Short Communication

Curcumin Promotes the Deoxygenated State of Hemoglobin

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ABSTRACT

Introduction: In beta-thalassemia, an imbalance in the production of beta subunits of hemoglobin leads to the oxidation and deposition of excess alpha-globin chains at the cell membrane, resulting in the hemolysis of erythrocytes and a disorder of erythropoiesis. Antioxidants, such as curcumin, may promote this progression. This study aims to investigate the antioxidant effect of curcumin on hemolysate samples from patients with beta-thalassemia.

Materials and methods: Pure curcumin was extracted and purified for use in studying its effect on the visual light absorbance of hemoglobin in hemolysate samples from beta-thalassemia patients compared to control samples. Changes in light absorbance at 540 and 700 nm wavelengths during exposure to curcumin were analyzed to examine the shift from oxyhemoglobin to deoxyhemoglobin.

Results: Curcumin was found to dissolve rapidly and to a high degree in ethanol at 1 mg/ml, but did not dissolve in distilled water at the same concentration. The curcumin addition to the hemolysate sample of a patient with beta-thalassemia resulted in a decrease in the light absorbance of the sample at 540 nm wavelength, with minimal changes observed in the control sample.

Conclusion: Curcumin deoxygenated the hemolysate samples from both the patient and control, causing hemoglobin precipitation to occur slowly. The study suggests a greater potential role for curcumin in deoxygenating hemoglobin in the hemolysate samples of beta-thalassemia patients compared to those of the normal control.

Introduction

Beta-thalassemia major is a genetic disorder resulting from decreased or absent expression of the beta globin gene due to mutations in the promoter, exons, and introns of the beta globin gene in the beta globin gene cluster on human chromosome 11 [1, 2]. An imbalance in the production of alpha-globin chains compared to beta-globin leads to oxidation and deposition of alpha-globin chains in the red blood cell membrane, followed by membrane destruction and hemolysis. Inhibition of alpha-globin chain oxidation prevents alpha-globin chain

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precipitation through the binding of the AHSP chaperone-like protein to the alpha chain, as observed in many studies. Some antioxidant compounds also exhibit similar effects [3, 4].

Curcumin is a yellow phenolic compound derived from the root of L. longa turmeric, a member of the ginger family. As a natural antioxidant, curcumin has numerous therapeutic effects, such as being an antioxidant, anti-inflammatory, anti-cancer, and antimicrobial agent. Likewise, curcumin acts as a lipoxygenase inhibitor, radical scavenger, histone acetylase inhibitor (EC: 3.5.1.98), iron chelator, neuroprotective agent, and inhibitor of certain reductase and dehydrogenase enzymes like aldehyde reductase, shikimate dehydrogenase, IMP dehydrogenase, thioredoxin reductase, and NAD (P)H dehydrogenase [5, 6].

The aim of this study was to investigate the effect of curcumin on the sedimentation of hemoglobin and alpha-globin chains in the blood of patients with severe beta-thalassemia. The study compared the effect of curcumin on the blood hemolysate of beta-thalassemia major patients to that of a healthy control group, examining wavelengths of 520, 540, and 700 nm.

Materials and Methods

Preparation of a Curcumin Solution: A commercial dietary supplement in the form of capsules with 95% purity containing 450 mg of curcumin per capsule was purchased from Karen Pharmaceutical Company. The capsules were dissolved by adding ethanol to the contents and separating them into the ethanol-containing phase in a tube. Pure curcumin dry powder was obtained after drying and evaporating the ethanol. Solutions of 1 mg/mL curcumin in ethanol and a mixture of 1 mg/mL curcumin in distilled water were also prepared.

Preparation of Hemolysate Samples: Blood samples were collected from patients with beta-thalassemia major who had not received a blood transfusion within the last month. The samples were collected in CBC collection tubes containing hemoglobin at concentrations of 7.9 mg/mL for patients and 13.4 mg/mL for controls. The blood was centrifuged at 5000 rpm for 15 minutes to obtain 1 mL of red blood cell sediment, which was then mixed with distilled water to prepare 2.5% and 5% hemolysate solutions. The absorbance of the samples was measured at wavelengths of 540 nm and 700 nm.

UV-Vis Absorbance Analysis: Curcumin samples in ethanol and distilled water were analyzed using a DR6000 UV-Vis (Hach, Germany) spectrophotometer at wavelengths of 418 nm. Absorbance at wavelengths of 520 nm, 540 nm, and 700 nm were measured for the hemolysate samples.

