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Effect of Silver Nanoparticles Synthesized from *Azadirachta indica* Leaves Extract on Sickle Erythrocyte Membrane Stability

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To synthesize silver nanoparticles (AgNPs) from *Azadirachta indica* leaf extract and determine its effect on membrane stability.
Place and Duration of Study: Department of Biochemistry, Sokoto State University, Sokoto, Nigeria, between January 2021 and April 2021.
Methodology: Simple characterization using uv-vis spectroscopy was conducted and the well-known method for osmotic fragility was employed.
Results: Surface plasmon excitation for conversion of Ag+ to AgNPs was successfully achieved at 320 nm. The result revealed increase in membrane stability with 67.50 % for sickle erythrocyte (HbSS), 33.33 % for carrier erythrocyte (HbAS) and 23.33 % for normal erythrocyte (HbAA); thereby decreasing the erythrocyte lysis in the order of HbSS>HbAS>HbAA.
Conclusion: AgNPs synthesized from *A. indica* reduces osmotic fragility and hence increases membrane stabilizing potential particularly against sickle erythrocyte.

Keywords: Azadirachta indica; erythrocyte; osmotic fragility; silver nanoparticles; sickle cell disease.

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1. INTRODUCTION

Metallic based nanoparticles have attracted scientists for decades which are now hugely utilized in the field of engineering and biomedical sciences [1]. The age-old application of silver in the making of utensils for drinking water and eating was probably due to its antibacterial nature. Nanotechnology now an emerging technology in this era due to its ability to modulate metals to nano-size which eventually changes their properties [2]. Materials in the nano-dimensions have very wide surface to volume ratio that gives them unique properties that differs from same material in bulk that are valuable in different fields such as electronics, photonics, biomedical, catalysis, etc. [3]. These properties of nanoparticles can be utilized in various areas including but not limited to of solar energy conversion, water treatment among others. Among the noble metals, silver is the most preferable in nanoparticles formation because of its antibacterial and catalytic properties with non-toxicity report in human cells [2], in comparison to other metals. Several methods have been used for the preparation of silver nanoparticles which can be physical, chemical or biological. Earlier methods used for the synthesis of silver nanoparticles were toxic and hazardous chemicals were used for their synthesis. Thus, the use of eco-friendly processes for the synthesis of silver nanoparticles is known as ‘‘Green synthesis’’. Green synthesis is preferred over conventional synthesis because it is ecofriendly, cost-effective, single-step method that can be easily scaled up and does not require high pressure, temperature or energy [4,5]. Research have reported the use of materials such as plant leaf, root, stem, bark, leaf, fruit, bud and latex extracts for the green synthesis [6].

Erythrocytes or red blood cells (RBCs) are the most abundant cell type in human blood. These cells are devoid of certain organelles which are important in other cell types to operate for their survival [7]. This unconventional nature has evolved so as to allow the accumulation of hemoglobin, a protein responsible for oxygen (O_2) delivery to the peripheral tissues. In a normal healthy individual, two million newly formed RBCs from the bone marrow get in to circulation, and at the same time approximately same quantity is cleared [8]. RBC production, otherwise called erythropoiesis, is tightly regulated process that produce new RBC in the bone marrow, and other related cells like endothelial cells (ECs), hematopoietic cells, osteoblasts, extra-cellular matrix proteins among others. Within the bone marrow, they are in directly associated with growth factors, cell adhesion molecules, and cytokines [9]. The RBC membranes undergo different morphological alterations and structural modifications right from maturation phase until it reaches the clearance phase. They undergo multiple and often tightly regulated processes for remodeling their structures which include the loss of some complex organelles and acquire the typical biconcave shape.

