Omega 3 and Azadirachta Indica J. Seed Oil Inhibit Hemoglobin Polymerization and Modulate Erythrocyte Membrane ATPases in Sickle Cell Disease (SCD)

Nantchouang NAL1, Kotue TC2*, Djouhou FM1, Ngo SF1, Pieme AC1, Kansci G1, Fokou E1

1Laboratory for Food Science and Metabolism - Department of Biochemistry – Faculty of Science – University of Yaoundé1, Cameroon
2Hematological Service at the Central Hospital, Yaounde, Cameroon

Keywords: Omega 3 - Azadirachta Indica J. seed oil - sickle cell disease - polymerization - Fe++/Fe+++ ratio - ATPase activities.

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*Corresponding author: Laboratory for Food Science and Metabolism - Department of Biochemistry – Faculty of Science – University of Yaoundé1, Cameroon, E-mail: ctkotue_bio@yahoo.fr / ckotue@uy1.uninet.cm

Abstract

Background: Functional foods/Nutraceuticals efficacy depends on both its composition and the physicochemical properties of the components that are used in their formulation. Vegetable oil can play an important role. Several studies are shown that omega 3 (0.2%) and Azadirachta indica J. seed oil (0.4%) have antisickling, antioxidant and anti-haemolytic properties.

Objective: The purpose of this present study was conducted to determine effects of omega 3 and Azadirachta indica J. seed oil on hemoglobin polymerization and erythrocyte membrane ATPases in Sickle Cell Disease (SCD).

Method: The ability for the omega 3 and Azadirachta indica J. seed oil to inhibit sickle cell hemoglobin polymerization and improve the Fe++/Fe+++ ratio of HbS blood were assed using spectroscopic method. The activities of three ATPases (Na+/K+; Ca++; and Mg++-ATPases) were investigated by the kinetic method following the release of inorganic phosphate (Pi) by ATP taking into account the determination of the total membrane proteins of erythrocytes by Bradford’s method.

Results: The lowest potential was obtained with Azadirachta indica J. seed oil (AO: 0.4% v/v) corresponding significantly (p <0.05) to the highest potential of inhibition of polymerization (83.11 ± 8%) compared to that obtained with omega 3 (0.2% v/v) estimated at 68.15±7%. The greatest Fe++/Fe+++ ratio was obtained (6.65±1.69) with AO (0.4% v/v) corresponding significantly (p <0.05) to the greatest rate of increase of oxyhemoglobin (79.05±3%) compared to that obtained with ω3 (0.2% v/v) estimated at 70.58±5%. As for the activities of Na+/K+; Ca++ and Mg++-ATPases expressed in μmole Pi/mg protein/hour x 10^3, there was an increase in the activities of Na+/K+ and Ca++-ATPases (20.38±24 and 190.48±3) with AO (0.4% v/v), but without any significant difference (p>0.05) compared to those obtained with ω3 (0.2% v/v) (188.66±9 and 235.61±52). However, for Mg++-ATPase, there was rather a significant decrease (p<0.05) in these activities ranging from 181.9±32 for ω3 (0.2% v/v) to 124.00±21 for AO (0.4% v/v).

Conclusion: The Azadirachta indica J. seed oil at a concentration of 0.4% v/v offered the best potential inhibition of hemoglobin S polymerization, oxyhemoglobin increase rate and the best ATPastic activities.

Keywords: Omega 3 - Azadirachta Indica J. seed oil - sickle cell disease - polymerization - Fe++/Fe+++ ratio - ATPase activities.

