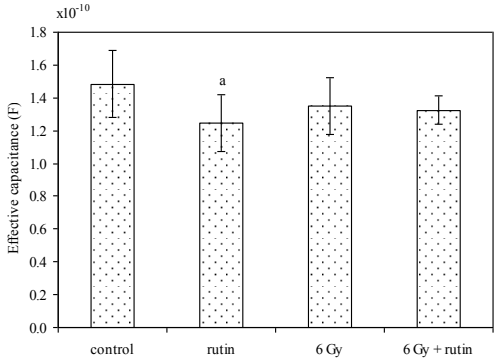

Fig. 5. Effective capacitance for control, treated with rutin, irradiated and treated after irradiation groups; a. significant difference *versus* control group at $P < 0.05$.

The polarized part in the membrane is composed of the proteins either integral or embedded in the hydrophobic lipid part. This polarization does not occur instantaneously, and the associated time constant is called the relaxation time τ [43]. It depends on the charge of the polarized part and its size. In this study, the relaxation time for the irradiated group showed a significant decrease as compared to control group.

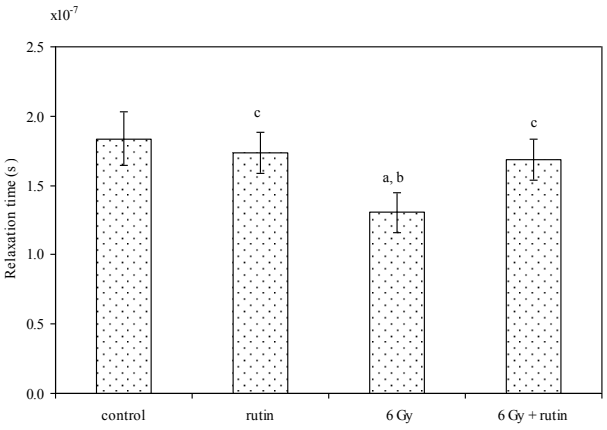

Fig. 6. Relaxation time for control, treated with rutin, irradiated and treated after irradiation groups; a. significant difference *versus* control group, b. significant difference *versus* positive control group and c. significant difference *versus* irradiated group at $P < 0.05$.

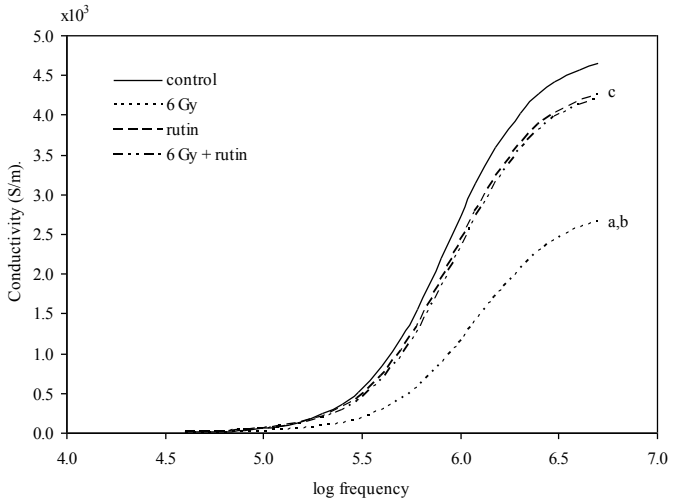

Fig. 7. AC conductivity for control, treated with rutin, irradiated and treated after irradiation groups; a. significant difference *versus* control group, b. significant difference *versus* positive control group, c. significant difference *versus* irradiated group at $P < 0.05$.

The positive control group and the treated group with rutin after irradiation showed a non-significant decrease to control group and a significant increase to irradiated group (Fig. 6). The results also showed a decrease in the AC conductivity after exposure to gamma radiation, while no significant change was observed for the positive control and the treated group with rutin after irradiation, compared to control (Fig. 7).

