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Effect of curcumin extract against oxidative stress on both structure and deformation capability of red blood cell

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ABSTRACT

The normal deformability of erythrocytes plays an important role in ensuring blood mobility, erythrocyte longevity, and microcirculation, which is the ability of erythrocytes to change shapes in response to external forces. However, the effects of curcumin extracts on the deformability of erythrocytes have not yet been evaluated. Accordingly, in this study, we explored the effects of pre-treatment with curcumin extract on erythrocyte deformation and erythrocyte band 3 (SLC4A1; EB3) expression. We also evaluated the associations between EB3 expression and erythrocyte deformability induced by hydrogen peroxide. Blood samples were divided into the control group, pre-treatment group (treated with curcumin extract or vitamin C), and negative control group, and oxidant stress parameters, antioxidant status, erythrocyte deformability and elasticity, and EB3 modifications were evaluated using immunoblotting and immunofluorescence staining. Hydrogen peroxide significantly increased oxidative stress parameters, modulus elasticity values and clustered EB3 levels and induced conjugation of membrane proteins to form high-molecular-weight complexes ($p < 0.05$). Erythrocyte deformability and elasticity were significantly decreased in the treated groups compared with those in the control group. Overall, our findings suggested that pre-treatment with curcumin extracts increased antioxidant status, reduced EB3 cross-linking, and improved erythrocyte deformability, to an even better extent than vitamin C. These results provide important insights into the effects of treatment with curcumin extracts on erythrocyte damage and suggest that curcumin may have applications in antioxidant therapy.

1. Introduction

Reactive oxygen species (ROS) are a byproduct of cellular metabolism and are generated during oxidative metabolism or via interactions with exogenous sources, such as xenobiotic compounds (Kehrer, 2000; Pandey and Rizvi, 2010; Pham-Huy et al., 2008). The imbalance between toxic ROS and the antioxidant defense system can lead to oxidative stress (Sinha et al., 2015). Moreover, erythrocytes (red blood cells) are continuously exposed to ROS (Arashiki et al., 2013), resulting in conformational and functional changes and ultimately leading to erythrocyte aging and removal from circulation (Mohanty et al., 2014). Owing to the susceptibility of erythrocytes to oxidative reactions, these cells are often used as a cell model for analysis of oxidative damage to biofilms (Honzel D, et al., 2008), and hydrogen peroxide, as a strong oxidant, is often used in oxidative damage models. Erythrocyte deformability (ED) is an important factor, that modulates the efficacy of tissue perfusion under conditions of increased shear stress in the microvasculature (Olumuyiwa-Akeredolu et al., 2017). Slight changes in the structures of erythrocytes will lead to the alterations in ED, which are required for erythrocytes to change their shape in response to mechanical forces in fluid flow or while passing through microcirculation (Chien, 1987; Kim et al., 2015; Mokken et al., 1992). Moreover, mechanical factors also play important roles in maintaining the unique properties of erythrocytes (Cluitmans et al., 2012). Following the discovery of the clinical importance of ED and the mechanical properties of erythrocytes, several methods for measuring this phenomenon have been developed.

Antioxidants are needed to mitigate the effects of oxidative damage. Natural antioxidants include the water-soluble antioxidant vitamin C and the fat-soluble antioxidant vitamin E. The toxicity of harmful oxygen-free radicals can be eliminated by reduction and neutralization of hydroxyl, alkoxyl, and lipid peroxyl ($ROO^{\cdot}$) radicals (Lü et al., 2010), and antioxidants such as vitamin C can rapidly react with O_2^-, HOO^-, and OH^- to generate semidehydroascorbic acid, remove singlet oxygen, and reduce sulfur-free radicals. Moreover, vitamin C is often used as a reference for

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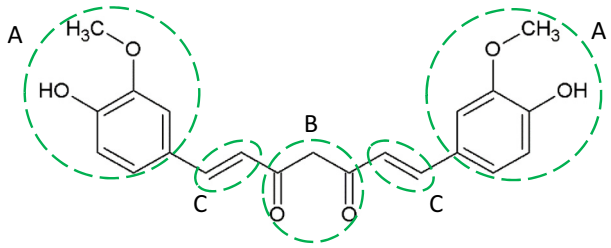

Fig. 1. The keto-form of curcumin (A) phenyl ring (B) β-diketone group (C) alkene.

identification and comparison of the free radical scavenging abilities of various drugs. Owing to its unique structure, curcumin is believed to be a strong antioxidant and has been used in traditional Chinese and Indian medicine (Hatcher et al., 2008; Jain et al., 2006). Curcumin contains a variety of functional groups, including the β-diketo group, carbon-carbon double bonds, and phenyl rings having various hydroxyl and methoxy substituents (Fig. 1) (Hatcher, et al., 2008; Menon et al., 2007; Wright, 2002).

Notably, treatment with either curcumin prevents reduction of the rate constant for $SO_4(=)$ uptake or protects -SH groups from oxidation (Morabito et al., 2015; Morabito et al., 2016). Additionally, oxidative stimulation of G proteins in the human brain membrane by metabolic per-oxidation, homocysteine, and hydrogen peroxide can be significantly decreased by curcumin via inhibition of lipid peroxidation in rat liver microsome preparations and rat brains (Jefremov, et al., 2007). Curcumin has also been shown to inhibit lipid peroxidation, and modulate superoxide anion radical, and hydrogen peroxide scavenging (Ak Tuba and Gulçin, 2008). Accordingly, we hypothesized that curcumin may maintain the redox balance in erythrocytes, which could prevent the development and progression of inflammation-related diseases and aging.

Accordingly, in order to clarify the responses of erythrocytes to natural antioxidant, we evaluated the antioxidant and free-radical scavenger properties of curcumin in erythrocytes.

2. Materials and methods

2.1. Ethics statement

Volunteers provided written informed consent to participate in the study. The research protocol was approved by Chongqing University and the Ethics Committee, and the study methodologies conformed to the standards set by the Declaration of Helsinki.

2.2. Preparation of blood samples

Venous blood samples were collected into anti-coagulation tubes containing heparin (15 IU/ml) from healthy adult men (18–30 years old). Blood samples were centrifuged at 2000 rpm, and 4 °C for 2.5 min, and the buffy coat and plasma were then removed. Erythrocytes were washed three times using phosphate-buffered saline (PBS, pH 7.4) with centrifugation at 2000 rpm for 2.5 min. Finally, erythrocytes were re-suspended in PBS (pH 7.4) to obtain human erythrocyte suspensions at different hematocrits (for assays or membrane cell extraction). All erythrocyte preparations, treatments, and measurements were carried out within 4–6 h after blood collection.

