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## Prospects of curcumin as an additive in storage solutions: a study on erythrocytes

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**Background/aim:** Curcumin, a naturally occurring antioxidant, shows a wide variety of medicinal properties. The possibility of utilizing curcumin as an additive in storage solutions of blood has not been explored. The purpose of this study was to analyze the effect of curcumin on erythrocytes during storage.

**Materials and methods:** Blood obtained from rats was stored (4 °C) for 20 days in citrate-phosphate-dextrose-adenine-1 solution. Samples were divided into four groups: 1) Controls; 2) Curcumin 10 mM; 3) Curcumin 30 mM; and 4) Curcumin 60 mM. Every fifth day, hemoglobin, superoxide, antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase (GSH-Px)), lipid peroxidation (conjugate dienes and malondialdehyde (MDA)), protein oxidation (advanced oxidation protein products (AOPP) and sulfhydryls (P-SH)), and hemolysis were analyzed.

**Results:** Hemoglobin was successfully maintained, while superoxide dismutase increased initially and decreased towards the end of storage. Superoxide, catalase, GSH-Px, conjugate dienes, and AOPP were lower in the curcumin groups than they were in the controls. MDA was higher in the curcumin groups than in the controls. P-SH increased in the curcumin groups, while hemolysis increased in all groups.

**Conclusion:** Curcumin maintained hemoglobin and modulated antioxidant enzymes throughout storage. However, curcumin could not protect all proteins and lipids from oxidative damage completely. This study opens up new avenues for using curcumin, in combination with other antioxidants, as a component in storage solutions.

**Key words:** Erythrocytes, storage, curcumin, antioxidant, lipid peroxidation, protein oxidation

### 1. Introduction

Different extracts and compounds from the *Curcuma longa* plant have been used extensively as culinary and medicinal ingredients. The rhizomes of these plants contain turmerin, essential oils, and curcuminoids (1). Curcuminoids are a group of phenolic compounds, constituting about 5% of the turmeric rhizome (2) such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin (3). Curcumin (diferuloylmethane) is the principle coloring agent (4), imparting an orange yellow color (5). The yellow color is attributed to its unique conjugated structure, including two methoxylated phenols and the enol form of a heptadiene-3, 5-diketone linking the two phenols (6). Curcumin has been shown to exhibit a wide plethora of properties, ranging from antioxidant to anticarcinogenic (7–11). The therapeutic effects of curcumin are due to its strong antioxidant property (6), which is in turn due to its unique structure (12). Curcumin has been found to be at least ten times more potent than Vitamin E (13). Curcumin is a powerful free radical scavenger (hydrogen

peroxide, singlet oxygen, hydroxyl, superoxide, peroxy, peroxynitrite, and nitrite radicals) (14–22) and neutralizes 2,2-diphenyl-1-picrylhydrazyl (20,22–24). Curcumin has both antioxidant and prooxidant effects in oxygen radical reactions, acting as a scavenger of hydroxyl radicals or a catalyst in the formation of hydroxyl radicals, depending on the experimental conditions (19,25–27). It protects oxyhemoglobin from nitrite-induced oxidation (7,10). Curcumin is an inhibitor of lipid peroxidation (10,28–32), reducing oxidative damage through its capacity to efficiently scavenge lipid peroxy radicals, thereby protecting membrane lipids (2,33). Curcumin increases intracellular glutathione (GSH) and regulates antioxidant enzymes (29,31).

In rat erythrocytes, curcumin inhibited reactive oxygen species (ROS) generation (25) and decreased osmotic fragility (34) and hemolysis (22). Banerjee et al. (35) studied the effects of curcumin on AAPH-induced hemolysis of red blood cells (RBCs), showing a concentration-dependent decrease in TBARS and

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hemolysis on treatment with curcumin. However, the effects of curcumin on storage-induced oxidative stress in erythrocytes have not been reported.

Hence, this study elucidates the action of curcumin on erythrocytes of stored blood by: 1) analyzing oxidant levels through hemoglobin (Hb), hemolysis, lipid peroxidation, and protein oxidation; and 2) determining antioxidant status through antioxidant enzymes-superoxide dismutase, catalase (CAT), and glutathione peroxidase (GSH-Px).