The plant extracts produce best capping materials for stabilizing nanoparticles [10]. In this work, the leaf extract of *A. indica* (commonly known as neem) was utilized for the green synthesis of silver nanoparticles. The plant is commonly used for treatment of certain ailments caused by bacteria, fungi, viruses since ancient times. The terpenoids and flavanones are the two most important phytochemicals present in neem which play a vital role in stabilizing the nanoparticle and also act as capping and reducing agents [11]. In this finding, AgNPs was synthesized from *A. indica* leaves and tested against the membrane stabilizing potential and was discovered to have increased the membrane stabilityby reducing the osmotic fragility preferentially on sickled erythrocytes.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The neem leaf was collected within Sokoto State University, behind one of the villages (Madorawa) in Bodinga local government area of Sokoto State, Nigeria. The neem leaves were washed with clean distilled water to remove the dust particles, and then placed in one side of the laboratory desk for air drying. Pestle and mortar were used for grinding the dried plant to powder form. The powder was stored for further analysis.

2.2 Preparation of Extract

Neem leaves powder (30 g)was transferred into 1000 ml beaker, then 400 ml of H_2O was added and left for 72 hours after which the extract was filtered using whatman filter paper.

2.3 Preparation and Characterization of AgNPs

Silver nitrate $AgNO_3$ was obtained from Sigma-Aldrich chemical company. All glass wares were

washed with distilled water and dried in oven. A stock solution of $AgNO_3$ (2 × 10-3 M) was prepared by dissolving 0.34 g/1000 ml in de-ionized water. Extract (100 ml) was added to 900 ml of the $AgNO_3$ stock solution and was kept in darkness and allowed to change colour to brown within 30 minutes. After that, the absorbance was taken using UV spectrophotometer at different wavelength of 20 nm intervals from 200 nm to 620 nm. Then, the content was evaporated and the powder obtained used to determine erythrocyte membrane stability using osmotic fragility test.

2.4 Blood Sample Collection

The blood samples; genotypes HbAA, HbAS and HbSS were collected into sterile EDTA (ethylene di-amine tetra acetic acid) bottle from Noma Hospital Sokoto, Hematology Laboratory.

2.5 Reagent Preparation

Stock solution of sodium chloride 1.0 % (w/v) NaCl: sodium chloride (1.0 g) was dissolved in distilled water and final volume made up to 100 ml. 0.1 M phosphate buffer solution pH 7.4, 13.65 g of Na_2HPO_4 and 2.24 g of KH_2PO_4 were dissolved in distilled water and the final volume made up to one liter. Working solution of buffered sodium chloride (0.00% - 0.90%) were prepared from the stock solution.

2.6 Determination of Membrane Stability; Osmotic Fragility Test

The method of Jaja et al., [12] was employed. This is based on lysis of erythrocyte cells when suspended in hypotonic saline solution due to endosmosis. The procedure is as follows: in an appropriately labeled duplicate centrifuge tubes containing 4.5 ml of different buffered saline concentration, 0.50 ml of AgNPs (1 g /5 ml) and 0.50 ml of suspended erythrocyte were added. The mixture was incubated at room temperature for 30 minutes, centrifuged at 2000 rpm for 5 minutes. The supernatant from each tube was collected, read spectrophotometrically at 540 nm against supernatant of 0.90% buffered saline concentration as blank.

3. RESULTS AND DISCUSSION

3.1 Results

Absorbance of silver nanoparticles (AgNPs) was successfully achieved by attaining plasmon resonance vibration at 320nm as shown in Fig. 1.

Percentage lysis of erythrocytes at 0.40% buffered saline concentration for control (without the addition of AgNPs) and in the presence of AgNPs synthesized from *A. indica* are presented in Table 1 and the bar chart representing the Percentage lysis and membrane stability of erythrocyte at 0.40% saline concentration is shown in Fig. 2.

