Chapter

Reactive Oxygen Species and Antioxidant Interactions in Erythrocytes

Vani Rajashekaraiah, Masannagari Pallavi, Aastha Choudhary, Chaitra Bhat, Prerana Banerjee, Ranjithvishal, Shruthi Laavanyaa and Sudharshan Nithindran

Abstract

There is a continuous generation of reactive oxygen species (ROS) in erythrocytes due to their microenvironment. Reactive oxygen species (ROS) and reactive nitrogen species are well known as both harmful and beneficial species. They help in activating the antioxidant enzymes. However, overproduction of ROS can cause fatal damage to cell structures, including lipids and membranes, proteins and cause oxidative stress. Erythrocytes have effective antioxidant defenses to maintain their structure and functions. They protect these cells from damage and maintain their activities. Studies have reported that antioxidant interventions in various situations have proved beneficial to erythrocytes. Therefore, they can be employed as *in vitro* models for antioxidant and free radical interactions and also are ideal cell models for translational studies.

Keywords: erythrocytes, oxidative stress, free radicals, antioxidants, reactive oxygen species

1. Introduction

The erythrocyte [red blood cell (RBC)] is an ideal cell to study free-radical-mediated alterations. Approximately 25 trillion erythrocytes course through the human circulatory system. The main function of erythrocytes is the transport of oxygen (O_2) and the mediation of carbon dioxide (CO_2) production [1]. Reactive oxygen species (ROS) are continuously produced within the erythrocytes due to high O_2 tension in arterial blood and heme iron content [2].

The mature erythrocyte contains a variety of enzymes, proteins, carbohydrates, lipids, anions, and cations, to balance the cell's metabolism and functions. An important consequence of erythrocyte imbalance is a reduced ability to deal with oxidative stress, which can lead to degenerative changes in hemoglobin, membrane, and enzymes [3].

Erythrocytes are exposed to circulating inflammatory mediators and related oxidative stress, which cause severe alterations in cellular membrane and functions in

a variety of pathological conditions. These alterations have been defined as "erythropathy" [4] and can be observed in conditions of cardiovascular injury [5, 6]. The loss of lipid asymmetry, and thus the exposure of phosphatidyl serine (PS) on the outer monolayer, contributes to the premature destruction of thalassaemic and sickle red cells [7, 8]. Sickle cell disease is distinguished by a change in erythrocyte shape from biconcave discs to elongated and sickle-shaped erythrocytes, consequently leading to loss of function and anemia [9].

Chronic obstructive pulmonary disease (COPD) causes changes in erythrocyte shape, redistribution of microfilaments such as actin and spectrin, and elevations in membrane rigidity [10]. Alterations were also observed in terms of erythrocyte morphology (leptocytes and elliptocytes), elevated membrane F2-isoprostanes and 4-hydroxynonenal (4-HNE) protein adducts, and oxidative damage to actin proteins in Rett syndrome (RTT) and autism spectrum disorder (ASD) [11, 12].

The changes in erythrocyte morphology and stiffness have also been reported in pathologies (type 2 diabetes, obesity, hypertension, and hypercholesterolemia) characterized by consistent oxidative damage followed by reshaping of the lipid distribution and architecture [13]. Erythrocytes participate in physiological and pathological processes associated with oxidative stress, such as aging, Down syndrome, neurodegenerative diseases such as Alzheimer's disease, erectile dysfunction, and cardiovascular disease [14].

The potential clinical application of these erythrocyte alterations as new biomarkers could be useful tools for monitoring a variety of oxidative-stress-related diseases.

2. Reactive oxygen species (ROS) in erythrocytes

Various physiological and pathological conditions, for example, aging, inflammation, and cell death develop through ROS generation. Several factors can lead to the generation of oxidizing radicals such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$) in erythrocytes [15].

Free radicals can be formed in three ways:

i. The cleavage of a covalent bond of a normal molecule, with each fragment retaining one of the paired electrons;

ii. The loss of a single electron from a normal molecule;

iii. The addition of a single electron to a normal molecule.

The latter, electron transfer, is a common process in biological systems [16].

Free radicals and ions are formed as illustrated below:

Radical formation by electron transfer: $A + e^- \rightarrow A^{\bullet -}$

Radical formation by homolytic fission: $X{:}\,Y \rightarrow X^{\bullet} + Y^{\bullet}$

Ion formation by heterolytic fission: $X{:}\,Y \text{ —} > X{:}^- + Y^+$

2.1 Nature of reactive oxygen species

ROS are defined as oxygen-containing species, which are highly reactive. O_2 undergoes one or two-electron reduction to form ROS, which reacts quickly with other compounds, attempting to capture the required electron in order to gain

stability. ROS are oxygen-centered molecules that include hydrogen peroxide, singlet oxygen, superoxide anion, hydroxyl radical, and nitric oxide (NO) [16]. ROS are constantly produced in small quantities by normal metabolic processes. The addition of one electron to O_2 forms $O_2^{\bullet-}$, whereas the addition of two electrons results in the production of H_2O_2.

There are two causes for $O_2^{\bullet-}$ generation in erythrocytes.

Firstly, Oxyhemoglobin (oxyHb) autoxidizes at a relatively slow rate to yield methemoglobin (metHb), and $O_2^{\bullet-}$, which, further produces H_2O_2. Hemoglobin (Hb) is constantly exposed to an intracellular and extracellular flux of H_2O_2. When oxyHb is exposed to H_2O_2, it undergoes oxidative modifications that have been proposed as selective signals for proteolysis in erythrocytes [17]. Secondly, the oxidation state of trivalent iron (Fe^{3+}) has lost an electron during its formation; consequently, $O_2^{\bullet-}$ has been generated from exogenous sources, such as drugs, etc. [18].

Hydrogen peroxide is hydrophilic; however, recent studies reported that aquaporins are not involved in facilitating H_2O_2 diffusion across RBC membranes; rather, diffusion occurs through the lipid fraction or an unidentified membrane protein [19]. While charged, $O_2^{\bullet-}$ can only cross membranes via transmembrane anion channels. MetHb, lipid peroxidation, and spectrin-Hb complexes increase with H_2O_2, which further generates a covalent complex of spectrin and Hb, leading to changes in cell shape, membrane deformability, phospholipid organization, and cell surface characteristics [20].

The Fenton reaction occurs when H_2O_2 reacts with ferrous iron to produce $OH^{\bullet}$. H_2O_2 can react with $O_2^{\bullet-}$ to generate $OH^{\bullet}$, the most active ROS that cannot travel far due to its short half-life of a few nanoseconds known as Haber-Weiss Reaction [21, 22].

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^- + OH^{\bullet} \text{ (Fenton Reaction)} \quad (1)$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet} \text{ (Haber-Weiss Reaction)} \quad (2)$$

ROS have the ability to act as both oxidizing and reducing agents. ROS are capable of directly attacking the red cell membrane and causing changes in lipid and protein structure [23]. ROS also alter mechanical properties, increase rigidity, and RBC interactions with other cells and coagulation factors, as well as stimulate microparticle (MP) generation and phosphatidylserine (PS) exposure [24]. Human red cell aging could be attributed to oxidative damage. RBC deformability, membrane permeability, and surface antigenicity abnormalities, on the other hand, have been recognized as defects in cellular properties that contribute to RBC senescence [25].

Nitric oxide, along with O_2 and CO_2, is the third gas transported by erythrocytes. Erythrocytes are the primary NO scavengers in circulation due to their high Hb concentration. NO is taken up by heme prosthetic groups of Hb-chain cysteine residues. NO is converted to nitrate by oxyhemoglobin ($HbFe^{+2}O_2$), whereas deoxyhemoglobin ($HbFe^{2+}$) binds to NO to form iron-nitrosylhemoglobin ($HbFe^{2+}NO$). NO consumption by erythrocytes can be regulated by $HbFe^{2+}NO$ formation under hypoxic conditions [26]. NO reaction with Hb greatly limits intravascular NO concentration. As a result, it is unlikely that NO is directly exported or produced by red blood cells as an intravascular signaling molecule. The rapid deoxygenation of NO by Hb results in the formation of nitrate and metHb, preventing NO diffusion from plasma to smooth muscle [15]. NO is produced in large amounts in inflammatory conditions and reacts

with $O_2^{\bullet -}$ to generate peroxynitrite [27]. Peroxynitrite oxidizes plasma components, releasing secondary radicals that promote tyrosine nitration, leading to gain or loss of protein function [28].