## 2. Materials and methods

Animal care and maintenance was in accordance with ethical committee regulations.

### 2.1. Chemicals

Hb reagent was obtained from Coral Clinical Systems. Thiobarbituric acid, epinephrine, glutathione reductase, GSH, curcumin, and bovine serum albumin were purchased from Sigma-Aldrich Chemicals. All other chemicals used were of reagent grade and organic solvents were of spectral grade.

### 2.2. Blood sampling

Animals were lightly anaesthetized with ether and restrained in dorsal recumbency as described earlier (36). In brief, the syringe needle was inserted just below the xiphoid cartilage and slightly to the left of midline. Blood was carefully aspirated from the heart into plastic collecting tubes with citrate-phosphate-dextrose-adenine-1 solution.

### 2.3. Experimental design

Blood was drawn from Wistar rats (4 months old) and stored over a period of time (15 days) at 4 °C in citrate-phosphate-dextrose-adenine-1 solution. Blood was collected from 20 animals and divided into the following four groups (with 5 animals in each group): 1) control group; 2) Cur 10-samples with curcumin as additive at a concentration of 10 mM; 3) Cur 30-samples with curcumin as additive at a concentration of 30 mM; and 4) Cur 60-samples with curcumin as additive at a concentration of 60 mM. Erythrocytes were isolated from stored blood at regular intervals (every fifth day) and the biomarkers of oxidative stress (OS) were studied.

### 2.4. Erythrocyte separation

Erythrocytes were isolated by centrifugation for 20 min at 1000 g at 4 °C. Plasma and buffy coat were removed using a micropipette. The cell pellet was washed three times with isotonic phosphate buffer, pH 7.4, centrifuged at 1000 g for 10 min, and finally suspended in an equal volume of isotonic phosphate buffer (37). This constituted the erythrocyte suspension.

### 2.5. Hb estimation

Hb was measured using Hemocor-D Kit (Coral Clinical Systems), which utilizes the cyanmethemoglobin method (38). Whole blood was incubated with Hb reagent for 3

min at room temperature and absorbance was measured at 540 nm. Hb concentration was represented in terms of g dL<sup>-1</sup>.

### 2.6. Superoxide

The superoxide generated in the samples was determined by the method of Olas and Wachowisz (39). Cytochrome c (160 μM) was added to equal volume of sample and incubated at 37 °C for 5 to 15 min. The samples were then centrifuged at 3500 rpm for 5 min. The absorbance of the supernatant was measured at 550 nm.

### 2.7. Antioxidant enzymes

#### 2.7.1. Superoxide dismutase (SOD, EC 1.15.1.1)

SOD was measured by the method of Misra and Fridovich (40). Hemolysate was added to carbonate buffer (0.05 M). Epinephrine was added to the mixture and measured (ELICO, Model SL 159) at 480 nm. SOD activity is expressed as the amount of enzyme that inhibits oxidation of epinephrine by 50%.

#### 2.7.2. CAT (EC 1.11.1.6)

CAT was determined by the method of Aebi (41). Briefly, hemolysate with absolute alcohol was incubated at 0 °C. An aliquot was taken up with 6.6 mM H<sub>2</sub>O<sub>2</sub> and decrease in absorbance was measured at 240 nm. An extinction coefficient of 43.6 M<sup>-1</sup>cm<sup>-1</sup> was used to determine enzyme activity.

#### 2.7.3. GSH-Px (EC.1.11.1.9)

GSH-Px was analyzed by the method of Flohe and Gunzler (42). Fifty microliters of 0.1 M phosphate buffer (pH 7.0), 100 μL of enzyme sample, 100 μL of glutathione reductase (0.24 units), and 100 μL of 10 mM GSH were mixed and preincubated for 10 min at 37 °C, followed by addition of 100 μL of 1.5 mM NADPH in 0.1% NaHCO<sub>3</sub>. The overall reaction was started by adding 100 μL of prewarmed hydrogen peroxide and decrease in absorption at 340 nm was monitored for 3 min.