DISCUSSION

Biological membranes play important roles in cells life which highly exceed their role of envelope for the cellular components. They rather regulate in and out the cell, the transport of ions and metabolites, and govern intercellular communications. The erythrocyte's membrane is composed of a double layer of asymmetrically organized lipid molecules. About one half of the mass of the envelope is made up of lipids, the other half being made up of proteins. About one half of the proteins (integral proteins) are embedded into the lipid bilayer. The rest of the proteins (peripheral proteins) are more loosely attached to the inner or outer surface of the erythrocyte membrane [8, 10]. The erythrocyte's membrane carries negative surface charge that is mainly attributed to sialic acid residues located on glycoproteins in the membrane glycocalyx. Reverberi *et al.*, 2007 [62] concluded that the erythrocyte deformability is the only parameter related to viability which shows sufficiently precocious and important changes. Deformability, as other erythrocytes properties, depends to a great extent on both mechanical and electrical properties of the cell membrane. It is found that the decrease of the surface charge of erythrocytes leads to the decrease in the deformation and orientation indices as well as to the increase in blood viscosity [83]. Hence, alteration of the electrical state of the cell membrane could result in a different flow behavior of erythrocytes in the circulation, causing the growth of pathogenesis of vascular diseases. Because the electrical surface properties of the cell membrane play a critical role in the whole cell adhesion process and in the resulting alteration in the membrane structure and function, the measurement of the passive electrical parameters, that is, the membrane conductivity, σ_s, and the membrane permittivity, ε_s, represents a significant tool in the understanding of the complex phenomenology occurring during cell adhesion [6]. The membrane capacitance determines the amount of charge that can be stored across a membrane when a cell is exposed to an electric field. Its value depends, mainly, on the level of folding of the cell membrane [21]. Each membrane subunit acts as a capacitor, the effective capacitance (C_{eff}) being determined by the relative positions of the subunits.

EFFECTS OF GAMMA RADIATION

Irradiation has been shown to induce biochemical changes in erythrocytes' membrane and to generate reactive oxygen species (ROS). Many studies have been performed on the effect of gamma radiation on the blood and erythrocytes. They showed that exposure to gamma radiation produces lipid peroxidation, cross linking in membrane proteins and change in the membrane permeability [74]. Many investigators reported a sodium gain [52] and a potassium loss [74] by the erythrocytes as a general effect of exposure to ionizing radiation. They stated that radiation can alter the metabolism or active transport (by inhibition of ATP-ase activity) and also may lead to loss of membrane sulfhydryl groups. The change in both relative permittivity and dielectric loss reflects the change in the protein part of the cell membrane. In this study, the two parameters showed a significant decrease after exposure to gamma radiation as the dose increased.

The exposure to 6 Gy gamma radiation was shown to decrease the AC conductivity of erythrocytes suspension which was attributed to permeability alteration of the cell membrane with the subsequent loss of ions, electrolytes, and intracellular components. Also, the results showed that the fragility of red blood cell membrane increased significantly compared to control groups [71]. In another study, exposure to 6 Gy gamma radiation resulted in significant changes in cellular antioxidant enzymes (reduced glutathione (GSH), catalase, and superoxide dismutase (SOD) and significant increase (75%) in the lipid peroxidation 24 h after exposure to radiation, which persisted until the 14^{th} day after irradiation [17]. Other studies showed that exposure to gamma radiation caused a decrease in relative permittivity and of the area under the loss curve and also of membrane effective capacitance. It resulted in an increase in the viscosity, consistency index, and yield stress, too. The obtained results could be attributed to the decrease of membrane surface charge after exposure to gamma radiation. Consequently, the decrease of the membrane surface charge will decrease the repulsion between the erythrocytes which results in increase of blood viscosity [15, 16, 71].

The radiation-induced permeability impairment can be the major cause of loss of the intracellular ions and electrolytes. It was reported that exposure to 6 Gy gamma radiation produced deformation of the erythrocytes structure and the appearance of echinocytes. This transformation was determined by the progressive appearance of regularly spaced spicules on the surface of the erythrocytes with the gradual transformation to ovoid shape [17, 74]. The radiation-induced irregularity in the membrane may result in the decrease of the relaxation time. The area under the loss peaks for whole blood and erythrocytes has decreased reflecting the partial degradation of the protein part, as well as the changes in the membrane conformation and spatial distribution of the protein into the lipid bilayer.

EFFECT OF RUTIN

The interaction of an antioxidant with the cells is dependent on its structure and the nature and physical state of the membrane. The ability of antioxidant to interact with and permeate the membrane is affected by electrostatic interactions, the formation of hydrogen bonds with polar groups of phospholipids, by hydrophobic interactions with fatty acyl chains, and by the molecular geometry of membrane phospholipids [65]. The most apolar antioxidants are buried deeper in the hydrophobic core of the membrane lipid bilayer, while antioxidants of low polarity are mainly located closer to the membrane polar water phase [3, 25, 51, 64]. This different location may result in changes in membrane fluidity that may sterically hinder the diffusion of free radicals, thereby decreasing the oxidative process throughout the membrane [4, 5, 51]. Recent studies have revealed that flavonoids alter bio-membrane organization which might then lead to modifications in membrane protein function [28, 61, 77].