3. Oxidative stress in erythrocytes

Erythrocytes are well endowed to combat oxidative stress due to their continuous contact with oxygen, as their inherent carrier function. When the concentration of ROS in cells or tissues exceeds the antioxidant protection, oxidative stress occurs [21]. RBC properties have been shown to change as a result of oxidative damage. Oxidative damage can also alter membrane permeability resulting in hemolysis [29, 30]. Oxidative cross-linking of spectrin can cause increased membrane rigidity and decreased erythrocyte deformability. Erythrocytes can be recognized by the immune system as a result of oxidative damage [31].

3.1 Extracellular hemoglobin as a source of oxidative stress

Extracellular Hb, which results from erythrocyte hemolysis or the infusion of cell-free Hb-based blood substitutes, can be a major source of oxidative stress. This potential source of oxidative stress is minimized under normal conditions by haptoglobin and hemopexin, which bind Hb and free heme, respectively. They inhibit the oxidative reactions of Hb and heme, allowing them to be removed from circulation. Elevated levels of free Hb and heme, which cannot be neutralized by haptoglobin and hemopexin, cause a variety of adverse clinical effects [32].

Autoxidation of Hb produces superoxide as well as methemoglobin. Hb is known to react with hydrogen peroxide to form ferrylhemoglobin, a strong oxidant [33]. Hb binds to RBC membrane proteins, especially under hypoxic conditions. The ROS produced by bound Hb may be inaccessible to cellular antioxidants, allowing the production of heme degradation products close to the membrane [34–36]. Extensive lipid peroxidation results in changes in fluidity such as a drop in membrane potential and an increase in permeability to different ions, which eventually leads to hemolysis. Thus, perturbations in erythrocyte function and structure can result in an increased flow of prooxidant generation that can lead to oxidative stress.

Oxidative stress and ROS accumulation in RBCs during aging may induce hemolysis. As a result, the plasma proteins haptoglobin and hemopexin can render free Hb and heme relatively inactive and deliver them safely to macrophages for phagocytosis [37, 38]. Oxidized Hb on the other hand exhibits impaired plasma clearance, due to its low affinity for haptoglobin protein.

Erythrocyte membranes exposed to oxidative stress undergo cellular component modifications such as oxidative denaturation of Hb, peroxidation of lipids, and high-molecular-weight cross-linked membrane proteins. The erythrocyte membrane is rich in sulfhydryl (–SH) groups, which help to maintain cellular oxidative balance [39]. Changes in the membrane elasticity may occur due to oxidative damage to the membrane –SH groups [40]. Oxidative damage to erythrocytes can occur and manifest itself in a variety of ways, including potassium release, increase in malondialdehyde (MDA), phosphatidylserine externalization [41], decrements in glutathione, superoxide dismutase, glutathione peroxidase, glutathione S-transferase, and glutathione reductase as well as total antioxidant activity of

plasma [42–44]. Oxidant stress is a key component to both normal RBC aging and pathological dysfunction [32].

Oxidative damage to a specific protein, particularly at the active site, can result in the progressive loss of a specific biochemical function [45]. Peroxidation causes globin cross-linking to proteins such as spectrin and band 3. These processes further lead to decreased phospholipid symmetry, formation of cross-linked spectrin and Hb, aggregation of band 3 protein, and increase in advanced glycation end products leading to deformability and morphologic and surface changes in the erythrocyte [15, 46, 47]. During erythrocyte aging, an irreversible oxidative complex is formed between the Hb globin chain and spectrin [20]. Iron release may be accompanied by the generation of senescent antigens (SCA) and oxidative alteration of membrane proteins [15, 48, 49]. Another event associated with red cell homeostasis disruption is an increased inflammatory state in the bloodstream, since erythrocytes are constantly exposed to inflammatory molecules transported in the vascular system, their membranes may be particularly vulnerable to their interaction. A large number of inflammatory mediators, including tumor necrosis factor-α, interleukins, interferons, and C-reactive protein (CRP), have been proposed as potential inflammatory response markers [50].

In general, erythrocytes respond to oxidative stress by activating tyrosine kinases [51, 52], resulting in tyrosine phosphorylation at the cytoplasmic domain of band 3 protein, which mediates interactions with ankyrin, leading to membrane destabilization. This also seen in β-thalassemia disease with the loss of stability between cytoskeleton and membrane complexes, such as band 3 protein [53]. Oxidative damage to band 3 has been linked to RBC aging including the exposure of senescent specific neo-antigens that bind autologous IgG triggering RBC removal [54].

Erythrocytes also contain NADH oxidases, which can generate endogenous ROS [55]. However, some forms of NADH oxidase were also detected in normal RBCs. L-isoaspartyl groups were discovered on erythrocyte membrane proteins in response to aging or pathological oxidative stress, such as glucose 6-phosphate dehydrogenase deficiency or Down syndrome [56–59]. Thus, L-isoaspartyl group accumulation in RBC proteins correlates with RBC dysfunction and pathology.

The effects of oxidative stress observed in various pathological conditions in erythrocytes are depicted in **Table 1**.

Diseases	**Oxidative stress markers**	**Reference**
Sickle cell disease	Variations in Superoxide dismutase	[60–63]
	Alterations in Catalase	[60, 62, 63]
	Glutathione—decreased	[64, 65]
	Changes in glutathione peroxidase	[61, 62]
	Vitamin E—decreased	[66, 67]
	Vitamin-C—decreased	[68]
	Lipid peroxidation—increased	[62, 63, 69, 70]
	Intracellular Ca^{2+}—increased	[71]
	Phosphatidylserine exposure—increased	[72–74]
	NADPH oxidase activity—increased	[55]
	ROS generation—increased	[55]

Diseases	Oxidative stress markers	Reference
β-Thalassemia	Band 3 tyrosine phosphorylation (P)—increased	[53]
	Phosphatidylserine exposure—increased	[53]
	Release of microparticles	[53]
	Membrane thiols—decreased	[53]
	K^+, Cl^-, and water loss	[53]
Diabetes (T2DM)	Lipid peroxidation—increased	[75]
	GSH/GSSG ratio—decreased	[75]
	Glutathione—decreased	[75]
	Phosphatidylserine externalization—increased	[75]
	Annexin binding—increased	[75]
	Caspase-3 activation—increased	[76]
Malaria	Lipid peroxidation—increased	[77]
	Glutathione—decreased	[77]
	Catalase—decreased	[77]
	Membrane stiffness—increased	[78]
Autism	TBARS—increased	[79]
	Xanthine oxidase—increased	[79]
	Superoxide dismutase—increased	[79]
	Superoxide dismutase—decreased	[80]
	Glutathione peroxidase—decreased	[80]
	Modulations in catalase	[79, 81]
Chronic kidney disease	Membrane fluidity—increased	[82]
	Osmatic fragility—increased	[82]
	RBC life span—decreased	[82]
	Antioxidant enzymes—decreased	[83–85]
	NADPH oxidase—increased	[86]
	Hemoglobin leakage—increased	[86]
	Nitrite ions—increased	[86]
	Peroxynitrite—increased	[86]
	Hemoglobin—decreased	[86]
	Circulating RBCs—decreased	[86]
Systemic sclerosis (SSc)	Anion exchange capability via band 3—decreased	[87]
	Blood viscosity—increased	[87]
	Membrane protein structure destabilization	[87]
	SO_4^{2-}—decreased	[87]

Diseases	Oxidative stress markers	Reference
Corona virus disease-19 (Covid-19)	Hematocrit levels—decreased	[88]
	RBCs amplitude—increased	[88]
	Glutathione—decreased	[89–91]
	Arginase 1—increased	[92]
	ROS—increased	[92]
	Interferon-γ—increased	[92]
	NO bioactivity—decreased	[92]
	Oxidized glutathione (GSSG)—increased	[93]
	Glycolytic metabolites—increased	[93]
	Phosphoglucomutase—decreased	[93]
	Carboxylic acids—increased	[93]
	Glutathione peroxidase—decreased	[91]
	Catalase—decreased	[91]
	Superoxide dismutase—decreased	[91]

Table 1.
Effects of oxidative stress in erythrocytes during diseases.