### 2.8. Lipid peroxidation

#### 2.8.1. Conjugate dienes

The primary marker of lipid peroxidation, conjugate dienes, was assessed by the method of Olas and Wachowisz (39). Briefly, the samples were diluted 5 times in ether : ethanol (1:3 v/v), vortexed for 1 min, and centrifuged at 8000 rpm for 20 min; the supernatant was analyzed in a spectrophotometer at 234 nm. The amount of conjugate dienes produced was calculated using the molar extinction coefficient of 29,500 M<sup>-1</sup>cm<sup>-1</sup>.

#### 2.8.2. Malondialdehyde (MDA)

MDA, a product of lipid peroxidation, was determined according to the method of Ohkawa et al. (43). The sample was added to 8.1% SDS, vortexed, and incubated at room temperature. This was followed by addition of 20% acetic acid and 0.6% thiobarbituric acid and placement in boiling water bath. The sample was allowed to cool and

butanol-pyridine was added and centrifuged. Absorbance of the colored layer was measured at 532 nm with 1, 1, 3, 3-tetramethoxy propane as a standard.

## 2.9. Protein oxidation

### 2.9.1. Advanced oxidation protein products (AOPP)

Spectrophotometric determination of AOPP levels was assayed as an index of dityrosine containing cross-linked protein products using a method proposed by Witko et al. (44). Hemolysate was diluted in phosphate buffered saline and 1.16 M/l of potassium iodide was added; this was followed by the addition of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm. AOPP was calculated by using the extinction coefficient of  $26 \text{ mM}^{-1}\text{cm}^{-1}$ .

### 2.9.2. Sulfhydryls (P-SH)

P-SH in lysate was measured as described by Habeeb (45). In brief, 0.08 mol/L sodium phosphate buffer containing 0.5 mg/mL  $\text{Na}_2\text{-EDTA}$  and 2% SDS were added to each assay tube; 0.1 mL of 5, 5' DTNB was also added. Absorbance was measured at 412 nm.

## 2.10. Hemolysis

A 5% suspension of packed erythrocytes in buffer was mixed with equal volume of 8 mM  $\text{H}_2\text{O}_2$ . The mixtures were incubated at 37 °C in a BOD incubator. Hemolysis was determined by measuring released Hb into the supernatant of the induced samples at 540 nm and expressed on the basis of the maximum absorbance (100%) in the aliquots of erythrocytes completely hemolyzed in distilled water (46).

## 2.11. Protein determination

Protein was determined in the lysate and membrane following a method described by Lowry et al. (47), using bovine serum albumin as standard.

## 2.12. Statistical analyses

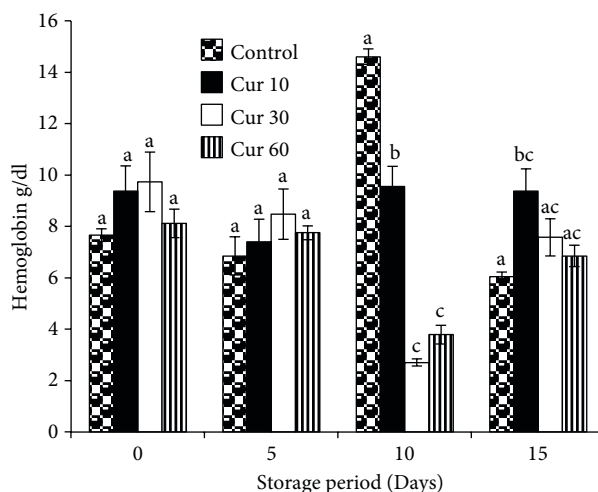
Results are represented as means  $\pm$  SE. Values between the groups were analyzed by two-way ANOVA and  $P < 0.05$  was considered significant. Bonferroni's post test was performed for all the assays using GraphPad Prism 6 software.

## 3. Results

### 3.1. Hb

Changes in Hb were significant during the storage period. Controls showed an increase of 100% (day 10) and a final reduction of 22% on day 15. Decrements of 20% (day 5) in Cur 10 and 72% (day 10) and 22% (day 15) in Cur 30 were observed. Cur 60 showed decreases of 53% and 16% on days 10 and 15, respectively.

Hb decreased by 35% (day 10) and increased by 55% (day 15) in Cur 10 against the controls. Cur 30 and Cur 60 showed reductions on day 10 by 70% against control and Cur 10 samples (Figure 1).