Methods: The absorbance of curcumin in ethanol and distilled water compared to blank distilled water was studied at 418 nm over 3 hours. A solution of 2.5% hemolysate with 0.5 g/ml curcumin was prepared by adding an equal volume of a mixture of 1 mg/ml curcumin in distilled water to the 5% hemolysate sample of patients or controls. The absorbance of each sample at wavelengths of 540 nm and 700 nm was recorded at 0, 5, and 15 minutes, and then every 15 minutes. Graphs were generated using Excel software.

Results and Discussion

The synergistic effect of ionic solution and organic solvents on the curcumin absorbance spectrum was shown in the visible and ultraviolet spectrum in **Figure** 1A, B. Curcumin is stably dissolved in ethanol and methanol but has low solubility in water as a solvent. The precipitation plot of curcumin in ethanol versus distilled water at a mixture of 1 mg/mL demonstrates the high potential of ethanol to dissolve curcumin. The absorbance of the curcumin solution in ethanol was higher than in water, and its dissolution was rapid. The absorbance of curcumin in distilled water was very low, with the mixture being heavily precipitated. Curcumin was stable in ethanol at a concentration of 1 mg/mL, but not completely soluble in distilled water.

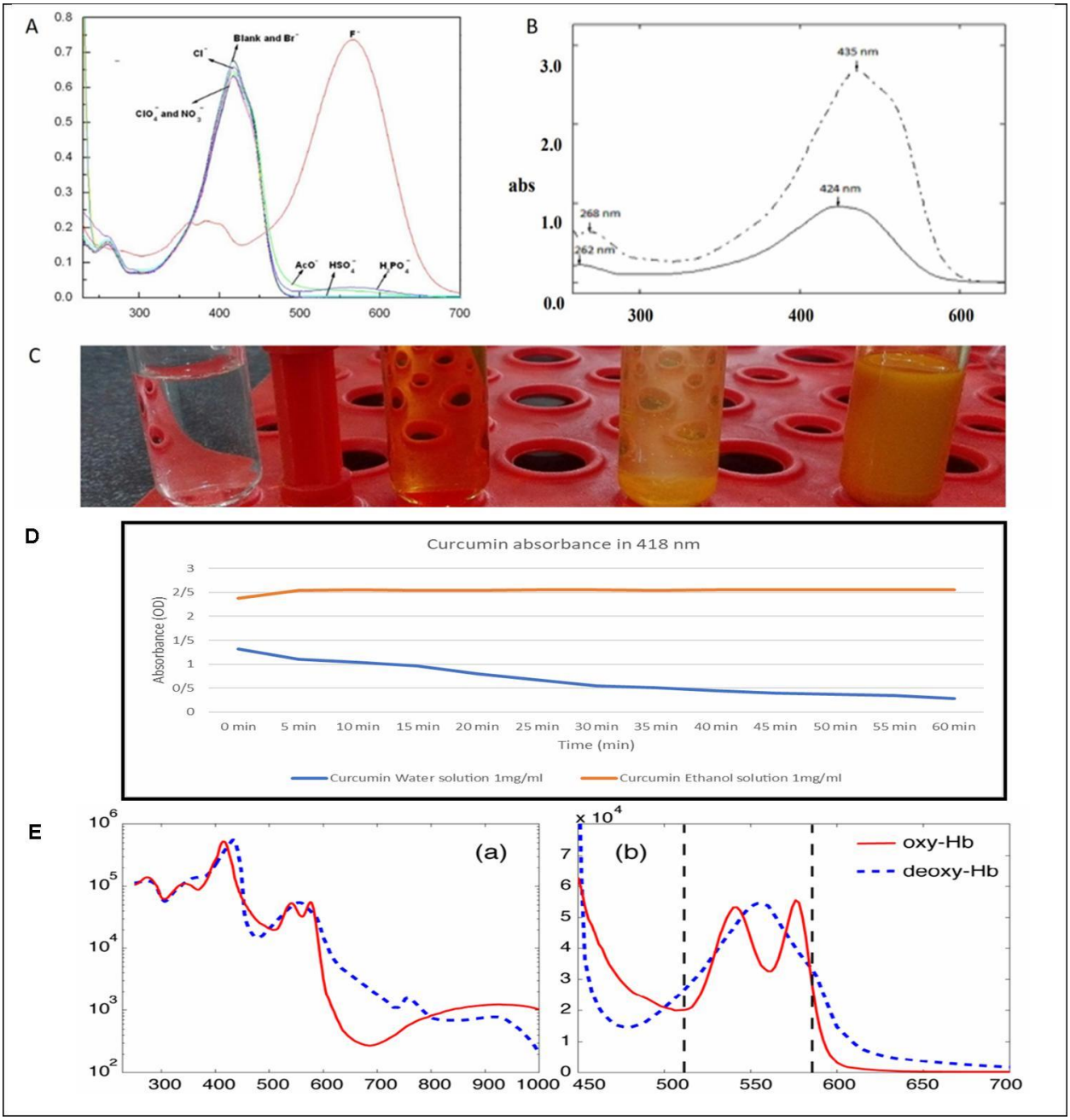

Figure 1 (A) Examines curcumin solutions containing diverse anions at varying concentrations in acetonitrile. These anion concentrations include 1.0 x 10^{-5} mol/L for F^-, 2.0 x 10^{-4} mol/L for AcO^-, and $H2PO4^-$, and 4.0 x 10^{-4} mol/L for $HSO4^-$, $ClO4^-$, $NO3^-$, Cl^-, and Br^-. (B) In methanol, as compared to DMSO, curcumin shows lower absorbance. DMSO produces higher absorbance levels for curcumin across all wavelengths in comparison to the methanol solution. Curcumin solutions display minimal absorbance at 700 nm, but exhibit low absorbance within the 520-540 nm spectrum, potentially affecting hemoglobin absorbance readings. The graph illustrates different concentrations of curcumin in different solvents from right to left [7, 8]. The comparison of curcumin absorbance in water and ethanol solvents during the first hour of incineration study is depicted in Figure (C, D). The comparison scale of oxygenated and deoxygenated hemoglobin in water solutions is illustrated in Figure (E)