2.3. Establishment of erythrocytes model and screening of hydrogen peroxide concentration

2.3.1. Selection of the suitable hydrogen peroxide concentration

In order to evaluate the effects of curcumin extracts from H_2O_2 induced oxidative stress, hemolysis assays were conducted as previously reported with some modifications (Zhao et al., 2017). H_2O_2 was used as an oxidative agent because it is rapidly converted by the Fenton reaction into ·OH radical which is highly reactive (Pandey and Rizvi, 2010). First, erythrocyte samples were prepared, and cells were tested in the presence of three different concentrations of peroxide (0.5, 4, and 8 mM). All cells were incubated for 12 h at 37 °C, and morphological changes in erythrocytes, hemolysis ratios and methemoglobin (MetHb) levels were measured. Moreover, the erythrocyte elongation index (EI) was measured to determine the deformability of the cells. In this experiment, we also used vitamin C as a standard drug and compared the results with those of curcumin extracts.

2.3.2. Establishment of the erythrocyte model

Cells were divided into three groups: the normal group, the treatment group and the negative control group. For each group, erythrocyte suspensions (5% in PBS, 500 μL) were pre-incubated with 500 μL ethanol-DMSO in PBS. For the normal and negative control groups, cells were then incubated with 500 μL PBS, whereas for the treatment groups, cells were incubated with 500 μL curcumin extract or vitamin C at different concentrations (1, 10 and 20 μM) at 37 °C for 30 min. Subsequently, 500 μL PBS was added to the normal group, and H_2O_2 solutions (pH 7.4) were added to the treatment and negative control groups. Cells were then incubated at 37 °C for 12 h. After incubation, a batch of the reaction mixture (100 μL) was diluted with 400 μL PBS and centrifuged at 2000 rpm at 4 °C for 5 min. The absorbance (A) of the supernatant at 540 nm was measured. Another portion of the reaction mixture (100 μL) was treated with 400 μL of distilled water to obtain complete hemolysis and centrifuged at 2000 rpm at 4 °C for 5 min. The absorbance (A_0) of the supernatant at 540 nm was measured.

The hemolysis ratio was measured using the following equation (Manna et al., 1999; Zhao et al., 2017)

$$Hemolysis\ \ Ratio = \frac{A}{A0} \times 100\%$$

2.4. Determination of oxidative stress in erythrocytes

Next, determination of Met-Hb was performed at 630 nm to evaluate the Met-Hb value in erythrocytes after exposure to oxidative stress. The activities of antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), and the levels of glutathione peroxidase (GPx) were measured to evaluate intracellular enzyme levels. The lipid peroxidation of erythrocyte membranes was estimated by measuring the levels of malondialdehyde (MDA), the secondary product of lipid peroxidation, according to the thiobarbituric acid (TBA) method. TBA reactive-substance levels were estimated by measuring the absorbance at 532 nm. All parameters were measured using kits from Nanjing Jian Cheng Bioengineering Institute (China, http://www.njjcbio.com/product.asp) according to the manufacturer's instructions.

2.5. Measurement of ED

To elucidate changes in the ED after exposure to oxidative stress, the elongation indexes of the cells were quantitatively measured at different shear rates (0.3 or 30 Pa), using a laser-assisted optical rotational cell deformability analyzer (Lorrca Maxsis, the Netherlands). Briefly, after treatment, the erythrocytes were washed three times using PBS, completely dissolved in a polyvinylpyrrolidone solution (pH 7.4), and subjected to analysis using the laser-assisted rotational cell deformability analyzer, which applies coquette geometry with a static bob and a rotating cylinder (cup) to create a simple shear flow, changes in erythrocyte

shapes and orientations can then be determined by measuring the diffraction of the laser light passing through the thin layer of blood suspension. The erythrocytes elongation index (EI) was calculated according to the following equation:

$$EI = \frac{(L - W)}{(L + W)}$$

where L and W represent the length and width of the diffraction pattern ellipse, respectively. Increased EI values indicate greater ED (Tang et al., 2014; Xiong et al., 2013).

2.6. Measurement of the Young's modulus of erythrocytes

Next, we further explored the correlations of biomechanical properties with changes in cytoskeletal membrane in erythrocytes after oxidative stress by measuring, the Young's modulus using an atomic force microscope (AFM). First, the surface of the glass slide was treated with a poly-L-lysine solution to enhance cell adhesion. Erythrocyte stiffness measurements were carried out by AFM in the contact mode, and a soft V-shaped silicon nitride cantilever with a nominal spring constant of 0.03 N/m was used. A pyramid probe (TR400PSA; Olympus, Japan) with a side-angle of 17.51 was attached to the cantilever tip, and measurements were recorded at the center of the cell with a tip scanning frequency of 1.0 Hz. During testing, the probe slowly approached and contacted the cell surface, and was then retracted; Next, the force-distance curves were obtained by recording the cantilever deflection and displacement of the probe driver during the approach-retract cycles. The force-distance curve is linear before the probe contacts the cell, and then the curve starts to climb from zero with increasing curvature. Non-linear force-distance curve reflects the mechanical properties of the cell. The Young's modulus of the cells was computed using the Hertz model (Radmacher et al., 1996). According to this model, the relationship between the applied force, F, and the indentation depth, σ, can be expressed as:

$$F = \sigma^2 \frac{\pi E}{2(1 - \upsilon^2)} \tan(\alpha)$$

where E is the elastic or Young's modulus, v is the Poisson ratio (assumed to be 0.5) and α is the opening angle of the probe. Young's modulus can be calculated from this expression. In our experiments, ten erythrocytes were randomly selected from each group, and individual erythrocytes were measured five times. All measurements were performed at 25 °C and the erythrocytes were completely immersed in PBS.

2.7. Electrophoresis and immunoblotting analyses of membranes

Sodium dodecyl sulfate-polyacrylamide gels electrophoresis (SDS-PAGE) and immunoblotting were conducted to explore protein clustering and to quantify the expression of EB3. Erythrocytes were incubated with cold lysis buffer mixture (containing 10 mM Tris, 1 mM $MgCl_2$, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1 mM ethylene-diamine-tetraacetic acid at 4 °C, pH 8.0) for 1 h. Erythrocyte membrane proteins were collected by centrifugation at 12,000 rpm for 12 min. Membranes were washed with lysis buffer, and the protein content of the membranes was then quantified using Bradford protein assays. Proteins were solubilized in Laemmli buffer in the absence or presence of 10 mM DL-dithiothreitol(DTT) at a volume ratio of 4:1. SDS-PAGE was conducted by heating the samples of 8 min at 100 °C, separating proteins on 8% polyacrylamide gels, and staining protein bands with colloidal Coomassie Blue.