**Figure 1.** Hemoglobin levels in erythrocytes of stored blood. Values are expressed as means  $\pm$  SE from 5 samples.

Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM).

Changes are analyzed by two-way ANOVA followed by Bonferroni's Post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.

## 3.2. Superoxide

The changes in superoxide were significant with storage. The superoxide levels in the controls decreased by 55% (days 10, 15, and 20) while in Cur 10 they decreased by 50% (day 5), 100% (days 10 and 15), and 75% (day 20). Decrements of 50% (Cur 30) and 30% (Cur 60) on day 5 and 85% (Cur 30 and Cur 60) on days 10 and 15 were observed.

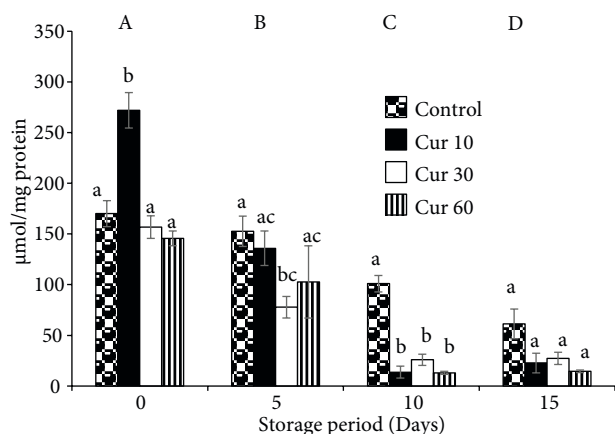
In Cur 10 superoxide levels were higher by 60% on day 0, while the levels were 90% lower on day 10 against the controls. Cur 30 showed decreases in superoxide levels of 50% (day 5) and 75% (day 10), whereas Cur 60 showed decreases of 85% (day 10) against the controls; the same levels were reduced by 50% in Cur 30 and Cur 60 on day 0 against Cur 10 (Figure 2).

## 3.3. Antioxidant enzymes

### 3.3.1. SOD

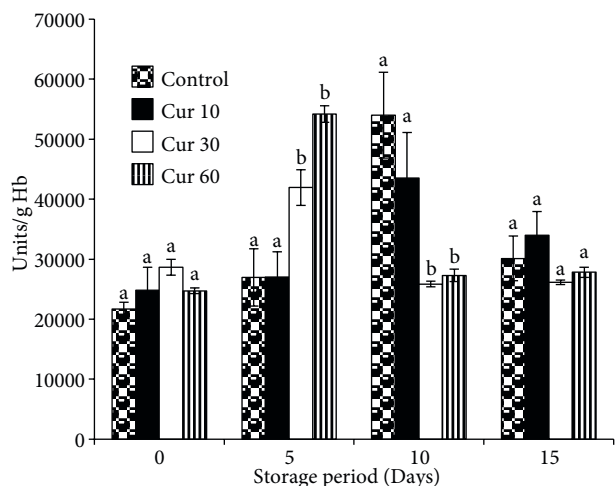
Significant changes in SOD activity were observed in all groups with storage. Increases by 24% and 39% on days 5 and 15, and 100% increase on day 10 were observed in the controls (day 0). SOD activity showed amplifications of 75% (day 10) and 40% (day 15) in Cur 10 and 46% (day 5) in Cur 30. Cur 60 showed 100% increase in SOD on day 5.

Significant changes in SOD activity were observed in Cur 30 (55% increase on day 5 and 45% decrease on



**Figure 2.** Superoxide levels in erythrocytes of stored blood. Values are expressed as means  $\pm$  SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni’s post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.

day 10) against the controls and Cur 10. On day 5, Cur 60 showed increases of 55% against controls and 100% against Cur 10, and a decrease of 40% on day 10 against controls and Cur 10 (Figure 3).



**Figure 3.** Superoxide dismutase activity in erythrocytes of stored blood. Values are expressed as means  $\pm$  SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni’s post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.

### 3.3.2. CAT

Changes in CAT activity with antioxidants and storage were found to be insignificant between the groups.