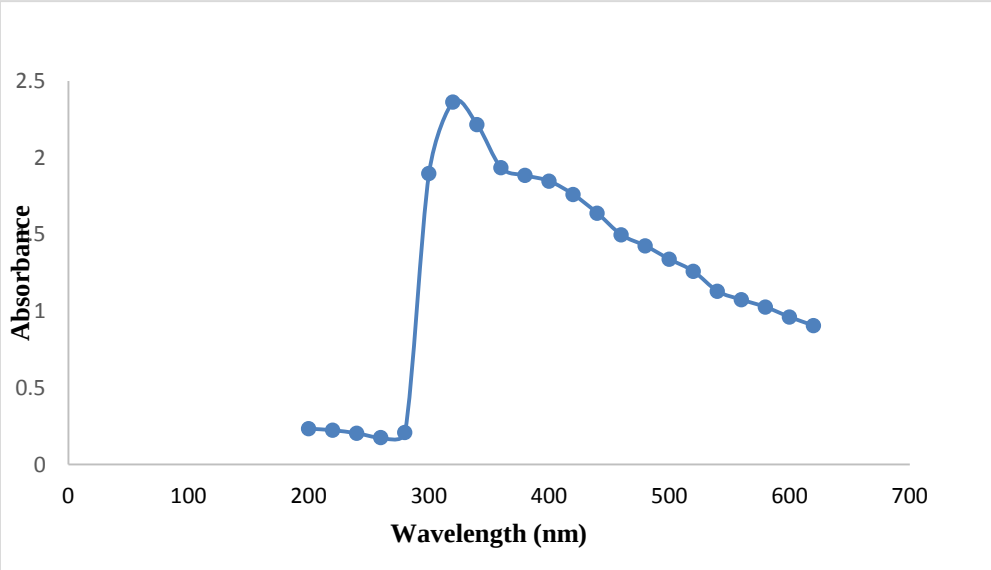

Fig. 1. UV-Vis spectroscopy of AgNPs using *A. indica* extract

Table 1. Percentage lysis of erythrocytes at 0.40% buffered saline concentration for control and in the presence of AgNPs synthesized from *A. indica*

Genotype	Absorbance of control	% Lysis of control	Absorbance of Sample + AgNPs	% Lysis of Sample + AgNPs	%Membrane Stability
HbAA	0.0060	100 %	0.0046 ± 0.001	76.67 %	23.33 %
HbAS	0.0060	100 %	0.0040 ± 0.001	66.67 %	33.33 %
HbSS	0.0040	100 %	0.0013± 0.0003	32.50 %	67.50 %

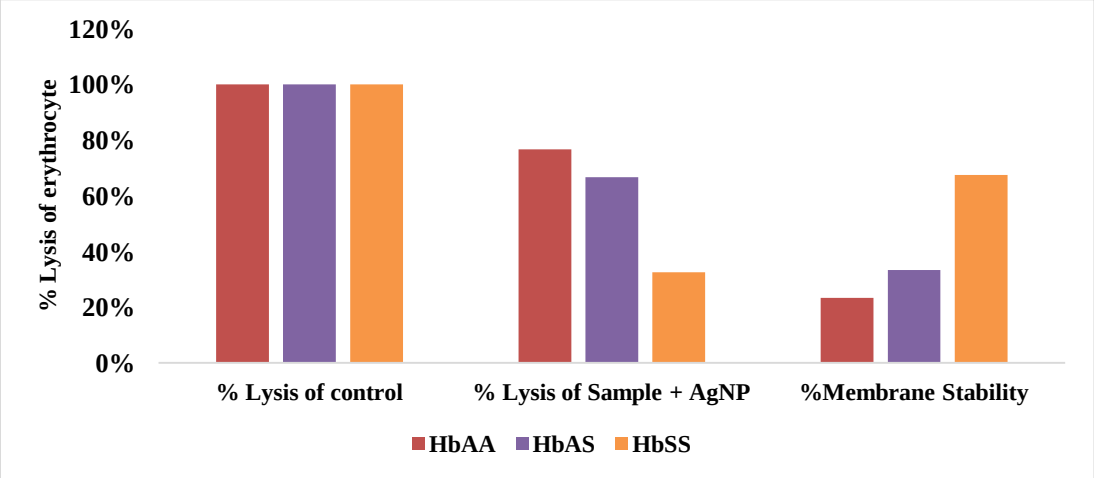

Fig. 2. Percentage lysis and membrane stability of erythrocyte at 0.4 % saline concentration