Rutin is relatively poorly absorbed in the intestines. Microflora of the lower gut hydrolyzes rutin to its aglycone quercetin, and the sugar residue, which are subsequently absorbed by the small intestine wall [11, 67, 80]. Hence, it can be expected that the effect on the erythocytes membrane in the blood stream to be a combination of both quercetin and rutin. Previous studies on the interaction of quercetin and rutin with the erythrocyte's membrane showed that quercetin was mainly located in the membrane hydrophobic core [3–5, 11, 25, 28, 48, 64, 67, 80], whereas, rutin was mainly located at the membrane surface [40].

The present results provide insight into the rutin membrane interaction and suggest that it may induce reorganization on the state-of-order of lipids. From a physiological aspect, this reorganization might be advantageous because the flavonoids' antioxidant activities are governed by their structural characteristics and their ability to interact with and penetrate lipid bilayers [40]. These induced changes in the membrane organization may explain the decrease in the effective capacitance of the cell membrane observed in this study. Non-significant changes in the relative permittivity, dielectric loss and the relaxation time reveal that rutin does not influence the membrane composition. Also the conductivity results can suggest that the induced change by rutin does not influence the membrane permeability.

THE MITIGATION EFFECT OF RUTIN

It is known that some herbal compounds have been utilized as radiation protection agents for managing radiation emergencies. For instance, the Chernobyl reactor liquidators (i.e., emergency workers) were fed with a special diet (antioxidant biofactor, AOB) containing rutin as the major ingredient. AOB reduced significantly the clastogenic factors [18, 19]. Also, rutin can interact with

free radicals and various protein systems to exhibit antioxidant, anti-inflammatory, anti-allergy, and antitumor activity [37]. The interaction of flavonoids with bilayers could be a relevant mechanism in the protection from membrane oxidation. Both in biological and in model membranes, the interaction between the flavonoids and the membrane bilayer results in either the binding at the lipid-water interface, or the distribution in the hydrophobic core of the membrane. The different location of these molecules is determined by their chemical properties [51]. Their partition in the non-polar region of the bilayer, and their capacity to interact with free radicals, inhibit the propagation of lipid peroxidation.

In the previous studies, the treatment with rutin for four consecutive weeks, after exposure to 6 Gy gamma radiation, resulted in improvement in liver function by decreasing serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and reduction in serum ammonia level [42]. In addition, the administration of rutin significantly modulated the alteration in cytokines levels and neurotransmitters content. The histo-pathological examinations of liver and brain tissues showed that administration of rutin has attenuated the radiation-induced damage and improved tissue architecture. In this study, the treatment with rutin after exposure to radiation was shown to decrease the damaging effects of radiation on the erythrocytes' membrane. After four weeks of irradiation, non-significant changes were observed in the relative permittivity, dielectric loss, AC conductivity, relaxation time, and effective capacitance. These results suggest the mitigation of the radiation-induced damage in the membrane surface charge, membrane permeability, total membrane dipole moments, and the membrane bilayer conformation and spatial distribution of the lipids and proteins.

REFERENCES

1. AFANAS'AV, I.B., A.I. DOROZHKO, A.V. BRODSKII, V.A. KOSTYUK, A.I. POTAPOVICH, Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation, *Biochem. Pharmacol.*, 1989, **38**, 1763–1769.
2. ANDRADE, T.U., B.T. EWALD, P.D. R FREITAS, D. LENZ, D.C. ENDRINGER, PAVONIA ALNIFOLIA A. ST. HIL.: *In vivo* hypotensive effect and *in vitro* ACE inhibitory activity, *International Journal of Pharmacy and Pharmaceutical Sciences,* 2012, **4**(1), 24–126.
3. AREIAS, F.M., A.C. REGO, C.R. OLIVEIRA, R.M. SEABRA, Antioxidant effect of flavonoids after ascorbate/Fe(2+)-induced oxidative stress in cultured retinal cells, *Biochem. Pharmacol.,* 2001, **62**, 111–118.
4. ARORA, A., T.M. BYREM, M.G. NAIR, G.M. STRASBURG, Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids, *Arch. Biochem. Biophys.*, 2000, **373**, 102–109.
5. BARROS, M.P., E. PINTO, P. COLEPICOLO, M. PEDERSEN, Astaxanthin and peridinin inhibit oxidative damage in Fe(2+)-loaded liposomes: scavenging oxyradicals or changing membrane permeability?, *Biochem. Biophys. Res. Commun.*, 2001, **288**, 225–232.