Cur 10, Cur 30, and Cur 60 showed decreases of 90% on days 5 and 10 when compared with the controls (Figure 4).

### 3.3.3. GSH-Px

GSH-Px activity was found to be insignificant with storage period.

GSH-Px decreased against the controls by 66% and 42% in Cur 10, 90% and 72% in Cur 30, and 88% and 74% in Cur 60 on days 10 and 15, respectively. Cur 30 showed a reduction of 72% in GSH-Px activity on day 5 when compared with Cur 10 (Figure 5).

### 3.4. Lipid peroxidation

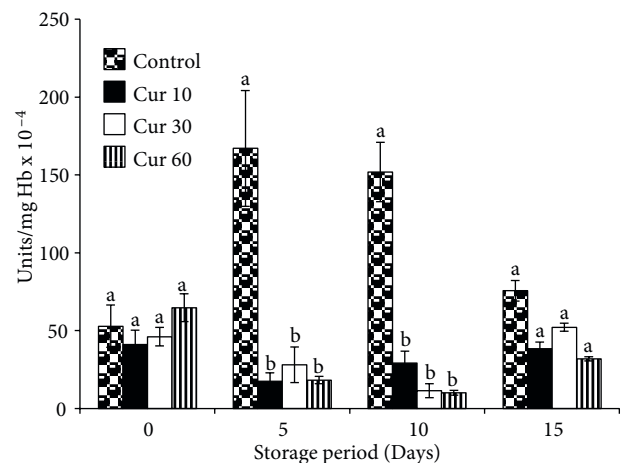
#### 3.4.1. Conjugate dienes

The levels of conjugate dienes changed significantly with storage. Conjugate dienes decreased in controls (40% on days 10 and 15), Cur 10 (30% on day 5 and 50% on days 10 and 15), Cur 30 (40% on days 10 and 15), and Cur 60 (60% on days 10 and 15).

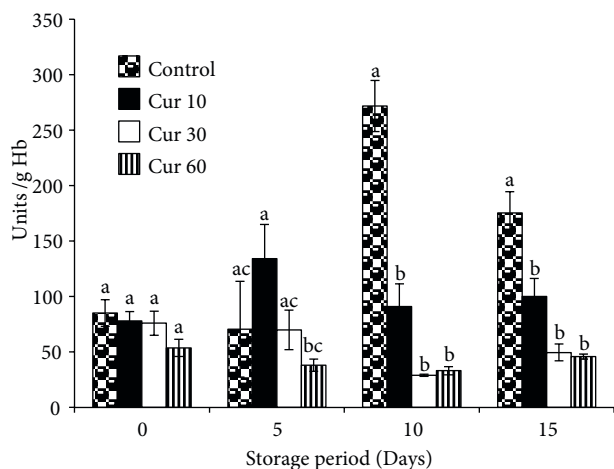
Conjugate dienes decreased against the controls by 20% on day 10 in Cur 10, 30% on day 5 and 45% on day 10 in Cur 30, and by 65% on day 10 in Cur 60. Cur 30 and Cur 60 showed decrements of 40% on day 0 against Cur 10 (Figure 6).

#### 3.4.2. MDA

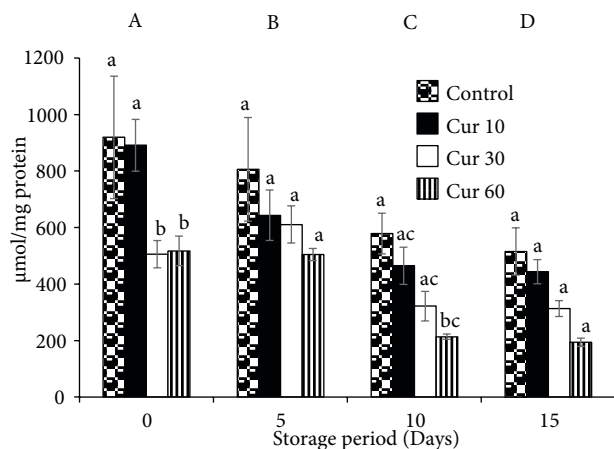
MDA was assayed as a measure of lipid peroxidation and significant changes were observed during storage.