Introduction

Vegetable oil is a rich source of nutraceuticals in addition to essential fatty acids. Triglyceride is the major component of the vegetable oil, and its fatty acids play a vital role in human health and nutrition. Minor components of vegetable oil such as lecithin, tocopherols and tocotriens, phytosterols and phytostanols, oryzanol, policosanol, squalene, carotenoids, and lignans also play a significant role in human. Results from epidemiologic and clinical studies have indicated that the consumption of omega-3 fatty acids, namely eicosapentaenoic acid (EPA) and docosaesaenoic acid (DHA) have positive effects in decreasing risk factors of cardiovascular diseases [1]. The findings from a previous retrospective and cross-sectional study suggest that omega-3 fatty acids might be beneficial in Sickle Cell Disease (SCD) [2, 3]. Omega-3 fatty acids EPA/DHA at 0.2% have antisickling, anti-haemolytic and antioxidant properties [4]. Several studies have reported that tiger nut oil (Cyperus esculentus) and black seed oil (Nigella sativa) are natural products resulting to considerable antisickling effects [5]. In addition, tiger nut and black seed oils increase the antioxidant capacity of HbS (sickle) blood samples [6]. A recent investigation has shown that Azadirachta indica J. seed oil used to manage Sickle Cell Disease (SCD) in North Cameroon at 0.4% has the best antisickling, anti-haemolytic and antioxidant properties [7, 8]. The omega-3 (linolenic acid) and vitamin E are in the composition of this oil...
In fact, the sickle cell haemoglobin (HbS) variant is caused by a point mutation affecting the coding sequence of the β-globin gene resulting in a substitution of glutamic acid by valine at the sixth position of β-globin chains [9,10]. Under low oxygen tension, deoxyHbS molecules polymerize into microfibrils parallel to each other with concomitant reduced solubility (forms gel) and resultant erythrocyte membrane deformity and damage [11]. The unidirectional active transport of ions such as Na⁺, K⁺ and Ca²⁺, has been reported to play major roles in maintaining the stability of the erythrocyte membranes [12,13]. Indeed, the presence of three different adenosine triphosphatases (Na⁺/K⁺, Ca²⁺ and Mg²⁺-ATPases) in human erythrocyte membranes had been reported by various workers [14, 15, 16]. The determinations of the activity levels of these ATPases in different human genotypes showed that (Na⁺/K⁺ and Ca²⁺-ATPases) were significantly lower in sickle cell erythrocytes, while Mg²⁺-ATPase was significantly higher than for normal erythrocytes [16, 17]. The new therapeutic approaches for the treatment and management of sickle cell disease include the usage of natural products that interact either non-covalently or covalently with HbS molecules to retard or inhibit hemoglobin aggregation and polymerization and/or modulate the activities of the three membrane-bound ATPases (increases for Na⁺/K⁺ and Ca²⁺-ATPases, and decrease for Mg²⁺-ATPase) [18, 19, 20].

The aim of this study, therefore, is to investigate the effects of omega 3 and Azadirachta indica J. seed oil on hemoglobin polymerization and erythrocyte membrane ATPases in sickle cell disease (SCD).

**Methodology**

**The Omega-3 Fatty Acids EPA/DHA and Azadirachta Indica J. Seed Oil Concentrations**

The omega-3 fatty acids EPA/DHA pure fish oil capsules were bought in a pharmacy. Each capsule contains 750 μL in proportion EPA/DHA (3v/2v). The following concentrations of 0.2% v/v for omega-3; 0.2% v/v and 0.4% v/v of the Azadirachta indica J. seed oil used to manage sickle cell disease (SCD) in North Cameroon were obtained by diluting the oils in normal saline diluted ethanol (1:4 in normal saline).

**Preparation of Blood Samples**

After receiving an ethical clearance with number CEN° 00650/CRERSHC/2019, consent from all blood donors was read and signed by all the patients participating to the study after being informed of the research objectives. Blood samples were collected from 19 confirmed HbS (homozygous) sickle cell patients (9 males and 10 females) between mean aged 26.52±9 years. Blood samples were siphoned from each patient in 2.5mL of distilled and deionized water in four different concentrations (1, 2, 3, 4) to produce a stock solution of 1:4 in normal saline. The stock solution was stored at the 6°C for the experiment.