For western blot analyses, the proteins were transferred to polyvinylidene fluoride membranes, immunostained with primary antibodies (rabbit polyclonal anti-EB3 antibodies, Abcam, UK) or (mouse polyclonal anti-β-actin antibodies, OriGene, USA), incubated with anti-rabbit and anti-mouse secondary antibodies, and detected by chemiluminescence (Thermo Scientific, USA, Thermo Fisher Scientific - CN https://www.thermofisher.com/cn/zh/home.html). Quantification of Coomassie Blue-stained gels and electrochemiluminescence-developed immunoblots was conducted by lengthwise scanning densitometry using a gel analyzer image-processing program (Azure Biosystem, USA).

2.8. Immunofluorescence and image analyses of EB3

To detect the cross-linking phenomenon of EB3 immunofluorescence staining method was conducted. Cells were fixed with 4% paraformaldehyde and 0.05% glutaraldehyde in PBS and permeabilized in the same solution containing 0.05% Triton X-100. After blocking with 5% bovine serum albumin (BSA) and 0.1% Tween 20 in PBS to block nonspecific protein binding, cells were treated with rabbit polyclonal anti- EB3 antibodies (Abcam, UK) at a ratio of 1:200 for 2 h at room temperature. Erythrocytes were washed at 3 min intervals three times with gentle shaking, incubated for 2 h with secondary antibodies (anti-rabbit Ig G-conjugated to fluorescein isothiocyanate) at a 1:200 dilution in 2% BSA in PBS, and then washed three times in PBS. Fluorescence was imaged using an Olympus IX71 microscope with a 63/1.25 oil immersion objective and equipped with a CCD camera (Olympus, Japan).

2.9. Reagents

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1E,6E-hepta diene-3,5-dione) molecular weight = 368.4 Da, "analytical standard" purity grade was purchased from Sigma-Aldrich (USA). L-ascorbic acid was purchased from Tianjin Fuchen Co. (China), and 30% H_2O_2 was purchased from KESHI (China).

2.10. Statistical analyses

Statistical analyses of the data were performed using Origin 8.5 Pro, (GraphPad Prism 5, USA). Data were analyzed by a multi-way analysis of variances (ANOVA). Data are presented as the mean ± standard deviation unless described otherwise. Differences with p values of less than 0.05 were considered significant.

3. Results

3.1. Optimal concentrations of hydrogen peroxide for induction of oxidative damage

After treatment with increasing concentrations of H_2O_2 (0.5–8 mM), hemolysis occurred and morphological changes in erythrocytes were observed. Acanthocytes (erythrocytes having acicular surfaces with irregular spacing and ridges of different in lengths and widths) were detected (Fig. 2A). Moreover, treatment with hydrogen peroxide increased the hemolysis ratio in a concentration--dependent manner. The group treated with 8 mM H_2O_2 showed significant increases in the hemolysis ratio and MetHb values ($p < 0.001$; Fig. 2B and C). Increasing the concentration of H_2O_2 also decreased the EI in all treated groups. However, only cells treated with 4 and 8 mM H_2O_2 showed significance decreases in EI at 0.3–30.0 Pa shear stress compared with the control group ($p < 0.001$; Fig. 2D).