The best wavelength for estimating hemoglobin concentration is 540 nm, as hemoglobin concentration correlates directly with absorbance in the 520-540 nm spectrum. Curcumin itself has low absorbance at 540 nm and very low absorbance at 700 nm, which does not interfere with hemolysate absorbance at 700 nm. High concentrations of hemolysate can affect the accuracy of absorbance detection, so a lower concentration (2.5%) was used for this absorbance test [9,10].

The oxygen saturation of hemoglobin is inversely proportional to the absorbance at 700 nm. At a wavelength of 700 nm, the absorbance of deoxygenated hemoglobin is several times that of oxygenated hemoglobin. In Figure 2A and 2C, the hemoglobin concentration shows a direct correlation with the absorbance intensity at 700 nm, where the absorbance of 5% of the hemolysate sample is higher than 2.5% in both the patient and control samples. However, despite the higher hemoglobin level, the absorbance at 700 nm was higher in the patient group than in the control group. Since the absorbance of deoxygenated hemoglobin at 700 nm is significantly higher than the absorbance of oxygenated hemoglobin, it can be concluded that the patient's hemoglobin tends to be deoxygenated. Therefore, the hemoglobin in the patient's blood appears to be less saturated with oxygen than in the control sample [11].

The binding of curcumin to hemoglobin overestimates the hemoglobin concentration in the sample. Curcumin itself has a low absorbance at 540 nm and a very low absorbance at 700 nm. At 700 nm, the absorbance of 2.5% hemolysate samples in the presence of curcumin was higher than that of 5% hemolysate samples without curcumin, in both patient and control samples. This indicates that curcumin can drive the deoxygenation of hemoglobin. The higher absorbance at 700 nm in curcumin-affected