**Sickle Cell Hemoglobin Polymerization Experiment**

Erythrocytes were isolated from 1200 μL HbS blood samples by centrifugation at a gravitational force of 1500 x g for 15 minutes using the bench centrifuge (5804R Centrifuge), following careful siphoning of the plasma with Pasteur pipette. The erythrocytes were by repeated inversion suspended in a volume of isotonic saline (0.9% NaCl) equivalent to the siphoned plasma. The erythrocyte suspension was freeze thawed at 4°C to produce a hemolyzate for the hemoglobin polymerization experiment. HbS polymerization was assessed by the turbidity of the polymerizing mixture at 700 nm using 2% Sodium metabisulphite as reductant or deoxygenating agent [21]. 0.5 mL of 2% solution of sodium metabisulphite (Na₂S₂O₃), 0.5 mL normal saline and 0.1 mL of hemoglobin solution (HbS) were pipetted into a cuvette shaken and the absorbance read at 700 nm at 30s, then at 180s. This represented the negative control. Distilled water was used as blank in all assays. In the main assay, 0.5 mL of 2% solution of sodium metabisulphite, 0.5 mL of omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v) and 0.1 mL of hemoglobin solution (HbS) were pipetted into a cuvette and the optical density reading taken as above. Phenylalanine at 4 mg/mL was used as positive control. The rates of the potential of inhibition of hemoglobin polymerization (PIP) in percentage were estimated after calculated the potential of inhibition of hemoglobin polymerization (PP) from the formula of average change in optical density/absorbance against time in seconds [22].

**Determination of the Fe³⁺/Fe²⁺ Rate of Sickle Cell Blood**

The Fe³⁺/Fe²⁺ rate of omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v) on the sickle cell blood was determined by the methods of [23] and reported by [24]. The procedure involves the lysing of 100 μL of whole blood from each patient in 2.5mL of distilled and deionized water in the presence of the omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v), using 0.9% NaCl and Phenylalanine at 4 mg/mL as negative and positive controls respectively and then determining the absorbance of hemoglobin and methemoglobin at their characteristic wavelengths of 540 nm and 630 nm, respectively.

**Effects of the Omega-3 and Azadirachta Indica J. Seed Oil on the Activities of Membrane ATPases of Sickle Cell Erythrocytes**

**Preparation of Erythrocyte Membrane**

This followed the method described by [25] reported by [20]. Briefly, the whole blood was centrifuged at 5, 000 g for 10 minutes and the resultant precipitate was washed with 0.15 M NaCl (pH 7.4). This ‘washing’ process was repeated three times, the final precipitate was lyzed by swelling in 5 mL NaHPO₄,2H₂O (pH 7.7), and then centrifuged at 5,000 g for 10 minutes. The resultant precipitate was washed with 10 mL Tris-HCl (pH 7.7) and suspended in 3 mL distilled water. The isolated membranes were stored at 4°C and used within 12 hours of collection of blood samples.

**Na⁺/K⁺; Ca²⁺ And Mg²⁺-ATPase Activities Assays**

The assays of the enzyme activities followed the procedure...
of [26], reported by [20] and monitored the inorganic phosphate released from ATP. Enzyme activities were expressed as µmol Pi/mg protein/hour x 10⁻³. The concentration of phosphate in 1 mL of the supernatant was measured by the method described by [27] reported by [20]. For this, 1.0 mL of 2.5% ammonium molybdate was added and after 10 minutes, the addition of 0.1 mL of 2% ascorbic acid followed. The mixture was kept at room temperature for 20 minutes for colour development. The absorbance of the final mixture was measured at 725 nm using spectrophotometer.

Protein Determination

The protein content of the membranes was determined by the method of [28], reported by [20] using Bovine Serum Albumin (BSA) as the protein standard.

Omega-3 and Azadirachta Indica J. Seed Oil Assays on the Different ATPases Activities

The reaction tubes, in addition to the contents the various ATPases, contained 0.5 mL of different concentrations of the omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil(AO: 0.2% and AO: 0.4% v/v) to produce the desired final concentrations in the total reaction mixture of 1 mL. 0.9% NaCl and Phenylalanine at 4 mg/mL as negative and positive controls respectively.