6. BASOLI, A., F. BORDI, C. CAMETTI, T. GILI, Structural alteration of erythrocyte cell membrane in presence of artificial prostheses: a radiowave dielectric spectroscopy study, *J. Biomed. Mater. Res*., 2002, **59**, 100–109.
7. BONINCONTRO, A., C. CAMETTI, A. ROSI, L. SPORTELLI, Electrical parameters of erythrocyte membranes deduced from radiowave conductivity measurements, *J. Membrane Science,* 1989, **41**, 345–354.
8. BRANTON, D., C.M. COHEN, J. TYLER, Interaction of cytoskeletal proteins on the human erythrocyte membrane, *Cell*, 1981, **24**, 24–32.
9. CINKILIC N., S.K. CETINTAS, T. ZORLU, O. VATAN, D. YILMAZ, T. CAVAS, S. TUNC, L. OZKAN, R. BILALOGLU, Radioprotection by two phenolic compounds: Chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes in vitro, *Food and Chemical Toxicology,* 2013, **53**, 359–363.
10. COHEN, C.M., The molecular organization of the red cell membrane skeleton, *Semin. Hematol.*, 1983, **20**, 141–158.
11. CRESPY, V., C. MORAND, C. BESSON, C. MANACH, C. DEMIGNE, C. REMESY, Quercetin, but not its glycosides, is absorbed from the rat stomach, *J. Agric. Food Chem.,* 2002, **50**, 618–621.
12. DAS, S., A.K. MANDAL, A. GHOSH, S. PANDA, N. DAS, S. SARKAR, Nanoparticulated quercetin in combating age related cerebral oxidative injury, *Curr. Aging Sci.*, 2008, **1**, 169–174.
13. DeWHALLEY, C.V., J.F. RANKIN, S.M. RANKIN, J.R. HOULT, W. JESSUP, D.S. LEAKE, Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages, *Biochem. Pharmacol*., 1990, **39**, 1743–1749.
14. EGERT, S., C. BOESCH-SAADATMANDI, S. WOLFFRAM, G. RIMBACH, M.J. MÜLLER, Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype, *J. Nutr.*, 2010, **140**, 278–284.
15. EL-MARAKBY, S.M., N.S. SELIM, O.S. DESOUKY, H.A. ASHRY, A.M. SALLAM, Effects of Poly-MVA on the rheological properties of blood after *in vivo* exposure to gamma radiation, *J. Radiation Research and Applied Sciences*., 2013, **6,** 21–30.
16. EL-MARAKBY, S.M., N.S. SELIM, O.S. DESOUKY, H.A. ASHRY, A.M. SALLAM, Prophylaxis and mitigation of radiation-induced changes in the electrical properties of erythrocytes by palladium lipoic acid nano-complex (Poly MVA), *Romanian J. Biophys.*, 2013, **23**(3), 171–190.
17. EL-MARAKBY, S.M., N.S. SELIM, O.S. DESOUKY, H.A. ASHRY, A.M. SALLAM, Radio-mitigation effect of Poly-MVA after exposure to acute dose of gamma radiation, *J. Taibah University for Science,* 2016, **10**(2), 251–265.
18. EMERIT, I., N. OGANISIAN, R. ARUYUNYIAN, A. POGOSSIAN, T. SARKISIAN, L. CERNJAVSKI, A. LEVY, J. FEINGOLD, Oxidative stress-related clastogenic factors in plasma of Chernobyl liquidators: protective effects of antioxidant plant phenols, vitamins and oligoelements, *Mutation Research*, 1997, **373**, 47–54.
19. EMERIT, I., R. ARUTYUNYAN, N. OGANESIAN, A. LEVY, L. CERNJAVSKI, T. SARKISIAN, A. POGOSIAN, K. ASRIAN, Radiation-induced clastogenic factors: anticlastogenic effects of *Ginkgo biloba* extract, *Free Radical Biology and Medicine,* 1995, **18**, 985–991.
20. GANESHPURKAR, A., A.K. SALUJA, The pharmacological potential of rutin, *Saudi Pharmaceutical Journal,* 2017, **25**(2), 149–164.
21. GASCOYNE, P.R.C., J.V. VYKOUKAL, Dielectrophoresis-based sample handling in general-purpose programmable diagnostic instruments, *Proceedings IEEE*, 2004, **92**, 22–42.
22. GHEORGHIU, E., The resting potential in relation to the equivalent complex permittivity of a spherical cell suspension, *Phys. Med. Biol.,* 1993, **38**, 979–988.