4. Antioxidant defense in erythrocytes

The antioxidant defense system of erythrocytes prevents oxidative cell damage. This implies that the erythrocyte antioxidant defenses operate in a balanced manner. As a result, an appropriate redox state, balanced antioxidant action is required for ROS homeostasis.

Antioxidants are molecules that prevent or delay cellular damage by inhibiting or quenching ROS reactions [94]. Antioxidants can be synthesized in the body or obtained from the environment, such as through diet. Erythrocytes are well equipped to fight against oxidative stress, i.e., mechanisms to scavenge and detoxify ROS, prevent their production, and sequester transition metals [95]. Erythrocytes contain both enzymatic and non-enzymatic antioxidants to combat oxidative stress.

4.1 Enzymatic antioxidants

Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and peroxiredoxin-2 (PRX-2). Their coordinated actions protect the erythrocytes from free-radical-mediated damage. Since there is no *de novo* synthesis of antioxidant enzymes in mature erythrocytes, their defense capacity is limited. Free radicals affect the capacities of antioxidative enzymes as well as the overall antioxidative system [96]. Under normal conditions, erythrocytes contain sufficient levels of scavenger enzymes such as Cu,Zn-SOD, CAT, and selenium-dependent GPX to protect from free radical injury.

Superoxide dismutase, a ubiquitous metal-containing enzyme, involves in the detoxification of $O_2^{\bullet-}$ into O_2 and H_2O_2.

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (3)$$

SOD family comprises CuSOD, ZnSOD, MnSOD, and extracellular SOD, which protect from particularly $O_2^{\bullet-}$. Cu,Zn-SOD catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2, which is later converted to water by CAT or GPX [21, 27, 97]. The activity of these enzymes in erythrocytes is highest than that of other tissues in the body [98].

Erythrocytes are well protected against ROS due to the abundance of Cu,Zn-SOD, which scavenges free radicals and thus prevents metHb formation [43]. Cu, Zn-SOD synthesis is induced by $O_2^{\bullet-}$ formation through the activation of regulatory genes [15]. SOD scavenges $O_2^{\bullet-}$ and inhibits the formation of peroxynitrite, thereby preventing injury and regulating the bioavailability of NO [27]. Erythrocytes contain an abundant quantity of Cu,Zn-SOD, which maintains intra-erythrocyte $O_2^{\bullet-}$ levels at concentrations as low as 10^{-13} mol/L [96].

Catalases catalyze the direct decomposition of H_2O_2 to water and O_2 [21].

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \quad (4)$$

Catalase and SOD react synergistically to protect each other [99]. CAT and GPX are equally active in the detoxification of H_2O_2 in normal erythrocytes [100]. At physiological concentrations, GPX acts as a primary defense in H_2O_2 degradation by reducing H_2O_2 while also converting GSH to its oxidized form (GSSG). However, under H_2O_2 overproduction, CAT exhibits increased enzymatic activity, as measured by the Michaelis-Menten constant (Km). The Km for CAT (2.4×10^{-4} M) is significantly greater than the Km for GPX (1×10^{-6} M), which indicates that CAT scavenges H_2O_2 efficiently at higher concentrations [101–103]. GPX is important in dealing with endogenous H_2O_2 produced by Hb autoxidation, whereas CAT becomes increasingly important when erythrocytes are exposed to increased H_2O_2 flux [15]. H_2O_2 readily crosses erythrocyte membranes and can protect other tissues against extracellular H_2O_2 by "absorbing" and destroying it. Reduced glutathione (GSH) is used by GPX to detoxify hydrogen peroxide during normal antioxidant defense system function. Furthermore, glutathione reductase is required to convert H_2O_2 to GSH, which contributes to H_2O_2 detoxification [104].

Glutathione-S-transferases are important in the detoxification of electrophilic xenobiotics. This enzyme catalyzes the conjugation of GSH with exogenous and endogenous toxic compounds or their metabolites, making them more water-soluble, less toxic, and easier to excrete. In addition, they are responsible for various resistance mechanisms such as chemotherapeutic or antibiotic drug resistance [15].

Glucose-6-phosphate dehydrogenase (G6PD) is an important antioxidant enzyme in erythrocytes, which is the regulatory enzyme of the pentose-phosphate pathway (PPP). As erythrocytes lack mitochondria, the PPP pathway is the only source of NADPH, and it plays an important role in NADPH-dependent antioxidant defense [105, 106]. G6PD is required to protect erythrocytes from oxidative damage. The lack of this protection can lead to severe hemolysis [107].

In addition to primary antioxidant defense systems that prevent the generation of free radicals or radical chain reactions, secondary systems have been proposed. Proteases that preferentially degrade oxidatively damaged proteins are among them. In erythrocytes, a multicatalytic proteolytic complex appears to be responsible for the degradation of oxidized intracellular proteins [47]. The presence of an 80-kDa serine protease in the oxidized erythrocyte membranes preferentially degrades oxidized proteins specifically protein hydrolase. When cells are oxidized, this cytoplasmic protein

becomes adherent to membranes, promoting membrane protein degradation. The protease is characterized by its inhibition by a serine protease inhibitor [108]. It is endogenously present in oxidized or aged erythrocyte membranes and plays a crucial role in the removal of the oxidation-induced membrane protein aggregates and in reducing the oxidation-induced anti-band 3 binding in aging. Oxidized protein hydrolase (OPH) acts as a secondary defense system by removing oxidized protein aggregates.

Peroxiredoxins (PRX), a class of thiol-containing enzymes, act as H_2O_2 and peroxynitrite scavengers in circulation. PRXs have a reductive capacity for hydroperoxides via a reductant thiol. Peroxiredoxins have been shown in studies to be catalytic peroxynitrite reductases. It has been also reported that PRX-II is present in the cytosol of erythrocytes. The catalytic cycle involves the reduction of oxidized PRX by thioredoxin and the reduction capacity of NADPH via NADPH-thioredoxin reductase [28, 109].

4.2 Non-enzymatic antioxidants

Endogenous non-enzymatic antioxidants are defined in two phases: lipophylic (vitamin E, carotenoids, ubiquinon, melatonin, etc.) and water-soluble (vitamin C, glutathione, uric acid, ceruloplasmin, transferin, haptoglobulin, etc.). Three antioxidant vitamins, A, C, and E, provide defense against oxidative damage. Vitamin C acts in the aqueous phase, whereas vitamin E acts in the lipid phase as a chain-breaking antioxidant. Vitamin C reduces $O_2^{\bullet-}$ and lipid peroxyl radical, but is also a well-known synergistic agent for vitamin E [2]. Uric acid is an endogenous antioxidant with metal-chelating properties and scavenges nitrogen radicals and superoxide in plasma, thereby blocking the generation of peroxynitrite. Uric acid in erythrocytes quenches the free radicals and ROS. Uric acid maintains the smooth membrane surface of RBCs, thus preventing echinocyte formation [110].

Additionally, erythrocytes have a plasma membrane redox system (PMRS) that transfers electrons from intracellular substrates to extracellular electron acceptors, which may be NAD+ or/and vitamin C [111].

Many studies have demonstrated the influence of different antioxidants on erythrocytes during oxidative stress (**Table 2**).