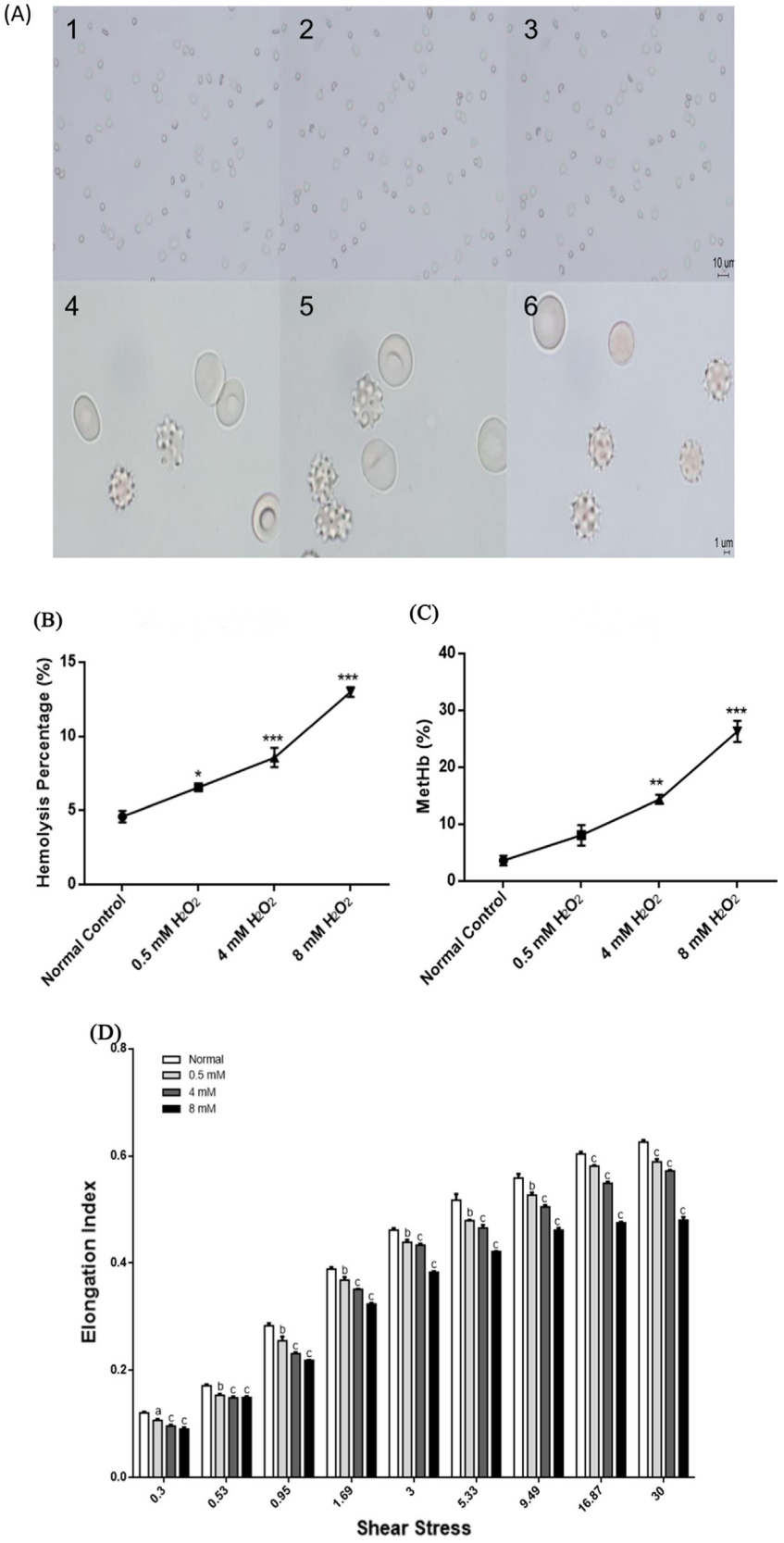

Fig. 2. Effects of hydrogen peroxide concentration on erythrocytes morphology, hemolysis rate, hemagglutinin-hemoglobin level and erythrocytes elongation index. (A) Morphology of erythrocytes after treatment of H_2O_2, (1)–(3) Different concentrations of hydrogen peroxide in20X magnification; (4)–(6) Different concentrations of hydrogen peroxide in100X magnification. (B) Measurement of Hemolysis Ratio. (C) Met-Hemoglobin Measurement. (D) Elongation Index of different concentration of H_2O_2, [a] significant differences ($p < 0.05$) between normal and tested group; [b] significant differences ($p < 0.01$) between normal and tested group; [c] significant differences ($p < 0.001$) between normal and tested group); (data is represented as mean ± standard deviation with n = 6,* $p < 0.05$; ** $p < 0.01$*** $p < 0.001$;).

3.2. Antioxidant status and oxidant stress parameters in erythrocytes

To determine the effects of pre-treatment with curcumin extracts on oxidative stress parameters in erythrocytes, we measured antioxidant status, hemolysis ratio, and MetHb, CAT, SOD, GPx, and MDA values (Fig. 3 A-F). Cells treated with H_2O_2 showed increased oxidative stress parameters and decreased antioxidant status, suggesting that hydrogen peroxide could cause severe oxidative damage to erythrocytes. H_2O_2 not only increased MDA levels and hemolysis rate, but also damaged cellular defense and

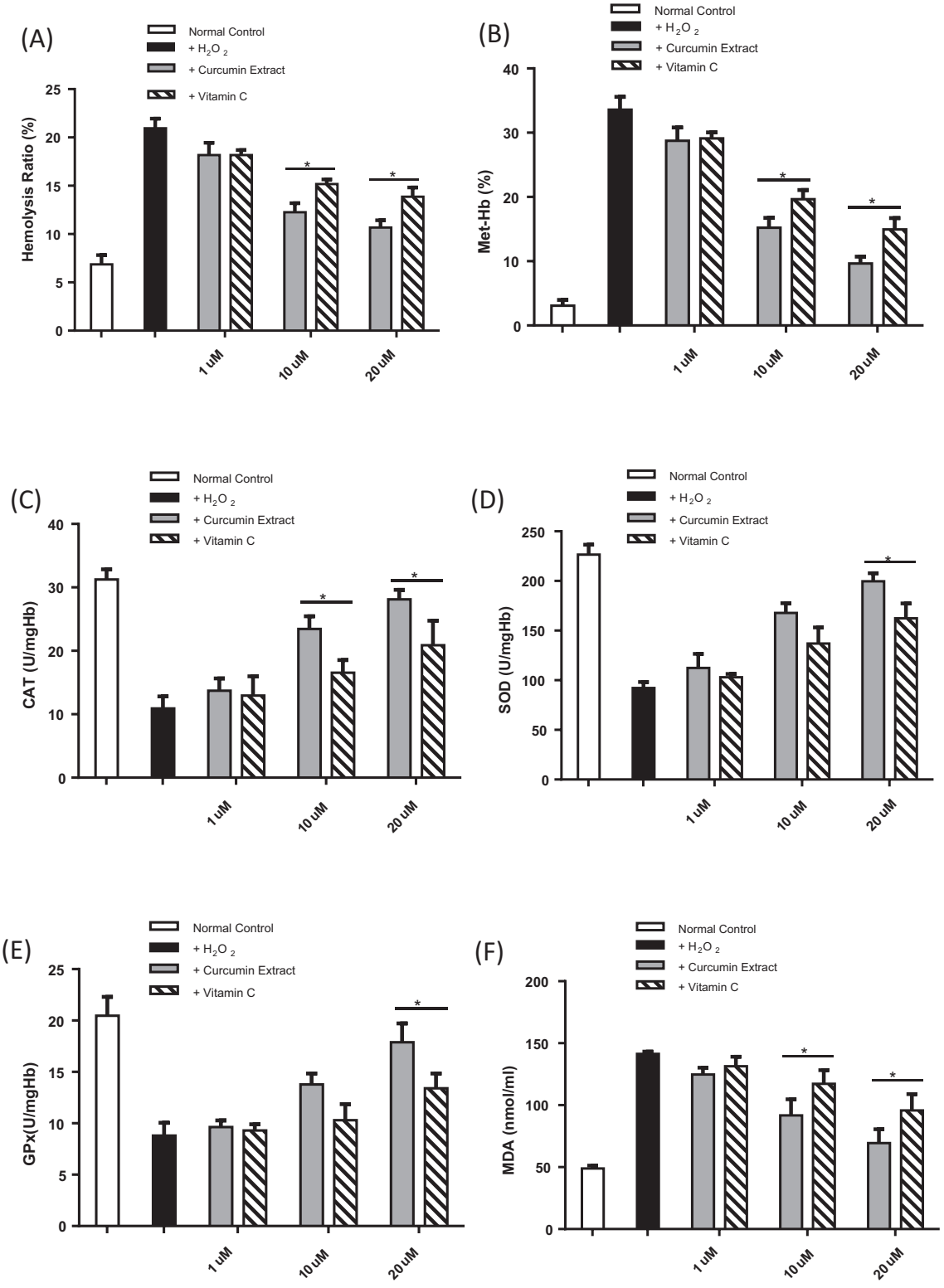

Fig. 3. Antioxidant status and oxidant stress parameters in erythrocytes (A) Hemolysis ratio. (B) Met-Hb content. (C) CAT. (D) SOD. (E) GPx and (F) MDA level of each treated group with or without curcumin extract at different concentration against H_2O_2 induced oxidative stress. (* significant differences ($p < 0.05$) between the group which added curcumin and vitamin C; data is represented as a mean ± standard deviation with n = 6).