23. GUERRERO, L, J. CASTILLO, M. QUIÑONES, S. GARCIA-VALLVÉ, L. AROLA, G. PUJADAS, B. MUGUERZA, Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies, *PLoS One,* 2012, **7**(11), e49493.
24. HARBORNE, J.B., Nature, distribution and function of plant flavonoids, *Prog. Clin. Biol. Res.*, 1986, **213**, 15–24.
25. HENDRICH, A.B., Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds, *Acta Pharmacol. Sin.,* 2006, **27**, 27–40.
26. HUK, I., V. BROVKOVYCH, J. NANOBASH VILI, G. WEIGEL, C. NEUMAYER, L. PARTYKA, S. PATTON, T. MALINSKI, Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study, *Br. J. Surg.,* 1998, **85**, 1080–1085.
27. INAM, M., M. ALTINIŞIK, M.D. BILGIN, The effect of quercetin on renal ischemia and reperfusion injury in the rat, *Cell Biochem. Funct.*, 2002, **20**(4), 291–296.
28. INGÓLFSSON, H.I., P. THAKUR, K.F. HEROLD, E.A. HOBART, N.B. RAMSEY, X. PERIOLE, D.H. DE JONG, M. ZWAMA, D. YILMAZ, K. HALL, T. MARETZKY, H.C. HEMMINGS, C. BLOBEL, S.J. MARRINK, A. KOÇER, J.T. SACK, O.S. ANDERSEN, Phytochemicals perturb membranes and promiscuously alter protein function, *ACS chemical biology*, 2014, **9**(8), 1788–1798.
29. IWAMOTO, S., H. KUMAGAI, Analysis of the dielectric relaxation of a gelatin solution, *Biosci. Biotechnol. Biochem.,* 1998, **62**, 1381–1387.
30. JANBAZ, K.H., S.A. SAEED, A.H. GILANI, Protective effect of rutin on paracetamol and CCl4-induced hepatotoxicity in rodents, *Fitoterapia*, 2002, **73**, 557–563.
31. JARRETT, D.G., R.G. SEDLAK, W.E. DICKERSON, G.I. REEVES, Medical treatment of radiation injuries – Current US status*, Radiation Measurements.,* 2007, **42**, 1063–1074.
32. JAVED, H., M.M. KHAN, A. AHMAD, K. VAIBHAV, M.E. AHMAD, A. KHAN, M. ASHAFAQ, F. ISLAM, M.S. SIDDIQUI, M.M. SAFHI, F. ISLAM, Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type, *Neuroscience*, 2012, **17**, 340–352.
33. JUZWIAK, S., J. WOJCINCKI, K. MOKRZICKI, M. MARCHLEWICZ, M. BIAŁECKA, L. WENDA-RÓZEWICKA, B. GAWROŃSKA-SZKLARZ, M. DROŹDZIK, Effect of quercetin on experimental hyperlipidemia and atherosclerosis in rabbits, *Pharmacol. Rep*., 2005, **57**, 604–609.
34. KAHRAMAN, A., N. ERKASAP, M. SERTESER, T. KÖKEN, Protective effect of quercetin on renal ischemia/reperfusion injury in rats, *J. Nephrol.*, 2003, **16,** 219–224.
35. KHAN, R.A., M.R. KHAN, S. SAHREEN, CCl4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat, *BMC Complementary and Alternative Medicine*., 2012, **12**, 178–184.
36. KIM, J.H., S.L. BROWN, A. KOLOZSVARY, Modification of radiation injury by ramipril, inhibitor of angiotensin converting enzyme, on optic neuropathy in the rat, *Radiat. Res.*, 2004, **161**, 137–142.
37. KOVAL'SKII, I.V., I.I. KRASNYUK, I.I. KRASNYUK JR., O.I. NIKULINA, A.V. BELYATSKAYA, YU. YA. KHARITONOV, N.B. FELDMAN, S.V. LUTSENKO, Molecular-biological problems of drug design and mechanism of drug action mechanisms of rutin pharmacological action, *Pharm. Chem. J.*, 2014, **48**(2), 73–76.
38. KREFT, S., M. KNAPP, I. KREFT, Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis, *J. Agric. Food Chem.*, 1997, **47**(11), 4649–4652.
39. LA CASA, C., I. VILLEGAS, C. ALARCO´ N DE LA LASTRA, V. MOTILVA, M.J. MARTI´NCALERO, Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions, *J. Ethnopharmacol*., 2000, **71**(1–2), 45–53.