Sample	OS inducer	Antioxidant	Results	Reference
Human RBC membrane	Hydrogen peroxide (H_2O_2) & ozone	1,3-dimethyluric acid and 1,3,7-trimethyluric acid	Prevented lipid peroxidation	[112]
Human RBCs	Hydrogen peroxide	β-Carotene & resveratrol	Increased SOD & catalase. decreased conjugate dienes & TBARS	[113]
Human RBCs	Tert-butyl hydroperoxide	Resveratrol	Increase in GSH & membrane –SH	[114]
Human RBCs	tert-butyl hydroperoxide	Capsaicin or L-ascorbic acid	Decrease in MDA & protein carbonyls	[115]
Human RBCs	Hydrogen peroxide	Tea polyphenols (TPP)	Protected RBCs and membranes against lipid peroxidation	[116]
Sickle cell human RBCs	Tert-butyl hydroperoxide	Flavonoids (quercetin & rutin), Ascorbic acid	Protected against oxidative stress in and lipid peroxidation	[117]

Sample	OS inducer	Antioxidant	Results	Reference
Human RBCs	Hydrogen peroxide	Oak barrel-aged red wine extract (SD95)	Protected against hemolysis, ROS & maintained MDA	[118]
Human RBCs	AAPH or H_2O_2 or t-BOOH	Ether and water fraction of honey	Ether fraction inhibited hemolysis and t-BOOH-induced lipid peroxidation	[119]
Human RBCs	Bisphenol A (BPA)	Quercetin	Decreased MDA levels and increased antioxidant enzymes	[120]
Human RBCs	Hydrogen peroxide	Melatonin	Restored band 3 expression levels, cell shape alterations & lower TBARS.	[121]
Rat RBCs	Tert-butyl hydroperoxide	Flavonoids (quercetin, catechin and naringenin)	Inhibited membrane lipid peroxidation and decreased glutathione oxidation	[122]
Human RBCs	Sodium fluoride (NaF)	3,4-dihydroxy benzaldehyde (DHB)	Increased AOPP, lipid peroxidation, restored PMRS & AO enzyme activity	[123]
Human RBCs	Hydrogen peroxide	β-carotene (BC) and resveratrol (RSV)	TBARS and conjugate dienes decreased in BC and RSV groups. SOD increased in RSV.	[113]

Table 2.
The effects of antioxidants on erythrocytes during oxidative stress.

There are *in vitro* studies on oxidative stress in erythrocytes reporting the protective effects of antioxidants from plant extracts (**Table 3**).

Sample	OS inducer	Antioxidant	Results	Reference
Rat RBCs	H_2O_2	Potato peel extract (PPE)	Inhibited morphological alterations RBCs	[124]
Human RBCs	2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)	Olive leaf extract (OLE)	Inhibited hemolysis, TBARS formation, and hemoglobin oxidation	[125]
Goat RBCs	Cu^{2+}-ascorbate	Terminalia arjuna (TA) bark extract	Decreased lipid peroxidation and protein carbonyl content. Increased GSH	[126]
Human RBCs	2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)	Quince (Cydonia oblonga) fruit pulp and peel extracts	Protection of erythrocyte membrane from hemolysis	[127]
Human RBCs	Hypochlorous acid (HClO)	Ugni molinae Turcz aqueous extract	Reduced hemolysis	[128]
Rat RBCs	Cadmium	Salicornia arabica lipid extract	Ameliorated antioxidant status and inhibited MDA levels	[129]

Sample	OS inducer	Antioxidant	Results	Reference
Human RBCs	Hypochlorous acid (HClO)	Pitavia punctata extract	Protecting the membrane integrity and inhibiting the oxidation of the LDL lipoprotein	[130]
Human RBCs	H_2O_2	Orchis latifolia and Centratherum anthelminticum extract	Protected membrane integrity resulting in a reduction of RBC hemolysis and lipid peroxidation	[131]

Table 3.
The modulations of antioxidants from plant extracts in erythrocytes.

5. Conclusion

Many physiological and pathological circumstances have the potential to cause oxidative stress and are possibly harmful in susceptible individuals. Furthermore, this increased risk of adverse reactions is generally reflected in the erythrocytes. The administration of antioxidants has the prospects of diminishing oxidative damage. Therefore, erythrocytes act as unique cell models for translational studies on oxidant and antioxidant interactions. However, they may not be helpful to study these effects in relation to mitochondria (a major source of ROS), as the mature erythrocytes lack the cell organelles.

Acknowledgements

The authors acknowledge Dr. Leela Iyengar, Anusha BA, Ms. Magdaline Christina, and JAIN (Deemed-to-be University) for their support.

Conflict of interest

The authors have no conflict of interest to disclose.

Author details

Vani Rajashekaraiah*, Masannagari Pallavi, Aastha Choudhary, Chaitra Bhat, Prerana Banerjee, Ranjithvishal, Shruthi Laavanyaa and Sudharshan Nithindran
Department of Biotechnology, School of Sciences, JAIN (Deemed-to-be University), Bangalore, India

*Address all correspondence to: vani.rs@jainuniversity.ac.in; tiwari.vani@gmail.com

IntechOpen

References

[1] Volpe EP. Blood and circulation. In: Dubuque WC, editor. Biology and Human Concerns. Dubuque: Wm.C.Brown Publishers; 1993. pp. 253-265

[2] Baynes JW. Oxygen and life. In: Baynes JW, Domoniczak MH, editors. Medical Biochemistry. Philadelphia: Elsevier; 2005. pp. 497-506

[3] Edwards JC, Fuller J. Oxidative stress in erythrocytes. Comparative Haematology International. 1996;**6**: 24-31. DOI: 10.1007/BF00368098

[4] Pernow J, Mahdi A, Yang J, Zhou Z. Red blood cell dysfunction: A new player in cardiovascular disease. Cardiovascular Research. 2019;**115**:1596-1605. DOI: 10.1093/cvr/cvz156

[5] Zhou Z, Mahdi A, Tratsiakovich Y, Zahorán S, Kövamees O, Nordin F, et al. Erythrocytes from patients with type 2 diabetes induce endothelial dysfunction via arginase I. Journal of the American College of Cardiology. 2018;**72**:769-780. DOI: 10.1016/j.jacc.2018.05.052

[6] Yang J, Zheng X, Mahdi A, Zhou Z, Tratsiakovich Y, Jiao T, et al. Red blood cells in type 2 diabetes impair cardiac post-ischemic recovery through an arginase-dependent modulation of nitric oxide synthase and reactive oxygen species. JACC: Basic to translational. Science. 2018;**3**:450-463. DOI: 10.1016/j.jacbts.2018.03.006

[7] Kuypers FA, Yuan J, Lewis RA, Snyder LM, Kiefer CR, Bunyaratvej A, et al. Membrane phospholipid asymmetry in human thalassemia. Blood. The Journal of the American Society of Hematology. 1998;**91**:3044-3051. DOI: 10.1182/blood.v91.8.3044.3044_3044_3051

[8] Setty BY, Kulkarni S, Stuart MJ. Role of erythrocyte phosphatidylserine in sickle red cell–endothelial adhesion. Blood. The Journal of the American Society of Hematology. 2002;**99**:1564-1571. DOI: 10.1182/blood.V99.5.1564

[9] Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. Nature Reviews Disease Primers. 2018;**4**:1-22. DOI: 10.1038/nrdp.2018.10

[10] Santini MT, Straface E, Cipri A, Peverini M, Santulli M, Malorni W. Structural alterations in erythrocytes from patients with chronic obstructive pulmonary disease. Pathophysiology of Haemostasis and Thrombosis. 1997;**27**:201-210. DOI: 10.1159/000217458

[11] Ciccoli L, De Felice C, Paccagnini E, Leoncini S, Pecorelli A, Signorini C, et al. Morphological changes and oxidative damage in Rett syndrome erythrocytes. Biochimica et Biophysica Acta (BBA)-General Subjects. 2012;**1820**:511-520. DOI: 10.1016/j.bbagen.2011.12.002

[12] Ciccoli L, De Felice C, Paccagnini E, Leoncini S, Pecorelli A, Signorini C, et al. Erythrocyte shape abnormalities, membrane oxidative damage, and β-actin alterations: An unrecognized triad in classical autism. Mediators of Inflammation. 2013;**2013**. Article ID 432616. DOI: 10.1155/2013/432616

[13] Gyawali P, Richards RS, Uba NE. Erythrocyte morphology in metabolic syndrome. Expert Review of Hematology. 2012;**5**:523-531. DOI: 10.1586/ehm.12.47