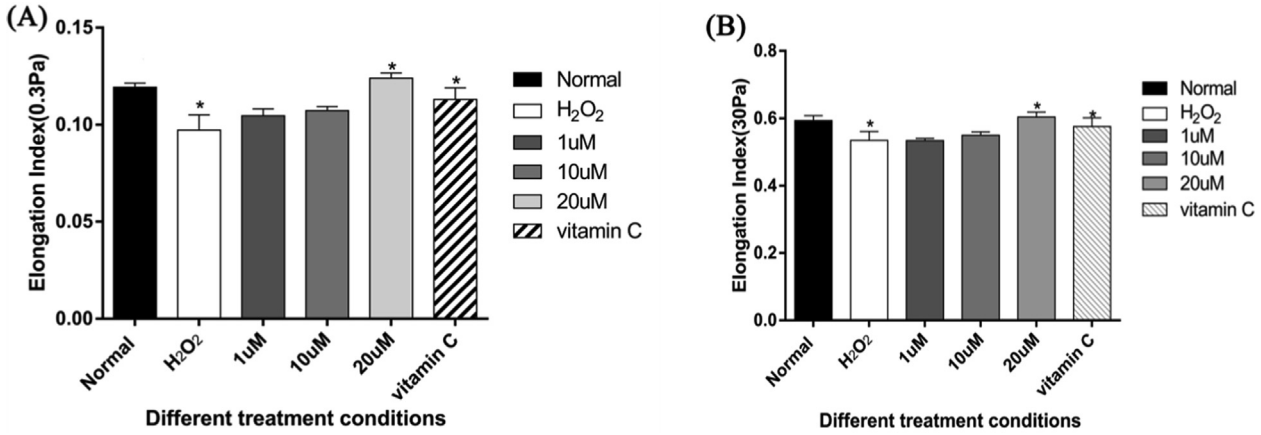

Fig. 4. The erythrocytes EI value at a shear stress of 0.3 Pa and 30 Pa with or without of curcumin extract after oxidative stress (Data is represented as a mean ± standard deviation with n = 6):Significnt different of normal control group compared with H_2O_2 group: *p < 0.05: Normal control group compared with Vitamin C group: *p < 0.05:20 μM curcumin group compared with normal control group: *p < 0.05: 20 μM curcumin group compared with Vitamin C group:*p < 0.05.

clearance functions, resulting in decreased levels of SOD, CAT and Gpx Pre-treatment with curcumin extracts (1, 10 and 20 μM) significantly decreased the hemolysis ratio and MetHb values compared with those in vitamin C-treated cells($p < 0.05$). Significant differences in CAT, SOD, and GPx levels were also observed between cells treated with curcumin extracts and cells treated with vitamin C ($p < 0.05$). Moreover, MDA levels were also significantly decreased in curcumin-treated cells compared with that in vitamin C-treated cells ($p < 0.05$).

3.3. Erythrocytes deformability at various shear stresses and Young's modulus values

In the H_2O_2-treated group, EI values of erythrocytes were significantly decreased a wider range of shear stresses (Fig. 4; $p < 0.05$ compared with control group). The group treated with curcumin extract or vitamin C showed increased EI values at low shear stress (0.3 Pa; Fig. 4A) and high shear stress(30 Pa, Fig. 4B). However, only 20 μM curcumin extract significantly increased the EI value compared with that in the H_2O_2-treated group (0.5713 ± 0.012 at 30 Pa; $p < 0.05$).

Table 1 describes the Young's modulus values of erythrocytes in each group. An obvious increase in the Young's modulus value was observed in the H_2O_2-treated group compared with that in the control group. In contrast, pre-treatment with curcumin (10, 20 μM) or vitamin C significantly decreased the Young's modulus compared with that in the H_2O_2 -treated group ($p < 0.05$).

3.4. Modifications of membrane protein structure

Erythrocytes lack nuclei and other organelles, and do not exhibit protein synthesis. Therefore, alterations in pre-existing proteins could result in recognizable changes in the membrane surface. In this work, we hypothesized that oxidative damage may lead to variations in the protein profiles of erythrocyte membranes. SDS–PAGE analyses of erythrocyte membrane proteins (Fig. 5) showed that a new high-molecular-weight (HMW) band appeared. However, the expression of the HMW band decreased significantly after incubation with DTT.

Table 1
The Young's modulus value of erythrocytes from each group.

Group	Young's Modulus (KPa)
Normal control	0.6476 ± 0.2290
+ H_2O_2	1.943 ± 0.4016
+ 1 μM curcumin extract	1.615 ± 0.2906
+ 10 μM curcumin extract	1.307 ± 0.2462***
+ 20 μM curcumin extract	1.102 ± 0.3340***
Vitamin C	1.334 ± 0.3799**

Note: Data is represented as a mean ± standard deviation, with n = 10.
** $p < 0.01$.
*** $p < 0.001$ compared with + H_2O_2 group.

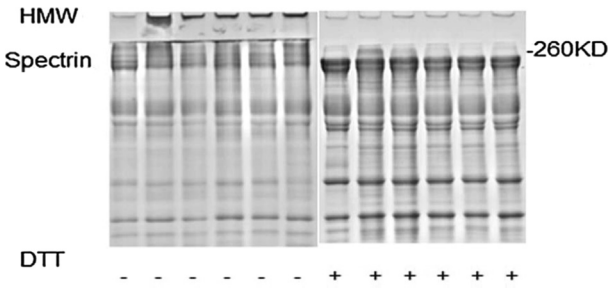

Fig. 5. SDS-PAGE analyses of erythrocytes membrane proteins in different groups. (1) Control (2) + H_2O_2 (3) + 1 μM curcumin extract (4) + 10 μM curcumin extract (5) + 20 μM curcumin extract (6) + Vitamin C(20 μM).

EB3 immunoblotting is shown in Fig. 6 A and C. Densitometric analyses showed differences in EB3 protein at 90–100 kDa and its aggregate products at more than 250 kDa (cluster band 3; Fig. 6C). For the HMW band, the EB3 aggregate product was reduced back to a 95 kDa band after incubation with DTT (Fig. 6A). Subsequent analyses of EB3 by immunofluorescence staining (Fig. 6E-2) verified the presence of EB3 aggregates in a small proportion of cells formed after oxidative stress. However, pre-treatment with curcumin extract decreased the number of echinocytes and visible aggregates (Fig. 6E-4; 5; and 6). Pre-treatment with vitamin C also resulted in similar improvements in erythrocytes, although irregularly-shaped erythrocytes were still observed.

4. Discussion

In the current study, we reported the effects of pre-treatment with curcumin extracts on erythrocyte antioxidant status, ED (membrane deformability and elasticity), and EB3 expression. Our results showed that curcumin extracts increased antioxidant capacity, helped to maintain EB3 normal structures, and improved ED performance to mitigate oxidative injury.