Statistical Analysis

The results were expressed as mean ± standard deviation. Data was analyzed using Analysis of Variance (ANOVA) of Kruuskall-Wallis with the software Sigma Start version 3.01A analysis software. Statistical data were considered significantly different at 95% confidence interval (p <0.05).

Results

The results illustrated in table I shows both the evaluation of the potential of hemoglobin polymerization (PP) and inhibition of hemoglobin polymerization (PIP) without (NE) and in the presence of omega 3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil(AO: 0.2% and AO: 0.4% v/v) and the phenylalanine (Phe: 4 mg/mL) used as positive control. Comparatively to negative control (56.5±3%), phenylalanine (Phe: 4 mg/mL); omega 3 (ω3: 0.2% v/v) and Azadirachta indica J. seed oil(AO: 0.2% and AO: 0.4% v/v) inhibited significantly (p<0.05) the HbS polymerization. Azadirachta indica J. seed oil at 0.4% v/v has shown the significantly higher value (p<0.05) of potential of inhibition of hemoglobin polymerization (83.11±8%).

Table II shows the effect of oils on the Fe²⁺/Fe³⁺ ratio and increase Fe²⁺/Fe³⁺ without (NE) and in the presence of the omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil(AO: 0.2% and AO: 0.4% v/v) and the phenylalanine (Phe: 4 mg/mL) used as positive control. Comparatively to negative control, phenylalanine (Phe: 4 mg/mL); omega 3 (ω3: 0.2% v/v) and Azadirachta indica J. seed oil(AO: 0.2% and AO: 0.4% v/v) improved significantly (p<0.05) the Fe²⁺/Fe³⁺ ratio of the sickle cell blood. The Azadirachta indica J. seed oil at 0.4% v/v has showed the significantly higher value (p<0.05) increase Fe²⁺/Fe³⁺ ratio (79.05±3%).

The modulation of Na⁺/K⁺; Ca²⁺ and Mg²⁺-ATPases activities in the absence (negative control) and presence of the omega 3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil(AO: 0.2% and AO: 0.4% v/v) and the phenylalanine (Phe: 4 mg/mL) used as positive control are shown by the figures 1; 2 and 3 respectively.

Figure 1 below shows the activity of the Na⁺/K⁺-ATPase erythrocyte membrane in the absence of the oil samples, of its modulation with the positive control (Phe: 4 mg/mL) and the oil samples omega 3 (ω3: 0.2%); AO (0.2%); then AO (0.4%) samples compared to the negative control. This figure shows a significant increase (p<0.05) in the activities of this enzyme expressed in µmol Pi/mg proteins/hour x 10⁻³. For the control (-) this activity was 123.30±33. In the presence of the positive control (Phe: 4 mg/mL), it increases considerably to 209.35±27. In addition, these activities were respectively 188.66±9, 196.84±16 and 203.82±41 for omega 3 (ω3: 0.2%); AO (0.2%) and AO (0.4%). However, no significant difference (p>0.05) was observed between the different values obtained despite the high activity recorded with phenylalanine 4 mg/mL. The greatest activity was obtained with AO (0.4%).

Figure 2 bellow shows the activity of Ca²⁺-ATPase membrane of sickle cell erythrocytes in the absence of phenylalanine (Phe: 4 mg/mL) and omega 3 (ω3: 0.2%); HA (0.2%); AO (0.4%) samples and its modulations with the latter. A modulation of the activity of Ca²⁺-ATPase expressed in µmol Pi/mg Proteins/hour x 10⁻³ was observed compared to the negative control. The greatest activity was obtained with Phe (4 mg/mL) used as a positive control (275.96±73), followed by omega-3 (ω3: 0.2%) (235.61±52); by AO: 0.4% (190.48±32) and finally by AO: 0.2% (178.21±39). However, a significant difference (p<0.05) was observed between the different values obtained compared to the negative control.