[14] Massaccesi L, Galliera E, Corsi Romanelli MM. Erythrocytes as markers of oxidative stress-related pathologies. Mechanisms of Ageing

and Development. 2020;**191**:111333. DOI: 10.1016/j.mad.2020.111333

[15] Cimen MYB. Free radical metabolism in human erythrocytes. Clinica Chimica Acta. 2008;**390**:1-11. DOI: 10.1016/j.cca.2007.12.025

[16] Cheeseman KH, Slater TF. An introduction to free radical biochemistry. British Medical Bulletin. 1993;**49**:481-493. DOI: 10.1093/oxfordjournals.bmb.a072625

[17] Giulivi C, Davies KJ. Mechanism of the formation and proteolytic release of H2O2-induced dityrosine and tyrosine oxidation products in haemoglobin and red blood cells. The Journal of Biological Chemistry. 2001;**276**:24129-24136. DOI: 10.1074/jbc.M010697200

[18] Hebbel RP, Eaton JW, Balasingam M, Steinberg MH. Spontaneous oxygen radical generation by sickle erythrocytes. The Journal of Clinical Investigation. 1982;**70**:1253-1259. DOI: 10.1172/jci110724

[19] Orrico F, Lopez AC, Saliwonczyk D, Acosta C, Rodriguez-Grecco I, Mouro-Chanteloup I, et al. The permeability of human red blood cell membranes to hydrogen peroxide is independent of aquaporins. Journal of Biological Chemistry. 2022;**298**:101503. DOI: 10.1016/j.jbc.2021.101503

[20] Snyder LM, Fortier NL, Trainor J, Jacobs J, Leb L, Lubin B, et al. Effect of hydrogen peroxide exposure on normal human erythrocyte deformability, morphology, surface characteristics, and spectrin-hemoglobin cross-linking. Journal of Clinical Investigation. 1985;**76**:1971-1977. DOI: 10.1172/JCI112196

[21] Al-Omar MA, Beedham C, Alsarra IA, Mohammed AAO. Pathological roles of reactive oxygen species and their defence mechanisms. Saudi Pharmaceutical Journal. 2004;**12**:1-18. ID: emr-69011

[22] Yan EB, Unthank JK, Castillo-Melendez M, Miller SL, Langford SJ, Walker DW. Novel method for in vivo hydroxyl radical measurement by microdialysis in fetal sheep brain in utero. Journal of Applied Physiology. 2005;**98**:2304-2310. DOI: 10.1152/japplphysiol.00617.2004

[23] Claster S, Chiu DT, Quintanilha A, Lubin B. Neutrophils mediate lipid peroxidation in human red cells. Blood. 1984;**64**:1079-1084

[24] Wang Q, Zennadi R. Oxidative stress and thrombosis during aging: The roles of oxidative stress in RBCs in venous thrombosis. International Journal of Molecular Sciences. 2020;**21**:4259. DOI: 10.339 0/ijms21124259

[25] Chiu D, Lubin B. Oxidative hemoglobin denaturation and RBC destruction: The effect of heme on red cell membranes. Seminars in Hematology. 1989;**126**:128-135

[26] Han TH, Qamirani E, Nelson AG, Hyduke DR, Chaudhuri G, Kuo L, et al. Regulation of nitric oxide consumption by hypoxic red blood cells. Proceedings of the National Academy of Sciences. 2003;**100**:12504-12509. DOI: 10.1073/pnas.2133409100

[27] Gunduz K, OzturkG SEY. Erythrocyte superoxide dismutase, catalase activities and plasma nitrite and nitrate levels in patients with Behçet disease and recurrent aphthous stomatitis. Clinical and Experimental Dermatology. 2004;**29**:176-179. DOI: 10.1111/j.1365-2230.2004.01488.x

[28] Romero N, Denicola A, Radi R. Red blood cells in the metabolism of

nitric oxide-derived peroxynitrite. IUBMB Life. 2006;**58**:572-580. DOI: 10.1080/15216540600936549

[29] Lubin B, Chiu DTY. Properties of vitamin E deficient erythroeytes following peroxidant injury. Pediatric Research. 1982;**16**:928-932. DOI: 10.1203/00006450-198211000-00005

[30] Durak I, Kavutcu M, Çimen MYB, Avc A, Elgün S, Öztürk HS. Oxidant/antioxidant status of erythrocytes from patients with chronic renal failure: Effects of hemodialysis. Medical Principles and Practice. 2001;**10**:187-190. DOI: 10.1159/000050367

[31] Low P, Waugh SM, Zinke K, Drenckhahan D. The role of hemoglobin denaturation and band 3 clustering in red blood cell aging. Science. 1985;**227**:531-533. DOI: 10.1126/science.2578228

[32] Rifkind JM, Mohanty JG, Nagababu E. The pathophysiology of extracellular hemoglobin associated with enhanced oxidative reactions. Frontiers in Physiology. 2015;**5**:500. DOI: 10.3389/fphys.2014.00500

[33] Everse J. Hsia N the toxicities of native and modified hemoglobins. Free Radical Biology and Medicine. 1997;**22**:1075-1099. DOI: 10.1016/s0891-5849(96)00499-6

[34] Bakonyi T, Radak Z. High altitude and free radicals. Journal of Sports Science and Medicine. 2004;**3**:64-69

[35] Nagababu E, Rifkind JM. Heme degradation during autoxidation of oxyhemoglobin. Biochemical and Biophysical Research Communications. 2000;**273**:839-845. DOI: 10.1006/bbrc.2000.3025

[36] Nagababu E, Chrest F, Rifkind J. Hydrogen-peroxide-induced heme degradation in red blood cells: The protective roles of catalase and glutathione peroxidase. Biochimica et Biophysica Acta (BBA)—General Subjects. 2003;**1620**:211-217. DOI: 10.1016/s0304-4165(02)00537-8

[37] Smith A, Morgan WT. Haem transport to the liver by haemopexin. Receptor-mediated uptake with recycling of the protein. Biochemical Journal. 1979;**182**:47-54. DOI: 10.1042/bj1820047

[38] Thomsen JH, Etzerodt A, Svendsen P, Moestrup SK. The haptoglobin-CD163-heme oxygenase-1 pathway for hemoglobin scavenging. Oxidative Medicine and Cell Longevity. 2013;**2013**:Article ID:523652. DOI: 10.1155/2013/523652

[39] Reglinski J, Hoey S, Smith WE, Sturrock RD. Cellular response to oxidative stress at sulfhydryl group receptor sites on the erythrocyte membrane. Journal of Biological Chemistry. 1988;**263**:12360-12366. DOI: 10.1016/S0021-9258(18)37763-9

[40] Wang X, Wu Z, Song G, Wang H, Long M, Cai S. Effects of oxidative damage of membrane protein thiol groups on erythrocyte membrane viscoelasticities. Clinical Hemorheology and Microcirculation. 1999;**21**:137-146

[41] Chin-Yee I, Arya N, d'Almeida MS. The red cell storage lesion and its implication for transfusion. Transfusion Science. 1997;**18**:447-458. DOI: 10.1016/S0955-3886(97)00043-X

[42] Racek J, Herynková R, Holeček V, Jerabek Z, Slama V. Influence of antioxidants on the quality of stored blood. Vox Sanguinis. 1997;**72**:16-19. DOI: 10.1046/j.1423-0410.1997.00016.x

[43] Dumaswala UJ, Zhuo L, Jacobsen DW, Jain SK, Sukalski KA.