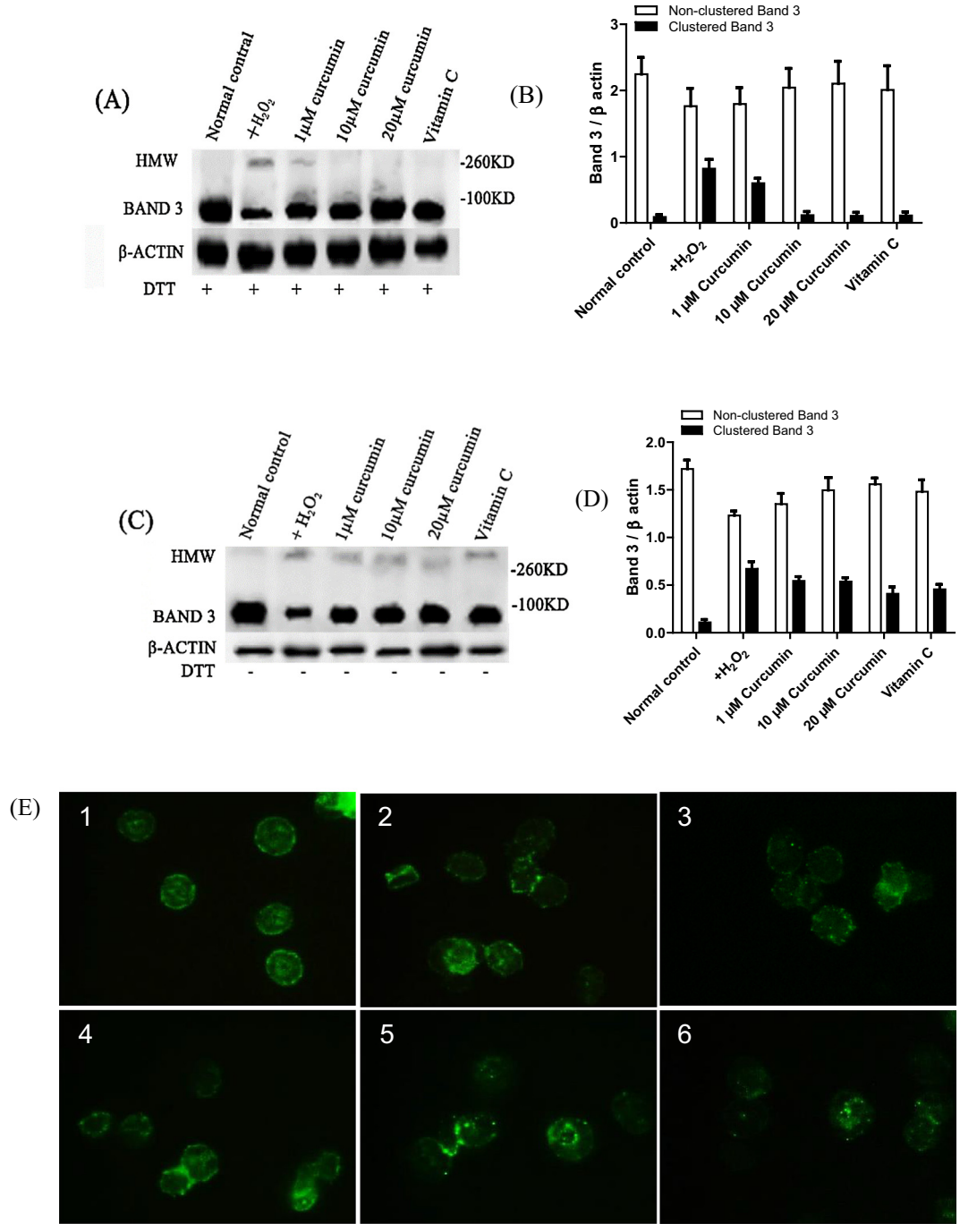

Fig. 6. Curcumin extract improved the erythrocyte membrane protein band 3 clusters. (A) Immunoblot of Band 3 protein (B) Quantification of band 3 cluster immunoblot density, normalized against β-actin in the presence of DTT. (C) In the absence of curcumin (D) Quantification of band 3 cluster immunoblot density, normalized against β-actin in the absence of DTT. (E) Immunofluorescence scanning of erythrocyte membrane protein band 3 clusters. (E-1) Normal; (E-2) + H_2O_2; (E-3) + Vitamin C; (E-4) + 1 μM curcumin extract; (E-5) + 10 μM curcumin extract; and (E-6) + 20 μM curcumin extract.

ED is important to facilitate the passage of erythrocytes through the microcirculation (Chien, 1987). However, our findings showed that oxidative stress caused some alterations in the erythrocyte membranes, most likely increasing membrane rigidity and altering in the organization of membrane phospholipids, which may affect the ability of erythrocytes to deform and the circulate. H_2O_2, a water-soluble oxidant, could quickly permeate the membrane and partition into the cytosol, and further causing lipid membrane oxidation and decreasing membrane fluidity (Hale et al., 2011). After such exposure to H_2O_2, membrane protein of erythrocytes may form a skeletal protein complex that could decrease in ED and result in morphological and surface changes to the erythrocytes (Fortier et al., 1988; Snyder et al., 1985).

Young's modulus, a measure of elasticity, is a physical quantity used in the biological field to characterize the surface properties of a cell or material, determined solely by the material or cell surface properties. The value of Young's modulus in cells reflects the degree of rigidity of the cell surface. Larger Young's modulus values indicate greater cell hardness and weaker ability to deform and carry oxygen to various parts of the body (Li et al., 2012; Lekka et al., 2012; Tang et al., 2014). Moreover, Young's modulus values can be altered by changing the affinity with membrane stent bind-

ing, and the value obtained in this process plays an important role in ED (Barns, 2017). In a recent study, deformability was found to be improved by treatment with curcumin extract or vitamin C with curcumin effectively preventing lipid peroxidation, at the cellular level (Cohly et al., 1998). Clearly, in our experiment curcumin extracts showed improved results compared with vitamin C. Thus, pre-treatment with curcumin could counteract lipid peroxidation to enhance membrane fluidity and facilitate the maintenance of erythrocyte elasticity.