In the absence of the positive control (Phe: 4 mg/mL) and of the various oil samples, the membrane Mg²⁺-ATPase of sickle cell erythrocytes showed an activity (µmol Pi/mg proteins /hour x 10⁻³) of 213.30±58. In the presence of Phe (4 mg/mL), this activity was 176.50±56 while in the presence of oil samples the activities were 181.90±32; 192.52±37 and 124±21 corresponding respectively to omega 3 (ω3: 0.2%); AO (0.2%) and AO (0.4%). However, apart from AO (0.2%), all values obtained with the positive control and the other oil samples are significantly (p<0.05) lower than the activity of the negative control. This reflects an inhibition of the activity of the Mg²⁺-ATPase by the different tests.
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Table I: Potential of hemoglobin polymerization (PP) and potential of inhibition of hemoglobin polymerization (PIP) without (NE) and in the presence of omega 3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v). Phenylalanine (Phe 4: mg/mL) was used as positive control

<table>
<thead>
<tr>
<th>Test</th>
<th>NE</th>
<th>Phe (4mg/mL)</th>
<th>ω3 (0.2% v/v)</th>
<th>AO (0.2% v/v)</th>
<th>AO (0.4% v/v)</th>
</tr>
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<tbody>
<tr>
<td>Potential of hemoglobin polymerization (%)</td>
<td>56.5±3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>17.99±7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.72±5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.54±8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potential of inhibition of hemoglobin polymerization (%)</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.24±8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.15±7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.63±5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.11±8&lt;sup&gt;c&lt;/sup&gt;</td>
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Values followed by different superscripts along the rows are significantly different (p<0.05)

Table II: Effect of oils on the Fe<sup>2+</sup>/Fe<sup>3+</sup> ratio and increase Fe<sup>2+</sup>/Fe<sup>3+</sup> without (NE) and in the presence of the omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v). Phenylalanine (Phe 4: mg/mL) was used as positive control

<table>
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<tr>
<th>Test</th>
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<th>ω3 (0.2% v/v)</th>
<th>AO (0.2% v/v)</th>
<th>AO (0.4% v/v)</th>
</tr>
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<tbody>
<tr>
<td>Fe&lt;sup&gt;2+&lt;/sup&gt;/Fe&lt;sup&gt;3+&lt;/sup&gt; ratio</td>
<td>1.34±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.67±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8±1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.65±1,68&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Increase Fe&lt;sup&gt;2+&lt;/sup&gt;/Fe&lt;sup&gt;3+&lt;/sup&gt; ratio (%)</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.1±8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.58±5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.99±6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.05±3&lt;sup&gt;c&lt;/sup&gt;</td>
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Values followed by different superscripts along the rows are significantly different (p<0.05)

Figure 1: Effect of oils on Na<sup>+</sup>/K<sup>+</sup>-ATPase activity without (negative control) and in the presence of the omega 3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v). Phenylalanine (Phe 4: mg/mL) was used as positive control. Values followed by different superscripts are significantly different (p<0.05)

Discussion

Research on formulations based on extracts from foods and edible plants is the new trend in the management of genetic diseases like sickle cell with a view to finding a cheaper alternative which sickle cell patients can access immediately. For this, different strategies have been considered to explain the modes of action of the omega-3 and the Azadirachta indica J. oil seed used by certain sickle cell families in the region of North Cameroon. They directly targeted polymerization, the fundamental basis of sickle cell formation and certain alterations in sickle cell red blood cells such as the oxidation of ferrous iron (Fe<sup>2+</sup>) to ferric iron (Fe<sup>3+</sup>) and the destabilization of the membrane ATPases activities. Indeed, the polymerization of hemoglobin S also called “gelation” is a physicochemical process based on non-covalent interactions governed by kinetic and thermodynamic factors. It is a reversible phenomenon [29].