Protein and lipid oxidation of banked human erythrocytes: Role of glutathione. Free Radical Biology and Medicine. 1999;**27**:1041-1049. DOI: 10.1016/s0891-5849(99)00149-5

[44] Knight JA, Voorhees RP, Martin L, Anstall H. Lipid peroxidation in stored red cells. Transfusion. 1992;**32**:354-357. DOI: doi.org/10.1046/j.1537-2995.1992.32492263451.x

[45] Dubinina EE, Gavrovskaya SV, Kuzmich EV, Leonova NV, Morozova MG, Kovrugina SV, et al. Oxidative modification of proteins: Oxidation of tryptophan and production of dityrosine in purified proteins using fenton's system. Biochemistry. 2002;**67**:343-350. DOI: 10.1023/a:1014840617890

[46] Caprari P, Scuteri A, Salvati AM, Bauco C, Cantafora A, Masella R, et al. Aging and red blood cell membrane: A study of centenarians. Experimental Gerontology. 1999;**34**:47-57. DOI: 10.1016/s0531-5565(98)00055-2

[47] Fujino T, Tada T, Hosaka T, Beppu M, Kikugawa K. Presence of oxidized protein hydrolase in human cell lines, rat tissues, and human/rat plasma. The Journal of Biochemistry. 2000;**127**:307-313. DOI: 10.1093/oxfordjournals.jbchem.a022608

[48] Morabito R, Romano O, La Spada G, Marino A. H2O2-induced oxidative stress affects SO4= transport in human erythrocytes. PLoS One. 2016;**11**:e0146485. DOI: 10.1371/journal.pone.0146485

[49] Van Zwieten R, Verhoeven AJ, Roos D. Inborn defects in the antioxidant systems of human red blood cells. Free Radical Biology and Medicine. 2014;**67**:377-386. DOI: 10.1016/j.freeradbiomed.2013.11.022

[50] Remigante A, Morabito R, Marino A. Band 3 protein function and oxidative stress in erythrocytes. Journal of Cellular Physiology. 2021;**236**:6225-6234. DOI: 10.1002/jcp.30322

[51] Minetti M, Mallozzi C, Di Stasi AM. Peroxynitrite activates kinases of the src family and upregulates tyrosine phosphorylation signaling. Free Radical Biology and Medicine. 2002;**33**:744-754. DOI: 10.1016/s0891-5849(02)00891-2

[52] Mladenov M, Gokik M, Hadzi-Petrushev N, Gjorgoski I, Jankulovski N. The relationship between antioxidant enzymes and lipid peroxidation in senescent rat erythrocytes. Physiological Research. 2015;**64**:891-896. DOI: 10.33549/physiolres

[53] De Franceschi L, Bertoldi M, Matte A, Santos Franco S, Pantaleo A, Ferru E, et al. Oxidative stress and beta-thalassemic erythroid cells behind the molecular defect. Oxidative Medicine and Cellular Longevity. 2013;**2013**:Article ID: 985210. DOI: 10.1155/2013/985210

[54] Kay MM. Generation of senescent cell antigen on old cells initiates IgG binding to a neo antigen. Cellular and Molecular Biology. 1993;**39**:131-153

[55] George A, Pushkaran KDG, Koochaki S, Malik P, Mohandas N, Zheng Y, et al. Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC,and plasma cytokines contributes to oxidative stress in sickle cell disease. Blood. 2013;**121**:2099-2107. DOI: 10.1182/blood-2012-07-441188

[56] Galletti P, Manna C, Ingrosso D, Iardino P, Zappia V. Hypotheses on the physiological role of enzymatic protein methyl esterification using human erythrocytes as a model system. Advances in Experimental Medicine

and Biology. 1991;**307**:149-160. DOI: 10.1007/978-1-4684-5985-2_14

[57] Galletti P, De Bonis ML, Sorrentino A, Raimo M, D'Angelo S, Scala I, et al. Accumulation of altered aspartyl residues in erythrocyte proteins from patients with Down's syndrome. The FEBS Journal. 2007;**274**:5263-5277. DOI: 10.1111/j.1742-4658.2007.06048.x

[58] Ingrosso D, Cimmino A, D'Angelo S, Alfinito F, Zappia V, Galletti P. Protein methylation as a marker of aspartate damage in glucose-6-phosphate dehydrogenase-deficient erythrocytes: Role of oxidative stress. European Journal of Biochemistry. 2002;**269**:2032-2039. DOI: 10.1046/j.1432-1033.2002.02838.x

[59] Janson CA, Clarke S. Identification of aspartic acid as a site of methylation in human erythrocyte membrane proteins. The Journal of Biological Chemistry. 1980;**255**:11640-11643. DOI: doi.org/10.1016/S0021-9258(19)70177-X

[60] Das SK, Nair RC. Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. British Journal of Haematology. 1980;**44**:87-92. DOI: 10.1111/j.1365-2141.1980.tb01186.x

[61] Gizi A, Papassotiriou I, Apostolakou F, Lazaropoulou C, Papastamataki M, Kanavaki I, et al. Assessment of oxidative stress in patients with sickle cell disease: The glutathione system and the oxidant antioxidant status. Blood Cells Molecules and Diseases. 2011;**46**:220-225. DOI: 10.1016/j.bcmd.2011.01.002

[62] Alsultan AI, Seif MA, Amin TT, Naboli M, Alsulima AM. Relationship between oxidative stress, ferritin and insulin resistance in sickle cell disease. European Review for Medical and Pharmacological Sciences. 2010;**14**:527-538

[63] Dasgupta T, Hebbel RP, Kaul DK. Protective effect of arginine on oxidative stress in transgenic sickle mouse models. Free Radical Biology and Medicine. 2006;**41**:1771-1780. DOI: 10.1016/j.freeradbiomed.2006.08.025

[64] Reid M, Badaloo A, Forrester T, Jahoor F. In vivo rates of erythrocyte glutathione synthesis in adults with sickle cell disease. Endocrinology and Metabolism: American Journal of Physiology. 2006;**291**:E73-E79. DOI: 10.1152/ajpendo.00287.2005

[65] Morris C, Suh J, Hagar W, Larkin S, Bland D, Steinberg M, et al. Erythrocyte glutamine depletion, altered redox environment, and pulmonary hypertension in sickle cell disease. Blood. 2008;**111**:402-410. DOI: 10.1182/blood-2007-04-081703

[66] Tatum VL, Chow CK. Antioxidant status and susceptibility of sickle erythrocytes to oxidative and osmotic stress. Free Radical Biology and Medicine. 1996;**25**:133-139. DOI: 10.3109/10715769609149918

[67] Chiu D, Lubin B. Abnormal vitamin E and glutathione peroxidase levels in sickle cell anemia: Evidence for increased susceptibility to lipid peroxidation in vivo. Journal of Laboratory and Clinical Medicine. 1979;**94**:542-548

[68] Jain SK, Williams DM. Reduced levels of plasma ascorbic acid (vitamin C) in sickle cell disease patients: Its possible role in the oxidant damage to sickle cells in vivo. Clinica Chimica Acta. 1985;**149**:257-261. DOI: 10.1016/0009-8981(85)90339-0

[69] Silva DG, Belini JE, Torres LS, Ricci JO, Lobo CC, Bonini-Domingos CR, et al. Relationship between oxidative stress, glutathione S-transferase polymorphisms and hydroxyurea

treatment in sickle cell anemia. Blood Cells Molecules and Diseases. 2011;**47**:23-28

[70] Klings ES, Farber HW. Role of free radicals in the pathogenesis of acute chest syndrome in sickle cell disease. Respiratory Research. 2001;**2**:280-285

[71] Jain SK, Shohet SB. Calcium potentiates the peroxidation of erythrocyte membrane lipids. Biochimica et Biophysica Acta. 1981;**642**:46-54. DOI: 10.1016/0005-2736(81)90136-x

[72] Wood KC, Granger DN. Sickle cell disease: Role of reactive oxygen and nitrogen metabolites. Clinical and Experimental Pharmacology and Physiology. 2007;**34**:926-932. DOI: 10.1111/j.1440-1681.2007.04639.x

[73] Repka T, Hebbel RP. Hydroxyl radical formation by sickle erythrocyte membranes: Role of pathologic iron deposits and cytoplasmic reducing agents. Blood. 1991;**78**:2753-2758. DOI: 10.1182/blood.V78.10.2753.2753

[74] Walter PB, Fung EB, Killilea DW, Jiang Q, Hudes M, Madden J, et al. Oxidative stress and inflammation in iron-overloaded patients with beta-thalassaemia or sickle cell disease. British Journal of Haematology. 2006;**135**:254-263. DOI: 10.1111/j.1365-2141.2006.06277.x