**Figure 4.** Catalase activity in erythrocytes of stored blood. Values are expressed as means  $\pm$  SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni’s post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.



**Figure 5.** Glutathione peroxidase activity in erythrocytes of stored blood. Values are expressed as means ± SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni's post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.



**Figure 6.** Lipid peroxidation in erythrocytes of stored blood in terms of conjugate dienes. Values are expressed as means ± SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni's post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.

Controls showed decreases of 10% on day 5. MDA in Cur 10 increased by 300% (days 5, 10, and 15) and in Cur 30 by 80% (day 5) and 33% (day 15) with respect to day 0. MDA

increased by 100% on day 5, but decreased by 50% on day 15 and 25% on day 10 in Cur 60.

A decrement of 75% (day 0) was observed in Cur 10, while an increment of 90% (day 5) was observed in Cur 30 against the controls. MDA in Cur 60 aggravated by 80% on day 5 and decreased by 70% on day 15 against the controls. Cur 30 showed a 2-fold increase in MDA against Cur 10 on day 0. Cur 60 showed 70% reduction in MDA on day 15 against Cur 10 and Cur 30 (Figure 7).

### 3.5. Protein oxidation

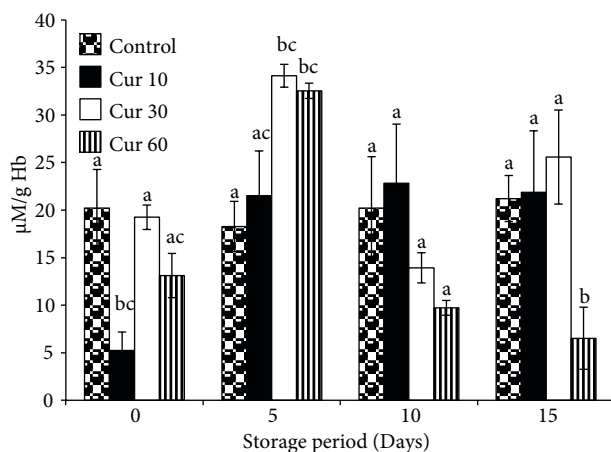
#### 3.5.1. AOPP

Significant changes were observed in AOPP with storage. Increases of 5-fold (day 5) and 3-fold (days 10 and 15) were observed in controls. AOPP decreased by 45% on all days in Cur 10. In Cur 30 and Cur 60, AOPP levels increased on day 0 by 30%, while they decreased on days 10 and 15 by 20% in Cur 30 and 35% in Cur 60.

AOPP in Cur 10 increased against the controls by 157% on day 0 and decreased by 75% (days 5, 10, and 15). On days 5, 10, and 15, Cur 30 and Cur 60 showed decrements of 80% against the controls (Figure 8).

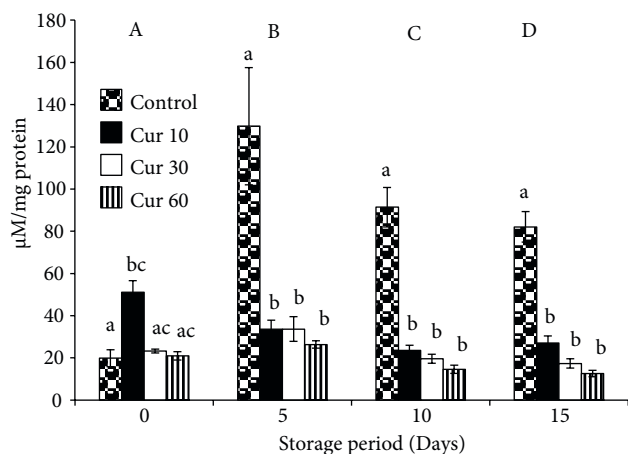
#### 3.5.2. P-SH

P-SH was significant in all groups with storage. Controls showed increments of 63%, 86%, and 100% with storage on days 5, 10, and 15, respectively. In Cur 10, P-SH levels decreased by 66% and 27%, while Cur 30 showed increments of 31% and 100% on days 10 and 15, respectively.



**Figure 7.** Lipid peroxidation in erythrocytes of stored blood in terms of malondialdehyde. Values are expressed as means ± SE from 5 samples.

Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni's post-test using Graphpad Prism software.  $P < 0.05$  was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.



**Figure 8.** Protein oxidation in erythrocytes of stored blood in terms of advanced oxidation protein products. Values are expressed as means ± SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni’s post-test using Graphpad Prism software. P < 0.05 was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.

P-SH was higher against the controls in Cur 30 (2-fold) and Cur 60 (5-fold) on day 15. On day 15, elevations of 4-fold and 7-fold were observed in Cur 30 and Cur 60 respectively, with respect to Cur 10. P-SH elevated by 80% on day 15 in Cur 60 against Cur 30 (Figure 9).

**3.6. Hemolysis**

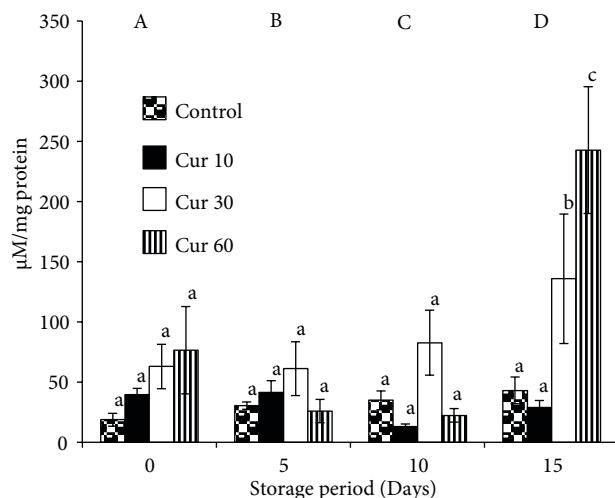
Significant changes in hemolysis were observed in all groups with storage. Hemolysis increased by 26%, 46%, and 65% (all days) in controls during storage. Levels of hemolysis increased in Cur 10 (35% on days 10 and 15), in Cur 30 (22% on day 5; 34% on day 10; and 50% on day 15) and in Cur 60 (35% on day 5; 44% on day 10; and 64% on day 15) with storage period.

**Table.** Hemolysis expressed as percentage (%).

Groups	Control	Cur 10	Cur 30	Cur 60
Day 5 <sup>A</sup>	25.50 ± 5.09 <sup>a</sup>	18.33 ± 7.64 <sup>a</sup>	32.67 ± 3.36 <sup>a</sup>	44.67 ± 9.39 <sup>a</sup>
Day 10 <sup>B</sup>	45.83 ± 9.82 <sup>a</sup>	42.00 ± 2.00 <sup>a</sup>	43.24 ± 3.16 <sup>a</sup>	53.57 ± 6.37 <sup>a</sup>
Day 15 <sup>C</sup>	64.94 ± 9.39 <sup>a</sup>	43.33 ± 7.60 <sup>a</sup>	58.67 ± 12.37 <sup>a</sup>	73.18 ± 11.55 <sup>a</sup>

Values are expressed as means ± SE from 5 samples.

Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni’s post-test using Graphpad Prism software. P < 0.05 was considered significant. Changes between groups (days) are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.



**Figure 9.** Protein oxidation in erythrocytes of stored blood in terms of protein sulfhydryls. Values are expressed as means ± SE from 5 samples. Cur 10 – Curcumin (10mM); Cur 30 – Curcumin (30mM); Cur 60 – Curcumin (60mM). Changes are analyzed by two-way ANOVA followed by Bonferroni’s post-test using Graphpad Prism software. P < 0.05 was considered significant. Changes between groups are represented in upper case, while changes within a group are represented in lower case. Those not sharing the same letters are significant.

The changes within the groups were found to be insignificant (Table).

**4. Discussion**

During storage, RBCs undergo structural and functional changes that may reduce function and viability after transfusion (48). The storage of erythrocytes away from their normal physiological environment leads them to exhibit various changes in relation to their physical and biochemical characteristics, which in turn reduce survival

and optimal functioning. These changes, collectively known as the red cell storage lesion, are characterized by changes in morphology, slowed metabolism with decrease in adenosine triphosphate, acidosis with decrease in concentration of 2,3-diphosphoglycerate, loss of function of cation pumps and consequent loss of intracellular potassium with accumulation of sodium intracellularly, oxidative damage to proteins, lipid peroxidation, and vesicle formation (49–54).