Curcuminoid supplementation may prevent membrane dysfunction in human erythrocytes owing to hyperglycemia-induced oxidative conditions (Yang et al., 2015). EB3 is has crucial structural roles in facilitating anion transport on the erythrocyte membrane and as an important binding site for cytoskeletal and erythrocyte proteins (Bruce et al., 2003; Kodippili et al., 2012; Pretorius et al., 2016). In the current study, our results further confirmed the protective roles of curcumin in inhibiting the formation of HMW protein. When oxidative stress has overcome the antioxidant defenses of erythrocytes, MetHb further produces ROS through the Fenton and Haber-Weiss reactions (Kehrer, 2000), and the generated hydroxyl radicals can directly attack the main chain of EB3, leading to its fragmentation (Rucci et al., 2010). Based on the literature, there are several possible explanations for this phenomenon. First, this protein complex is formed during hydrogen peroxide-induced oxidative stress owing to the binding of MetHb to the cytoplasmic domain of EB3 or the attachment site of ankyrin from its cytoplasmic domain of EB3. This then induced conformational changes, free crosslinking, and EB3 clustering (Arashiki et al., 2013; Ferru et al., 2011). Alternatively, the formation of HMW may result in attack of free thiol groups of membrane proteins by ROS, resulting in the formation of -S-S- covalent crosslinks and protein aggregation. This phenomenon may occur in membrane EB3 through the formation of reducible inter- and/or intramolecular disulfide bonds. Combined with our previous findings, we suggest that EB3 may be impaired by hydrogen peroxide-induced oxidative stress (Xiong, et al., 2013). Therefore, antioxidant therapy is crucial.

In this study, we found that curcumin was an effective scavenger of ROS in vitro and may function indirectly as an antioxidant via the activity of inflammatory enzymes or by enhancing glutathione synthesis (Henrotin et al., 2010). Notably, the antioxidant activity of curcumin may be related to the phenolic OH group (Barclay et al, 2000; Priyadarsini et al, 2003). However, curcumin has also been reported to act as an H atom donor by donating H atoms from the central methylenic group rather than phenolic group (Jovanovic and collaborator, 1999). Additionally, most studies have suggested that the hydrogen atom transfer mechanism of curcumin protect the membrane -SH groups from oxidation (Litwinienko and Ingold, 2004; Morabito et al., 2015). However, the mechanisms through which curcumin exert antioxidant effects are still unclear.

5. Conclusion

Our current findings provided insights into the effects of oxidative on the structural characteristics and deformability of erythrocytes. Preventing erythrocyte damage maintaining the biological and mechanical properties of erythrocytes, elucidating the link between oxidative damage and EB3 expression are essential for improving outcomes in circulation-related diseases. Therefore, pre-treatment with curcumin extracts may have promising applications owing to the ability of this preparation to maintain the fluidity and rigidity of erythrocytes and therefore facilitating ED under the influence of mechanical force. This will allow the cells to pass through wide or narrow capillaries to supply oxygen to tissue.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

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References

Ak, T., Gulçin, I., 2008. Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 174, 27–37.
Arashiki, N., Naoki, K., Sumie, M., Narla, M., Yuichi, T., 2013. Membrane peroxidation, and methemoglobin formation are both necessary for band 3 clustering: mechanistic insights into human erythrocytes senescence. Biochemistry 52, 5760–5769.
Barclay, L.R.C., Vinqvist, M.R., Mukai, K., Goto, H., Hashimoto, Y., Tokunaga, A., Uno, H., 2000. On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant mechanism and activity. Org. Lett. 2, 2841–2843.
Barns, S., Balanant, M.A., Sauret, E., Flower, R., Saha, S., Gu, Y.G., 2017. Investigation of red blood cell mechanical properties using AFM indentation and coarse-grained particle method. Biomed. Eng. Online 16, 140.
Bruce, L.J., Beckmann, R., Ribeiro, M.L., Peters, L.L., Chasis, J.A., Delaunay, J., Mohandas, N., Anstee, D.J., Tanner, M.J., 2003. A band 3–based macro complex of integral and peripheral proteins in the RBC membrane. Blood 101, 4180–4188.
Chien, S., 1987. Red cell deformability and its relevance to blood flow. Ann Rev Physiol. 49, 177–192.
Cluitmans, J.C.A., Hardeman, M.R., Dinkla, S., Brock, R., Bosman, G.J., 2012. Red blood cell deformability during storage: towards functional proteomics and metabolomics in the Blood Bank. Blood Transfus. 10, s12–s18.
Cohly, H.H.P., Taylor, A., Angel, M.F., Salahudeen, A.K., 1998. Effect of turmeric, turmerin, and curcumin on H_2O_2-induced renal epithelial (LLC-pk1) cell injury. Free Radic. Biol. Med. 24, 49–54.
Fortier, N., Snyder, L.M., Garver, F., Kiefer, C., McKenney, J., Mohandas, N., 1988. The relationship between in vivo generated hemoglobin skeletal protein complex and increased red cell membrane rigidity. Blood 71, 1427–1431.
Ferru, E., Giger, K., Pantaleo, A., Campanella, E., Grey, J., Ritchie, K., Vono, R., Turrini, F., Low, P.S., 2011. Regulation of membrane-cytoskeletal interactions by tyrosine phosphorylation of erythrocytes band 3. Blood 117, 5998–6006.
Hale, J.P., Winlove, C.P., Petrov, P.G., 2011. Effect of hydroperoxides on red blood cell membrane mechanical properties. Biophys. J. 101, 1921–1929.
Hatcher, H., Planalp, R., Cho, J., Torti, F.M., Torti, S.V., 2008. Curcumin: from ancient medicine to current clinical trials. Cell. Mol. Life Sci. 65, 1631–1652.
Henrotin, Y., Clutterbuck, A.L., Allaway, D., Lodwig, E.M., Harris, P., Mathy-Hartert, M., Shakibaei, M., Mobasheri, A., 2010. Biological actions of curcumin on articular chondrocytes. Osteoarth Cartilage. 18, 141–149.
Honzel, D., Carter, S.G., Redman, K.A., Schauss, A.G., Endres, J.R., Jensen, G.S., 2008. Comparison of chemical and cell-based antioxidant methods for evaluation of foods and natural products: generating multifaceted data by parallel testing using. J. Agric. Food Chem. 56, 8319–8325.
Jain, S.K., Rains, J., Jones, K., 2006. Effect of curcumin on protein glycosylation, lipid peroxidation, and oxygen radical generation in human red blood cells exposed to high glucose levels. Free Radic. Biol. Med. 41, 92–96.
Jefremov, V., Zilmer, M., Zilmer, K., Bogdanovic, N., Karelson, E., 2007. Antioxidative effects of plant polyphenols: from protection of G protein signaling to prevention of age-related pathologies. N.Y. Acad. Sci. 1095, 449–456.
Jovanovic, S.V., Steenken, S., Boone, C.W., Simic, M.G., 1999. H-atom transfer is a preferred antioxidant mechanism of curcumin. J. Am. Chem. Soc. 121, 9677–9681.
Kehrer, J.P., 2000. The Haber-Weiss reaction and mechanism of toxicity. Toxicology 149, 43–50.
Kim, J., Lee, H., 2015. Advances in the measurement of red blood cell deformability: a brief review. J. Cell. Biotechnol. 1, 63–79.
Kodippili, G.C., Spector, J., Hale, J., Giger, K., Hughes, M.R., McNagny, K.M., Birkenmeier, C., Peters, L., Ritchie, K., Low, P.S., 2012. Analysis of the mobilities of band 3 populations associated with ankyrin protein and junctional complexes in intact murine erythrocytes. J. Biol. Chem. 287, 4129–4138.
Lekka, M., Gil, D., Pogoda, K., Dulinska-Litewka, J., Jach, R., Gostek, J., Klymenko, O., Prauzner-Bechcicki, S., Stachura, Z., Wiltowska-Zuber, J., Okon, K., Laidler, P., 2012. Cancer cell detection in tissue sections using AFM. Arch. Biochem. Biophys. 518, 151–156.
Li, M., Liu, L., Xi, N., Wang, Y., Dong, Z., Xiao, X., Zhang, W., 2012. Atomic force microscopy imaging and mechanical properties measurement of red blood cells and aggressive cancer cells. Sci. China Life Sci. 55, 968–973.