[75] Calderon-Salinas JV, Munoz-Reyes EG, Guerrero-Romero JF, Rodriguez-Moran M, Bracho-Riquelme RL, Carrera-Gracia MA, et al. Eryptosis and oxidative damage in type 2 diabetic mellitus patients with chronic kidney disease. Molecular and Cellular Biochemistry. 2011;**357**:171-179. DOI: 10.1007/s11010-011-0887-1

[76] Maellaro E, Leoncini S, Moretti D, Bello BD, Tanganelli I, De Felice C, et al. Erythrocyte caspase-3 activation and oxidative imbalance in erythrocytes and in plasma of type 2 diabetic patients. Acta Diabetologica. 2011;**50**:489-495. DOI: 10.1007/s00592-011-0274-0

[77] Das BS, Nanda NK. Evidence for erythrocyte lipid peroxidation in acute falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999;**93**:58-62. DOI: 10.1016/s0035-9203(99)90180-3

[78] Glenister FK, Coppel RL, Cowman AF, Mohandas N, Cooke BM. Contribution of parasite proteins to altered mechanical properties of malaria-infected red blood cells. Blood. 2002;**99**:1060-1063. DOI: 10.1182/blood.v99.3.1060

[79] Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli E, Yetkin O, et al. Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. European Archives Psychiatry and Clinical Neuroscience. 2004;**254**:143-147. DOI: 10.1007/s00406-004-0456-7

[80] Yorbik O, Sayal A, Akay C, Akbiyik D, Sohmen T. Investigation of antioxidant enzymes in children with autistic disorder. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2002;**67**:341-343. DOI: 10.1054/plef.2002.0439

[81] Altun H, Şahin N, Kurutaş EB, Karaaslan U, Sevgen FH, Fındıklı E. Assessment of malondialdehyde levels, superoxide dismutase, and catalase activity in children with autism spectrum disorders. Psychiatry and Clinical Psychopharmacology. 2018;**28**:408-415. DOI: 10.1080/24750573.2018.1470360

[82] Vos FE, Schollum JB, Coulter CV, Doyle TCA, Duffull SB, Walker RJ. Red blood cell survival in longterm dialysis patients. American Journal of Kidney

Diseases. 2011;**58**:591-598. DOI: 10.1053/j.ajkd.2011.03.031

[83] Nguyen-Khoa T, Massy ZA, de Bandt JP, Kebede M, Salama L, Lambrey G, et al. Oxidative stress and haemodialysis: Role of inflammation and duration of dialysis treatment. Nephrology Dialysis Transplantation. 2001;**16**:335-340

[84] Knap B, Prezelj M, Buturović-Ponikvar J, Ponikvar R, Bren AF. Antioxidant enzymes show adaptation to oxidative stress in athletes and increased stress in hemodialysis patients. Therapeutic Apheresis and Dialysis. 2009;**13**:300-305. DOI: 10.1111/j.1744-9987.2009.00728.x

[85] Shainkin-Kestenbaum R, Caruso C, Berlyne GM. Reduced superoxide dismutase activity in erythrocytes of dialysis patients: A possible factor in the etiology of uremic anemia. Nephron. 1990;**55**:251-253. DOI: 10.1159/000185970

[86] Gwozdzinski K, Pieniazek A, Gwozdzinski L. Reactive oxygen species and their involvement in red blood cell damage in chronic kidney disease. Oxidative Medicine and Cellular Longevity. 2021;**2021**:Article ID 6639199. DOI: 10.1155/2021/6639199

[87] Solas AB, Kutzsche S, Vinje M, Saugstad OD. Cerebral hypoxemia-ischemia and reoxygenation with 21% or 100% oxygen in new born piglets: Effects on extracellular levels of excitatory amino acids and microcirculation. Pediatric Critical Care Medicine. 2001;**2**:340-345. DOI: 10.1097/00130478-200110000-00011

[88] Dhinata KS. Common change of complete blood count parameters in COVID-19: A literature review. Journal of Medicine and Health. 2021;**3**:198-207. DOI: 10.28932/jmh.v3i2.3097

[89] Bartolini D, Stabile AM, Bastianelli S, Giustarini D, Pierucci S, Busti C, et al. SARS-CoV2 infection impairs the metabolism and redox function of cellular glutathione. Redox biology. 2021;**45**:102041. DOI: 10.1016/j.redox.2021.102041

[90] Kumar P, Osahon O, Vides DB, Hanania N, Minard CG, Sekhar RV. Severe glutathione deficiency, oxidative stress and oxidant damage in adults hospitalized with COVID-19: Implications for GlyNAC (glycine and N-acetylcysteine) supplementation. Antioxidants. 2021;**11**:50. DOI: 10.3390/antiox11010050

[91] Muhammad Y, Kani YA, Iliya S, Muhammad JB, Binji A, El-Fulaty Ahmad A, et al. Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: A cross-sectional comparative study in Jigawa. Northwestern Nigeria. SAGE open medicine. 2021;**9**:2050312121991246. DOI: 10.1177/2050312121991246

[92] Mahdi A, Collado A, Tengbom J, Jiao T, Wodaje T, Johansson N, et al. Erythrocytes induce vascular dysfunction in COVID-19. Basic to Translational Science. 2022;**7**:193-204. DOI: 10.1016/j.jacbts.2021.12.003

[93] Thomas T, Stefanoni D, Dzieciatkowska M, Issaian A, Nemkov T, Hill RC, et al. Evidence of structural protein damage and membrane lipid remodeling in red blood cells from COVID-19 patients. Journal of Proteome Research. 2020;**19**:4455-4469. DOI: 10.1021/acs.jproteome.0c00606

[94] Young IS, Woodside JV. Antioxidants in health and disease. Journal of Clinical Pathology. 2001;**54**:176-186. DOI: 10.1136/jcp.54.3.176

[95] Masella R, Benedetto R, Vary R, Filesi C, Giovannini C. Novel

mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione-related enzymes. The Journal of Nutritional Biochemistry. 2005;**16**:577-586. DOI: 10.1016/j.jnutbio.2005.05.013

[96] Nikolic-Kokic A, Blagojevic D, Spasic MB. Complexity of free radical metabolism in human erythrocytes. Journal of Medical Biochemistry. 2010;**29**:189-195. DOI: 10.2478/v10011-010-0018-7

[97] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiological Reviews. 1979;**59**:527-605. DOI: 10.1152/physrev.1979.59.3.527

[98] Spasi MB. Antioxidative defence in mammals—A review. Jugoslovenska medicinska biohemija. 1993;**12**:1-9

[99] Kellogg EW, Fridovich I. Liposome oxidation and erythrocyte lysis by enzymically generated superoxide and hydrogen peroxide. The Journal of Biological Chemistry. 1977;**252**:6721-6728. DOI: doi.org/10.1016/S0021-9258(17)39909-X

[100] Gaetani GF, Galiano S, Canepa L, Ferraris AM, Kirkman HN. Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood. 1989;**73**:334-339

[101] Zińczuk J, Maciejczyk M, Zaręba K, Pryczynicz A, Dymicka-Piekarska V, Kamińska J, et al. Pro-oxidant enzymes, redox balance and oxidative damage to proteins, lipids and DNA in colorectal cancer tissue. Is oxidative stress dependent on tumour budding and inflammatory infiltration? Cancers. 2020;**12**:1636. DOI: 10.3390/cancers12061636

[102] Day BJ. Catalase and glutathione peroxidase mimics. Biochemical Pharmacology. 2009;**77**:285-296. DOI: 10.1016/j.bcp.2008.09.029

[103] Maciejczyk M, Zalewska A, Gryciuk M, Hodun K, Czuba M, Płoszczyca K, et al. Effect of normobaric hypoxia on alterations in redox homeostasis, nitrosative stress, inflammation, and lysosomal function following acute physical exercise. Oxidative Medicine and Cellular Longevity. 2022;**2022**:4048543. DOI: 10.1155/2022/4048543