40. LÓPEZ-REVUELTA, A., J.I. SÁNCHEZ-GALLEGO, A. HERNÁNDEZ-HERNÁNDEZ, J. SÁNCHEZ-YAGÜE, M. LLANILLO, Membrane cholesterol contents influence the protective effects of quercetin and rutin in erythrocytes damaged by oxidative stress, *Chemico-Biological Interactions*, 2006, **161**, 79–91.
41. MAMANI-MATSUDA, M., T. KAUSS, A. AL-KHARRAT, J. RAMBERT, F. FAWAZ, D. THIOLAT, D. MOYNET, S. COVES, D. MALVY, M.D. MOSSALAYI, Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decreased macrophage inflammatory mediators, *Biochem. Pharmacol.*, 2006, **72**, 1304–1310.
42. MANSOUR, S.Z., S.M. EL-MARAKBY, F.S.M. MOAWED, Ameliorative effects of rutin on hepatic encephalopathy-induced by thioacetamide or gamma irradiation, *Journal of Photochemistry & Photobiology, B: Biology,* 2017, **172**, 20–27.
43. MARTINSEN, O.G., S. GRIMNES, H.P. SCHWAN, Interface phenomena and dielectric properties of biological tissue, *Encyclopedia of Surface and Colloid Science*, Marcel Deckker, Inc., 2002, pp. 2643–2652.
44. McANULTY, S.R., L.S. MCANULTY, D.C. NIEMAN, J.C. QUINDRY, P.A. HOSICK, M.H. HUDSON, L. STILL, D.A. HENSON, G.L. MILNE, J.D. MORROW, C.L. DUMKE, A.C. UTTER, N.T. TRIPLETT, A. DIBARNARDI, Chronic quercetin ingestion and exercise-induced oxidative damage, *Applied Physiology, Nutrition, and Metabolism*, 2008, **33**(2), 254–262.
45. MELLOU, F., H. LOUTRARI, H. STAMATIS, C. ROUSSOS, F.N. KOLISIS, Enzymatic esterification of flavonoids with unsaturated fatty acids: effect of the novel esters on vascular endothelial growth factor release from K562 cells, *Process Biochem*., 2006, **41**, 2029–3204.
46. MIKLAVČIČ, D., N. PAVŠELJ, F.X. HART, Electric properties of tissues, in: *Wiley Encyclopedia of Biomedical Engineering*, 2006, John Wiley & Sons, Inc., pp. 1–12.
47. MOREL, I., G. LESCOAT, P. COGREL, O. SERGENT, N. PASDELOUP, P. BRISSOT, P. CILLARD, J. CILLARD, Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures, *Biochem. Pharmacol.*, 1993, **45**, 13–19.
48. MORIDANI, M.Y., J. POURAHMAD, H. BUI, A. SIRAKI, P.J. O'BRIEN, Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers, *Free Radic. Biol. Med.,* 2003, **34**, 243–253.
49. MOULDER, J.E., B.L. FISH, E.P. COHEN, ACE inhibitors and AII receptor antagonists in the treatment and prevention of bone marrow transplant nephropathy, *Curr. Pharm. Des.,* 2003, **9**(9), 737–749.
50. NASSIRI-ASL, M., S.R. MORTAZAVI, F. SAMIEE-RAD, A.A. ZANGIVAND, F. SAFDARI, S. SAROUKHANI, E. ABBASI, The effects of rutin on the development of pentylenetetrazole kindling and memory retrieval in rats, *Epilepsy Behav*., 2010, **18**(1–2), 50–53.
51. OTEIZA, P.I., A.G. ERLEJMAN, S.V. VERSTRAETEN, C.L. KEEN, C.G. FRAGA, Flavonoid membrane interactions: a protective role of flavonoids at the membrane surface?, *Clin. Dev. Immunol.*, 2005, **12**, 19–25.
52. OTHMAN, A.I., M.A. EL-MISSIRY, Effect of radiation on sodium and water transport in rat erythrocytes and possible repair using olive oil, *Isotopes and Rad. Res*., 1990, **22**(1), 27–29.
53. PARK, J.H., C.S. KIM, B.C. CHOI, K.Y. HAM, The correlation of the complex dielectric constant and blood glucose at low frequency, *Biosensors and Bioelectronics*, 2003, **19**, 321–324.
54. PATIL, S.L., H.M. SOMASHEKARAPPA, K.P. RAJASHEKHAR, Evaluation of the radioprotective action of rutin in mice exposed to gamma-radiation, *International Journal of Biological & Pharmaceutical Research*, 2012, **3**(1), 12–18.
55. PATIL, S.L., N.B. RAO, H.M. SOMASHEKARAPPA, K.P. RAJASHEKHAR, Antigenotoxic potential of rutin and quercetin in Swiss mice exposed to gamma radiation, *Biomed. J.*, 2014, **37**(5), 305–313.