Key: Control = No AgNPs added
HbAA = Normal erythrocyte
HbAS = Carrier erythrocyte
HbSS = Sickle erythrocyte

3.2 Discussion

The orthodox medicine used for the treatment of sickle cell disorder stimulates HbF synthesis, antagonist of HbS gene, although effective but, is mutagenic [13]. Other report emerged as some anti-sickling agents are prolonging the delay time of hemoglobin polymerization [14]. The phytotherapy research is updated trends in use to manage tropical diseases and other genetic disorder such as sickle cell diseases with the aim of finding cheaper and alternative medicines which wide populations can have easy access when compared to orthodox ones [15]. This research observed that AgNPs synthesized from aqueous extract of neem leaves reduced the percentage lysis of human erythrocyte and increased the membrane stability. The result obtained from preliminary characterization revealed that the AgNPs synthesized from aqueous extract of (*A. indica*) in which Ag+ was converted to Ag0successfully by changing colour of the solution to brown. This was further confirmed by attaining surface plasmon resonance vibration at 320 nm. Upon osmotic fragility test, it was confirmed that the synthesized nanoparticles showed effectiveness in increasing erythrocyte membrane strength on sickle erythrocyte through the decrease in percentage lysis of HbSS at 0.40% buffered saline concentration.

However, AgNPs was found to have higher lysis reducing effect on sickled erythrocytes. During this process, AgNPs are able to diffuse freely in to the cell membrane and interact with cholesterol integrated in the membrane bilayer forming cholesteryl ester, thereby increasing the stability of the membrane.The usual observed trend is due to the ability of AgNPs or other agent to interact with erythrocyte membrane or probably due to their interaction with any amino acids involved in polymerization reaction [16]. It was also reported that, increase in surface area to volume ratio increases the resistance of erythrocyte to lysis [17,18]. Other plant materials were tested for their ability to prevent the sickle cell from lysis using similar method. The in vitro anti-lytic activity of Cajanuscaja seeds and Terminalia catappa leaves extracts can be attributed to phytochemicals present in these plants [1]. The author further confirmed that plant

extracts rich in anthraquinone, steroidal glycosides, cardiac glycosides, flavonoids and terpens derivatives inhibit osmotically induced hemolysis of erythrocyte. Similarly, Imaga et al., [15] reported that Carica papaya leaf extract protect the sickled erythrocyte membrane against osmotically induce lysis. Chikeze, [19], revealed that *A. indica* and *V. amygdalina* inhibit HbS polymerization which is one of the anti-sickling parametres. Also, leaves, fruits and stem extracts of *A. indica* were reported to have anti-sickling inhibitory effect [20]. Therefore, AgNPs Synthesized from aqueous extract of *A. indica* can be one of the alternatives for managing sickle cell disease crisis and other genetic disorders associated with erythrocyte lysis.

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

From the result which indicates that AgNPs was synthesized from *A. indica* and Ag+ was converted to nanoparticles by attaining the plasmon resonance vibration at 320 nm and showed effectiveness of decreasing the percentage lysis of sickle erythrocytes by increasing its membrane rigidity. The membrane stabilizing effect is more pronounced in HbSS than HbAS and the least stability was observed in normal erythrocyte (HbAA). Therefore, AgNPs synthesized from aqueous extract of *A. indica* confers an increased membrane stabilizing effect on the HbSS erythrocytes. This provides a scientific basis for the medicinal use of AgNPs from *A. indica* for the management of sickle cell disease.

4.2 Recommendation

It is recommended that further investigations should be done to ascertain the mechanism behind the cell membrane and the AgNPs in conferring its stability.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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