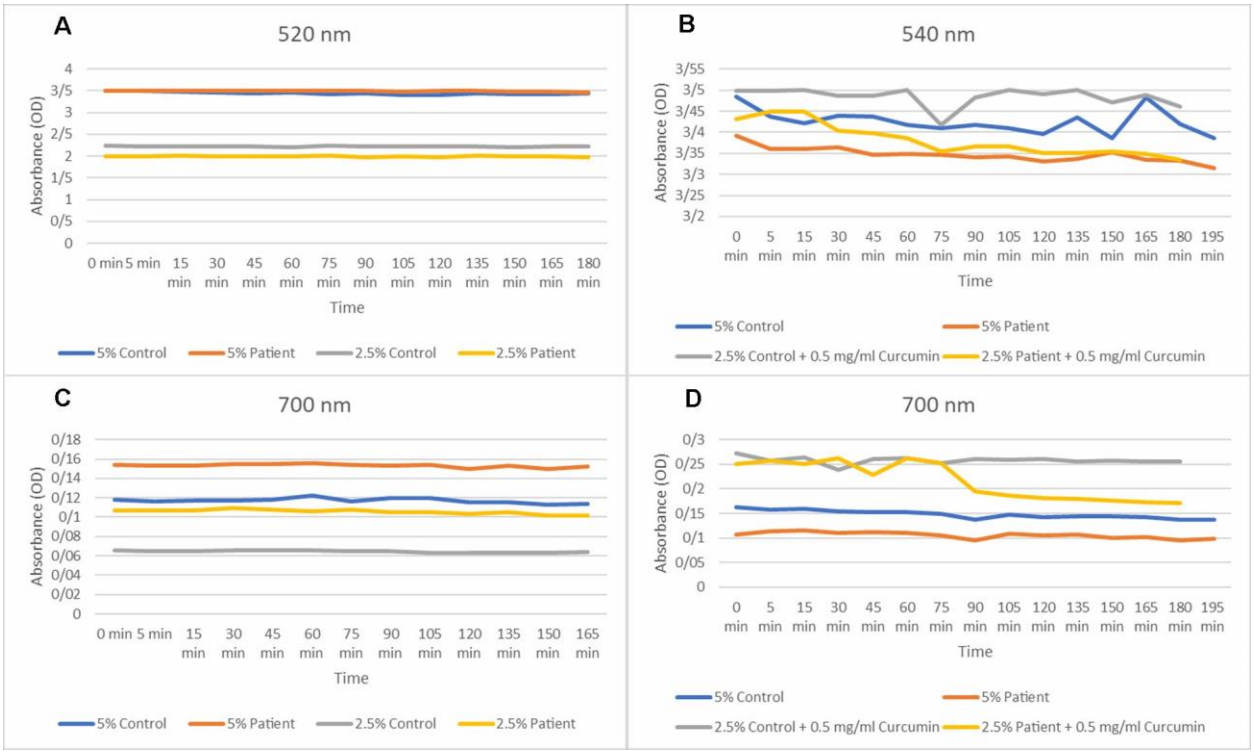

Figure 2 shows the light absorbance of hemolysate samples exposed to curcumin (B, D) and unexposed to curcumin (A, C). The patient group had beta-thalassemia major, while the control group was normal

control and patient hemolysate samples than in unaffected samples suggests an increase in deoxygenation of hemoglobin in curcumin-affected patient and control samples. It can be interpreted that curcumin decreases the ratio of oxygenated hemoglobin at the beginning of the test (Figure 2D). Despite the difference in hemoglobin concentration, the absorbance at 700 nm of a 2.5% patient and control hemolysate sample is the same in the presence of curcumin during the first 75 minutes after the start of the test. This shows the dominating effect of the absorption of deoxygenated hemoglobin in the 700 nm (Figure 2D). At 75 minutes from the start of the test, the absorbance at 700 nm of the affected hemolysate (2.5%) of the patient sample was lower compared to the control. On the other hand, in this test, the patient curcumin-affected hemolysate shows a decrease in absorbance at 540 nm during the test, indicating a slight precipitation of hemoglobin (Figure 2C). This means that curcumin deoxygenates and precipitates the hemoglobin of hemolysate samples from thalassemia patients. The curcumin effect on blood samples from beta-thalassemia patients is not observed in the control blood sample (Figure 2C, D) [11].

The curcumin-affected hemolysate samples lead to a transition to the deoxygenated state in the hemoglobin of healthy individuals and patients. Although this study was performed on blood samples from thalassemia patients, due to curcumin's antioxidant activity, it can be expected to have the same effect on blood samples from patients with sickle cell anemia. This study contradicted previous findings, which suggested that curcumin might stabilize hemoglobin in its oxygenated state [12-15].

Conclusion

The solubility of curcumin in an aqueous solvent is very low, causing it to quickly leave the solvent when introduced into the bloodstream. This limits its effectiveness in spreading throughout the bloodstream. The effects of curcumin on hemolysate samples were observed during the progressive deoxygenation of hemoglobin in both normal and patient samples, as indicated by variances in absorbance at 700 nm. Curcumin promotes deoxygenation of hemoglobin in both patient and normal control samples. It was also found to gradually precipitate hemoglobin in the hemolysate samples.

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