56. PATIL, S.L., S.H. MALLAIAH, R.K. PATIL, Antioxidative and radioprotective potential of rutin and quercetin in Swiss albino mice exposed to gamma radiation, *J. Med. Phys*., 2013, **38**(2), 87–92.
57. PEREZ-VIZCAINO, F., J. DUARTE, R. JIMENEZ, C. SANTOS-BUELGA, A. OSUNA, Antihypertensive effects of the flavonoid quercetin, *Pharmacol. Rep.*, 2009, **61**, 67–75.
58. PETHIG, R., *Dielectric and Electronic Properties of Biological Materials*, Wiley, New York, 1979.
59. PETHIG, R., M.S. TALARY, Dielectrophoretic detection of membrane morphology changes in Jurkat T-cells undergoing etoposide-induced apoptosis, *IET Nanobiotechnol*., 2007, **1**, 2–9.
60. POP, G.A.M., W.J. HOP, L. MORARU, M. VAN DER JAGT, J. QUAK, D. DEKKERS, Z. CHANG, F.J. GIJSEN, D.J. DUNCKER, C.J. SLAGER, Blood electrical impedance closely matches whole blood viscosity as parameter of hemorheology and inflammation, *Appl. Rheol*., 2003, **13**(6), 305–312.
61. RAGHUNATHAN, M., Y. ZUBOVSKI, R.M. VENABLE, R.W. PASTOR, J.F. NAGLE, S. TRISTRAM-NAGLE, Structure and elasticity of lipid membranes with genistein and daidzein bioflavonoids using X-ray scattering and MD simulations, *The Journal of Physical Chemistry B*, 2012, **116** (13), 3918–3927.
62. REVERBERI, R., M. GOVONI, M. VERENINI, Deformability and viability of irradiated red cells, *Ann. Ist. Super. Sanita.*, 2007, **43**(2), 176–185.
63. RICHETTI, S.K., M. BLANK, K.M. CAPIOTTI, A.L. PIATO, M.R. BOGO, M.R. VIANNA, C.D. BONAN, Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish, *Behav. Brain Res.*, 2011, **217**(1), 10–15.
64. SAIJA, A., M. SCALESE, M. LANZA, D. MARZULLO, F. BONINA, F. CASTELLI, Flavonoids as antioxidant agents: importance of their interaction with biomembranes, *Free Radic. Biol. Med.,* 1995, **19**, 481–486.
65. SÁNCHEZ-GALLEGO, J.I., A. LÓPEZ-REVUELTA, J.L. SARDINA, Á. HERNÁNDEZ-HERNÁNDEZ, J. SÁNCHEZ-YAGÜE, M. LLANILLO, Membrane cholesterol contents modify the protective effects of quercetin and rutin on integrity and cellular viability in oxidized erythrocytes, *Free Radical Biology & Medicine*, 2010, **48**, 1444–1454.
66. SATYAMITRA, M., S. MANTENA, C.K.K. NAIR, S. CHANDNA, B.S. DWARAKANATH, P. UMA DEVI, The antioxidant flavonoids, orientin and vicenin enhance repair of radiation-induced damage, *SAJ Pharmacy and Pharmacology*, 2014, **1**(1), 2–9.
67. SCHOLZ, S., G. WILLIAMSON, Interactions affecting the bioavailability of dietary polyphenols *in vivo*, *Int. J. Vitam. Nutr. Res.,* 2007, **77**, 224–235.
68. SCHWAN, H.P., Electrical properties of blood and its constituents: Alternating current spectroscopy, *Annals of Hematol*., 2004, **46**, 185–197.
69. SCHWEDHELM, E., R. MAAS, R. TROOST, R.H. BÖGER, Clinical pharmaco-kinetics of antioxidants and their impact on systemic oxidative stress, *Clin. Pharmacokinet*., 2003, **42**, 437–459.
70. SELIM, N.S., Comparative study on the effect of radiation on whole blood and isolated red blood cells, *Romanian J. Biophys*., 2010, **20**(2), 127–136
71. SELIM, N.S., O.S. DESOUKY, S.M. ALI, I.H. IBRAHIM, H.A. ASHRY, Effect of gamma radiation on some biophysical properties of red blood cell membrane, *Romanian J. Biophys.*, 2009, **19**(3), 171–185.
72. SEUFI, A.M., S.S. IBRAHIM, T.K. ELMAGHRABY, E.E HAFEZ, Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats *via* oxidant/antioxidant activity: molecular and histological evidences, *J. Exp. Clin. Cancer Res.*, 2009, **28**(80), 1–8.
73. SEZDI, M., M. BAYIK, Y. ÜLGEN, Storage effects on the Cole-Cole parameters of erythrocyte suspensions, *Physiol. Meas*., 2006, **27**, 623–635.
74. SOLIMAN, M.S., Whole body gamma radiation effects on rheological behaviour (deformability of rat erythrocytes), *Egypt. J. Rad. Sci. Applic.*, 2004, **17**(2) 345–363.