When HbS was treated with phenylalanine 4 mg/mL (used as a positive control) and with Omega 3 (ω3: 0.2%); AO (0.2%) and AO (0.4%) samples, a marked change in the polymerization was observed. This reflects the direct influence of these substances
Omega 3 and Azadirachta Indica J. Seed Oil Inhibit Hemoglobin Polymerization and Modulate Erythrocyte Membrane Atpases in Sickle Cell Disease (SCD)

Figure 2: Effect of oils on Ca$^{2+}$-ATPase activity without (negative control) and in the presence of the omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v). Phenylalanine (Phe 4: mg/mL) was used as positive control. Values followed by different superscripts are significantly different (p<0.05)

Figure 3: Effect of oils on Mg$^{2+}$-ATPase activity without (negative control) and in the presence of the omega-3 (ω3: 0.2% v/v), Azadirachta indica J. seed oil (AO: 0.2% and AO: 0.4% v/v). Phenylalanine (Phe 4: mg/mL) was used as positive control. Values followed by different superscripts are significantly different (p<0.05)

On the gelation of HbS. With phenylalanine used as a positive control, the polymerization inhibition potential was 58.24 ± 8%. Furthermore, the greatest potential for polymerization inhibition (83.11 ± 8%) was obtained with AO (0.4%). This value is less than 94.17 ± 0.1% and close to the 84 ± 0.12% obtained respectively by [30] with the lipid fraction of Telferia occidentalis seed and by [31] with the lipid fraction of Mucuna sloanei seed. This PIP is greater than 72.08% and 77.31% obtained by [32] with the lipid fractions of Xylopia aethiopica and Monoda myristica seeds. This inhibition may be due to the greater influence of HA (0.4%) on the balance between the relaxed (R) and tense (T) forms of the hemoglobin tetramer, which translates into an increase in the percentage of oxygen saturation of HbS. The same is true for omega 3 (ω3: 0.2%). The inhibition of polymerization by AO (0.4%) and omega 3 (ω3: 0.2%) seems to justify the mode of action by which these oils inhibit sickling as shown by [4, 8].

The substitution of glutamic acid in position 6 by a valine in the β chain of hemoglobin causes its precipitation in red blood cells with sickling, promoting the oxidation of ferrous iron (Fe$^{2+}$) to ferric iron (Fe$^{3+}$) unable to fix oxygen [33]. In the absence of any treatment with HbS, the Fe$^{2+}$/Fe$^{3+}$ ratio was 1.34 ± 0.2 and 3.7 ± 0.52 for phenylalanine (4 mg/mL) used as a positive control. At concentrations of 0.2% and 0.4%, the oil extracted from the
seeds of *Azadirachta indica* J. significantly improves (p <0.05) this ratio, to 4.8 ± 1 and 6.65 ± 1.68 respectively, corresponding to the oxygen-hemoglobin increase rate (TAO) of 70.99 ± 6% and 79.05 ± 3%. As for omega 3 (ω3: 0.2%), its TAO (70.58 ± 5%) is not significantly different (p <0.05) from that of AO (0.2%). These values are greater than 50.04 ± 4% obtained by [30] with the lipid fraction of *Telferia occidentalis* seed. This increase may be due to the fact that these oil samples would prevent the oxidation of ferrous iron (Fe^{2+}) and thus increase the affinity of hemoglobin S for oxygen. Indeed, it has been shown that AO (0.4%) and omega 3 (ω3: 0.2%) had large chelating activities [4, 8]. It may also be due to vitamin E recognized as a powerful antioxidant. Indeed, it has been shown that this oil contains 486.51 ± 0.7 μg/g of vitamin E [8].