Erythrocytes are constantly exposed to a flux of oxygen. The dissociation of oxyhemoglobin is not a perfect process and can lead to the generation of superoxide radical and methemoglobin (metHb). MetHb is usually rapidly converted back into Hb by metHb reductase. However, during storage, there is a reduction in the conversion of metHb into free Hb and increased free iron is released. The results obtained in our study showed that the addition of curcumin was beneficial to the erythrocytes and maintained Hb throughout storage. Curcumin has been shown to protect Hb from oxidation and oxyhemoglobin from nitrite-induced oxidation (7,10).

Superoxide radicals are toxic and play an important role in the ROS cascade and its effects in erythrocytes. These radicals can get continuously oxidized, resulting in the formation of other ROS, in turn causing damage to proteins and lipids (55). Erythrocytes contain abundant Cu-Zn SOD to scavenge the superoxide produced (56), which was observed in the controls. The samples showed further decreases in superoxide levels, indicative of successful assistance of curcumin in eliminating superoxides. This result was corroborated by our previous results on ROS (57).

The enzymatic antioxidants include the first line of defense against free radicals in erythrocytes. Curcumin regulates and modulates the antioxidant enzymes (30–32) and this was observed in our results of SOD, CAT, and GSH-Px. The scavenging of superoxide radicals by curcumin (11,15,16,25) was elucidated by a decrease in superoxide and SOD in the samples as storage prolonged. The addition of curcumin assisted the erythrocytes by scavenging the  $H_2O_2$  (21) and thereby preventing the excessive formation of  $\cdot OH$ .

The polyunsaturated fatty acids present in erythrocytes are vulnerable to OS (58). Lipid peroxidation leads to the production of hydroperoxides and endoperoxides, which in turn, upon fragmentation, produce reactive carbonyl species (RCS) in the form of aldehydes such as MDA. These RCS have far-reaching damaging effects on targets within and outside membranes (59). The results of the primary products of lipid peroxidation, i.e. conjugate dienes, are indicative of the protective effect of curcumin on lipids. However, as storage progressed, the reduction in all groups could also be due to the formation of the

secondary products of lipid peroxidation, i.e. MDA. MDA was maintained in controls with storage, indicating the efficient antioxidant defense of erythrocytes. However, curcumin could not scavenge the free radicals efficiently and inhibit lipid peroxidation completely.

Proteins are also prone to attack by free radicals. Protein oxidation in erythrocytes is varied and leads to changes in the erythrocyte membrane, in turn leading to changes in morphology (60–63). AOPP are formed by the action of chloraminated oxidants, which produce dityrosine containing cross-linked protein products (44). AOPP levels in the groups with additives were much lower than those in the control group, substantiating that curcumin was beneficial to proteins. P-SH increased in samples with higher concentrations of curcumin, indicating that few free radicals were successfully scavenged by curcumin (15,16,18–22,25,29); this also indicated the reversibility of the disulfide groups to reduced SH. Similar results were observed in plasma from stored blood (64). In erythrocytes, Band 3, a protein containing six SH groups, is prone to oxidative damage. Damage to this protein by oxidation of SH groups to disulfides causes reversible membrane changes in erythrocytes (65,66). All these changes are associated with modifications in membrane properties and in turn, affect the functions and efficiency of erythrocytes.

Hemolysis increased in all groups during storage due to lipid peroxidation and redistribution of oxidized band 3 in the membrane (67). The increase in hemolysis during storage can be correlated with: 1) results of lipid peroxidation; and 2) the formation of other products of protein oxidation, and hence the insufficient protection by curcumin against those forms of protein oxidation and lipid peroxidation.

Curcumin could successfully maintain Hb and modulate the antioxidant enzymes throughout storage. However, curcumin could not protect all proteins and lipids from oxidative damage completely. This study forms a benchmark for the possibility of using curcumin in storage solutions. Further studies on curcumin, in combination with other antioxidants, need to be explored to prevent oxidative damage during storage. This would form the basis for development of effective storage solutions, and thereby prolong the shelf life of erythrocytes.

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