75. SU, J.F., C.J. GUO, J.Y. WEI, J.J. YANG, Y.G. JIANG, Y.F. LI, Protection against hepatic ischemia-reperfusion injury in rats by oral pretreatment with quercetin, *Biomed. Environ. Sci.,* 2003, **16**, 1–8.
76. TRUMBECKAITE, S., J. BERNATONIENE, D. MAJIENE, V. JAKSTAS, A. SAVICKAS, A. TOLEIKIS, The effect of flavonoids on rat heart mitochondrial function, *Biomed. Pharmacother.*, 2006, **60**, 245–248.
77. TSUCHIYA, H., Membrane interactions of phytochemicals as their molecular mechanism applicable to the discovery of drug leads from plants, *Molecules,* 2015, **20**(10), 18923–18966.
78. ÜLGEN, Y., M. SEZDI, Electrical parameters of human blood, *Proceedings of the 20th annual international conference of the IEEE engineering in medicine and biology society*, 1998, **20**, 2983–2986.
79. VALERIO, D.A., S.R. GEORGETTI, D.A. MAGRO, R. CASAGRANDE, T.M. CUNHA, F.T. VICENTINI, S.M. VIEIRA, M.J.V. FONSECA, S.H. FERREIRA, F.Q. CUNHA, W.A. VERRI JR., Quercetin reduces inflammatory pain: inhibition of oxidative stress and cytokine production, *J. Nat. Prod.*, 2009, **72,** 1975–1979.
80. WALLE, T., A.M. BROWNING, L.L. STEED, S.G. REED, U.K. WALLE, Flavonoid glucosides are hydrolyzed and thus activated in the oral cavity in humans, *J. Nutr.*, 2005, **135**, 48–52.
81. WASELENKO, J.K., T.J. MacVITTIE, W.F. BLAKELY, N. PESIK, A.L. WILEY, W.E. DICKERSON, H. TSU, D.L. CONFER, C.N. COLEMAN, T. SEED, P. LOWRY, J.O. ARMITAGE, N. DAINIAK, Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group, *Ann. Intern. Med*., 2004, **140**, 1037–1051.
82. WEISS, J.F., M.R. LANDAUER, History and development of radiation protective agents, *Int. J. Radiat. Biol.*, 2009, **85**, 539–573.
83. WEN, Z., W. YAO, L. XIE, Z. YAN, K. CHEN, W. KA, D. Sun, Influence of neuraminidase on the characteristics of microrheology of red blood cells, *Clinical Hemorheology and Microcirculation,* 2000, **23**(1), 51–57.
84. YAMAMOTO, Y., E. OUE, Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet, *Biosci. Biotechnol. Biochem.*, 2006, **70**, 933–939.
85. ZHAO, W.I., J. SUN, H. XIANG, Y.Y. ZENG, X.B. LI, H. XIAO, D.Y. CHEN, R.L. MA, Synthesis and biological evaluation of new flavonoid fatty acid esters with anti-adipogenic and enhancing glucose consumption activities, *Bioorg. Med. Chem*., 2011, **19**(10), 3192–3203.