Membrane transporters such as Ca^{2+}-activated K⁺ channel and KCl cotransporter contribute to dehydration [34]. In the erythrocyte membrane, ATPases drive energy-dependent transport of electrolytes an activity that is abnormally high in sickle cell anemia resulting to sickling of the red cells [35]. All the ATPase activities of the erythrocyte membranes of sickle cells expressed in μmol Pi/mg proteins/hour × 10^4 have been studied by treating the hemolysates of the erythrocyte membranes with Phe: 4 mg/mL and omega 3 (ω3: 0, 2%); AO (0.2%) and AO (0.4%). Phenylalanine, a known anti-sickling agent, at 4 mg/mL, activated both Na⁺, K⁺- and Ca^{2+}-ATPases, but inhibited the Mg^{2+}-ATPase activity HbS erythrocytes membrane preparations.

In the absence of oil samples, the activity of Na⁺/K⁺-ATPase was 123.30 ± 33. The greatest activity (209.35 ± 27) was obtained in the presence of phenylalanine (Phe: 4 mg/mL) used as a positive control. With oil samples, the greatest activity (203.82 ± 21) was obtained with AO (0.4%) and omega 3 (ω3: 0.2%) followed by AO (0.4%) (190.48 ± 32). These values which are significantly higher (p <0.05) than that of the negative control is less than 220.1 ± 0.9 obtained by [20] with the aqueous extract of *Zantoxylum macrophylla* and tends towards the reference range [272.4-276.6].

In the absence of oil samples, the activity of Ca^{2+}-ATPase was 144.26 ± 61. The greatest activity (275.96 ± 73) was obtained in the presence of phenylalanine (Phe: 4 mg/mL) used as a positive control. With the oil samples, the greatest activity (235.61 ± 52) was obtained with omega 3 (ω3: 0.2%) followed by AO (0.4%) (190.48 ± 32). These values which are significantly greater (p <0.05) than the value of the negative control are less than 258.8 ± 0.8 obtained by [20] with the aqueous extract of *Zantoxylum macrophylla*, but nevertheless tends towards the reference range [290.5 - 296.7].

As for the Mg^{2+}-ATPase from the erythrocyte membrane, phenylalanine (4 mg/mL), omega 3 (ω3: 0.2%); AO (0.2%) and AO (0.4%) significantly decrease (p <0.05) its activities compared to the negative control. The lowest activity (124.00 ± 21) was obtained with AO (0.4%) which tends more to restore the normal activity of Mg^{2+}-ATPase. This value is less than 135.4 ± 1.9 obtained by [20] with the aqueous extract *Zantoxylum macrophylla* and tends towards the reference range [101.2 - 105.8].

These different modulations may be due to the restoration of the membrane structure of sickle cell erythrocytes by these oils. Indeed, the composition and organization of membrane lipids are maintained throughout the life cycle of the cell. In the event of the polymerization, there is therefore a destabilization of the membrane structure in the event of a decrease in phospholipids [36]. The modulation of the activities of the three membrane-bound ATPases (increases for Na⁺/K⁺ and Ca^{2+}-ATPases, and decreases for Mg^{2+}-ATPase) by the omega 3 and *Azadirachta indica* J. seed oil noted in this study suggest vital roles for the natural product in the maintenance of the shape/volume of erythrocytes. It further provides some clues at the molecular level on the possible roles of the ATPases in the sickling of erythrocytes, and the role of the omega 3 and *Azadirachta indica* J. seed oil in reversing this sickling [7, 8] thereby supporting its use in the therapy of the sickle cell patients.

**Conclusion**

Omega-3 at 0.2% and *Azadirachta indica* J. seed oil used in the management of SCD in the North Region of Cameroon at 0.4% inhibit the polymerization, increase rates of oxygen-hemoglobin of hemoglobin S and modulate the membrane ATPasic activities. These results justify the antisickling, antioxidant and membrane stability properties of these oils. Nutraceutical capsules/functional foods formulation with these oils for SCD management will be the next point of focus within this research.

**References**

Omega 3 and Azadirachta Indica J. Seed Oil Inhibit Hemoglobin Polymerization and Modulate Erythrocyte Membrane ATPases in Sickle Cell Disease (SCD)