[104] Fowkes SW. Antioxidant intervention in Down's syndrome. Smart Drug News. 1996;**4**:1-12

[105] Faut M, Paiz A, Martin S, de Viale LC, Mazzetti MB. Alterations of the redox state, pentose pathway and glutathione metabolism in an acute porphyria model. Their impact on heme pathway. Experimental Biology and Medicine. 2013;**238**:133-143. DOI: 10.1177/1535370212473702

[106] Pandolfi PP, Sonati F, Rivi R, Mason P, Grosveld F, Luzzatto L. Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. EMBO Journal. 1995;**14**:5209-5215. DOI: 10.1002/j.1460-2075.1995.tb00205.x

[107] Hattangadi SM, Lodish HF. Regulation of erythrocyte lifespan: Do reactive oxygen species set the clock? The Journal of Clinical Investigation. 2007;**117**:2075-2077. DOI: 10.1172/JCI32559

[108] Fujino T, Tada T, Beppu M, Kikugawa K. Purification and characterization of a serine protease in erythrocyte cytosol that is adherent to

oxidized membranes and preferentially degrades proteins modified by oxidation and glycation. The Journal of Biochemistry. 1998;**124**:1077-1085. DOI: 10.1093/oxfordjournals.jbchem.a022224

[109] Dubuisson M, Vander Stricht D, Clippe A, Etienne F, Nauser T, Kissner R, et al. Human peroxiredoxin 5 is a peroxynitrite reductase. FEBS Letters. 2004;**571**:161-165. DOI: 10.1016/j.febslet.2004.06.080

[110] Song Y, Tang L, Han J, Gao Y, Tang B, Shao M, et al. Uric acid provides protective role in red blood cells by antioxidant defense: A hypothetical analysis. Oxidative Medicine and Cellular Longevity. 2019;**2019**: Article ID: 3435174. DOI: 10.1155/2019/3435174

[111] Rizvi SI, Jha R, Maurya PK. Erythrocyte plasma membrane redox system in human aging. Rejuvenation Research. 2006;**9**:470-474. DOI: 10.1089/rej.2006.9.470

[112] Nishida Y. Inhibition of lipid peroxidation by methylated analogues of uric acid. Journal of Pharmacy and Pharmacology. 1991;**43**:885-887. DOI: 10.1111/j.2042-7158.1991.tb03204.x

[113] Revin VV, Gromova NV, Revina ES, Samonova AY, Tychkov AY, Bochkareva SS, et al. The influence of oxidative stress and natural antioxidants on morphometric parameters of red blood cells, the hemoglobin oxygen binding capacity, and the activity of antioxidant enzymes. BioMed Research International. 2019;**2019**. Article ID 2109269. DOI: 10.1155/2019/2109269

[114] Pandey KB, Rizvi SI. Biomarkers of oxidative stress in red blood cells. Biomedical Papers. 2011;**155**:131-136. DOI: 10.5507/bp.2011.027

[115] Luqman S, Rizvi SI. Protection of lipid peroxidation and carbonyl formation in proteins by capsaicin in human erythrocytes subjected to oxidative stress. Phytotherapy Research. 2006;**20**:303-306. DOI: 10.1002/ptr.1861

[116] Grinberg L, Newmark H, Kitrossky N, Rahamim E, Chevion M, Rachmilewitz E. Protective effects of tea polyphenols against oxidative damage to red blood cells. Biochemical Pharmacology. 1997;**54**:973-978. DOI: 10.1016/s0006-2952(97)00155-x

[117] Cesquini M, Torsoni M, Stoppa G, Ogo S. T-BOOH-induced oxidative damage in sickle red blood cells and the role of flavonoids. Biomedicine & Pharmacotherapy. 2003;**57**:124-129. DOI: 10.1016/s0753-3322(03)00018-0

[118] Tedesco I, Russo M, Russo P, Iacomino G, Russo GL, Carraturo A, et al. Antioxidant effect of red wine polyphenols on red blood cells. The Journal of Nutritional Biochemistry. 2000;**11**:114-119. DOI: 10.1016/S0955-2863(99)00080-7

[119] Blasa M, Candiracci M, Accorsi A, Piacentini M, Piatti E. Honey flavonoids as protection agents against oxidative damage to human red blood cells. Food Chemistry. 2007;**104**:1635-1640. DOI: 10.1016/j.foodchem.2007.03.014

[120] Sangai NP, Patel CN, Pandya HA. Ameliorative effects of quercetin against bisphenol A-caused oxidative stress in human erythrocytes: An in vitro and in silico study. Toxicology Research. 2018;**7**:1091-1099. DOI: 10.1039/c8tx00105g

[121] Morabito R, Remigante A, Marino A. Melatonin protects band 3 protein in human erythrocytes against H2O2-induced oxidative stress.

Molecules. 2019;**24**:2741. DOI: 10.3390/molecules24152741

[122] Veiko AG, Sekowski S, Lapshina EA, Wilczewska AZ, Markiewicz KH, Zamaraeva M, et al. Flavonoids modulate liposomal membrane structure, regulate mitochondrial membrane permeability and prevent erythrocyte oxidative damage. Biochimica et Biophysica Acta (BBA)—Biomembranes. 2020;**1862**:183442. DOI: 10.1016/j.bbamem.2020.183442

[123] Anjum R, Maheshwari N, Mahmood R. 3,4-Dihydroxybenzaldehyde mitigates fluoride-induced cytotoxicity and oxidative damage in human RBC. Journal of Trace Elements in Medicine and Biology. 2022;**69**:126888. DOI: 10.1016/j.jtemb.2021.126888

[124] Singh N, Rajini PS. Antioxidant-mediated protective effect of potato peel extract in erythrocytes against oxidative damage. Chemico-biological Interactions. 2008;**173**:97-104. DOI: 10.1016/j.cbi.2008.03.008

[125] Lins PG, Pugine SM, Scatolini AM, de Melo MP. In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. Heliyon. 2018;**4**(9):e00805. DOI: 10.1016/j.heliyon.2018.e00805

[126] Ghosh AK, Mitra EL, Dutta MO, Mukherjee DE, Basu AN, Firdaus SB, et al. Protective effect of aqueous bark extract of Terminalia arjuna on Cu^{2+} – ascorbate induced oxidative stress in vitro: Involvement of antioxidant mechanism(s). Asian Journal of Pharmaceutical and Clinical Research. 2013;**6**:196-200

[127] Magalhães AS, Silva BM, Pereira JA, Andrade PB, Valentão P, Carvalho M. Protective effect of quince (Cydonia oblonga Miller) fruit against oxidative hemolysis of human erythrocytes. Food and Chemical Toxicology. 2009;**47**:1372-1377. DOI: 10.1016/j.fct.2009.03.017

[128] Suwalsky M, Orellana P, Avello M, Villena F. Protective effect of Ugni molinae Turcz against oxidative damage of human erythrocytes. Food and Chemical Toxicology. 2007;**45**:130-135. DOI: 10.1016/j.fct.2006.08.010

[129] Hammami N, Athmouni K, Lahmar I, Ben Abdallah F, Belghith K. Antioxidant potential of Salicornia arabica lipid extract and their protective effect against cadmium induced oxidative stress in erythrocytes isolated from rats. Journal of Food Measurement and Characterization. 2019;**13**:2705-2712. DOI: 10.1007/s11694-019-00191-8

[130] Castro RI, Forero-Doria O, Soto-Cerda L, Peña-Neira A, Guzmán L. Protective effect of pitao (Pitavia punctata (R. & P.) Molina) polyphenols against the red blood cells lipoperoxidation and the in vitro LDL oxidation. Evidence-Based Complementary and Alternative Medicine. 2018;**2018**:1049234. DOI: 10.1155/2018/1049234

[131] Jawaid SA, Jain S, Bhatnagar M, Purkayastha S, Ghosal S, Avasthi AS. Free radical scavenging and antioxidant impact of Indian medicinal plant extracts on H2O2 mediated oxidative stress on human erythrocytes. American Journal of Phytomedicine and Clinical Therapeutics. 2014;**2**:1052-1069