Litwinienko, G., Ingold, K.U., 2004. Abnormal solvent effects on hydrogen atom abstraction. 2. Resolution of thecurcumin antioxidant controversy. The role of sequential proton loss electron transfer. J. Org. Chem. 69, 5888–5896.
Lü, J., Lin, P., Yao, Q., Chen, C., 2010. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. J. Cell Mol. Med. 40, 840–860.
Manna, C., Galletti, P., Cucciolla, V., Montedoro, G., Zappia, V., 1999. Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. J. Nutr. Biochem. 10, 159–165.
Menon, V.P., Sudheer, A.R., 2007. Antioxidant and anti-inflammatory properties of curcumin. Adv. Exp. Med. Bio. 595, 105.
Mohanty, J.G., Nagababu, E., Rifkind, J.M., 2014. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front. Physiol. 5, 84.
Mokken, F.C., Kedaria, M., Henny, C.P., Hardeman, M.R., Gelb, A.W., 1992. The clinical importance of erythrocytes deformability, a hemorrheological parameter. Ann. Hematol. 64, 113–122.
Morabito, R., Falliti, G., Geraci, A., La Spada, G., Marino, A., 2015. Curcumin protects –SH groups and sulphate transport after oxidative damage in human erythrocytes. Cell. Physiol. Biochem. 36, 345–357.
Morabito, R., Romano, O., La spada, G., Marino, A., 2016. H_2O_2-induced oxidative stress affects SO_4= transport in human erythrocytes. PLoS ONE 11, e0146485.
Olumuyiwa-Akeredolu, O.O., Soma, P., Buys, A.V., Debusho, L.K., Pretorius, E., 2017. Characterizing pathology in erythrocytes using morphological and biophysical membrane properties: relation to impaired hemorheology and cardiovascular function in rheumatoid arthritis. Biomembranes 1859, 2381–2391.
Pandey, K.B., Rizvi, S.I., 2010. Marker of oxidative stress in erythrocytes and plasma during aging in humans. Oxidat. Med. Cell Longevity 3, 2–12.
Pham-Huy, L.A., He, H., Pham-Huy, C., 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 4, 89–96.
Pretorius, E., Olumuyiwa-Akeredolu, O.O., Mbotwe, S., Bester, J., 2016. Erythrocytes and their role as health indicator: Using structure in a patient-orientated precision medicine approach. Blood Rev. 30, 263–274.
Priyadarsini, K.I., Maity, D.K., Naik, G.H., Kumar, M.S., Unnikrishnan, M.K., Satav, J.G., Mohan, H., 2003. Role of phenolic OH and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radic. Biol. Med. 35, 475–484.
Radmacher, M., Fritz, M., Kacher, C.M., Cleveland, J.P., Hansma, P.K., 1996. Measuring the viscoelastic properties of human platelets with the atomic force microscope. Biophys. J. 70, 556–567.
Rucci, A., Ensinck, M.A., Mufarrege, N., Cotorruelo, C., Borrás, S.G., Racca, L., Biondi, C., Racca, A., 2010. Modifications of band 3 and oxidation level of membrane proteins in senescent erythrocytes. Clin. Biochem. 43, 1171–1173.
Sinha, A., Chu, T.T.T., Dao, M., Chandramohanadas, R., 2015. Single-cell evaluation of red blood cell bio-mechanical and nano-structural alterations upon chemically induced oxidative stress. Sci. Rep. 5, 9768.
Snyder, L.M., Fortier, N.L., Trainor, J., Jacobs, J., Leb, L., Lubin, B., Chiu, D., Shohet, S., Mohandas, N., 1985. Effect of hydrogen peroxide exposure on normal human erythrocytes deformability, morphology, surface characteristics, and spectrin-hemoglobin cross-linking. J. Clin. Invest. 76, 1971–1977.
Tang, F., Lei, X., Xiong, Y., Wang, R., Mao, J., Wang, X., 2014. Alteration Young's moduli by protein 4.1 phosphorylation play a potential role in the deformability development of vertebrate erythrocytes. J. Biomech. 47, 3400–3407.
Wright, J.S., 2002. Predicting the antioxidant activity of curcumin and curcuminoids. J. Mol. Struct. 591, 207–217.
Xiong, Y., Li, Y., Xiong, Y., Zhao, Y., Tang, F., Wang, X., 2013. Cluster of erythrocytes band 3: a potential molecular target of exhaustive exercise-induced dysfunction of erythrocytes deformability. Can. J. Physiol. Pharmacol. 91, 1–8.
Yang, W., Fu, J., Yua, M., Wang, D., Rong, Y., Yao, P., Nüsslerd, A.K., Yan, H., Liu, L., 2015. Effects of three kinds of curcuminoids on anti-oxidative system and membrane deformation of human peripheral blood erythrocytes in high glucose levels. Cell. Physiol. Biochem. 35, 789–802.
Zhao, M., Yang, Q., Lin, L., Sun, B., Wang, Y., 2017. Intracellular antioxidant activities of selected cereal phenolic extracts and mechanisms underlying the protective effects of adlay phenolic extracts on H_2O_2-induced oxidative stress in human erythrocytes. J. Funct. Foods 31, 160–171.