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Efficacy Of Plant Based Silver Nanoparticles In Preventing Toxicity Of Mercuryon Blood Cells

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ABSTRACT

This study explores the green synthesis of silver nanoparticles (AgNPs) using Neem leaves and Aloe vera leaves, characterized through UV-vis spectrophotometry and scanning electron microscopy. The research has a threefold objective: synthesizing and characterizing AgNPs, investigating their impact on blood contaminated with mercury toxin, and examining AgNPs binding with bio-coated red blood cells. While AgNPs couldn't prevent mercury-induced hemolysis, they effectively bound to RBCs in blood groups B+ and O+, with partial hemolysis in A+. These findings suggest potential biomedical applications. This research contributes to the broader fields of Nanotechnology, Biotechnology, and Environmental science, providing valuable insights into the potential of green-synthesized AgNPs and addressing the urgent need for innovative solutions to mercury contamination and its impact on human health and the environment.

KEYWORDS: Silver Nanoparticles (AgNPs), Green synthesis, Mercury detoxification, Red Blood Cells, Aloe-vera, Tulsi

Introduction

The synthesis of nanomaterials is currently one of the most active areas in Nanoscience. Special attention has been dedicated to nanomaterials that help improve the human quality of life. A remarkable example is the silver nanoparticles (AgNPs) which are known by their inhibitory and bactericidal effects (Vélez et al, 2018). AgNPs can be produced with various sizes and shapes depending on the fabrication method which can be physical, chemical, biological and hybrid. The chemical methods use toxic chemicals which are not friendly to the environment, making them unsuitable for biomedical applications. Specifically, the widely used chemical reduction methods (Bhui and Misra, 2012) usually employ toxic and perilous chemicals that are responsible for various biological risks. On the other hand, physical methods are expensive and incompatible with sizeable production of nanoparticles. Therefore, to avoid toxic and hazardous chemicals, the green synthesis methods have been developed, attracting significant interest because they are environment friendly, rapid, facile, and energy efficient (Mohapatra et al, 2015). Green synthesis using huge biological molecules derived from plant extracts (Ahmed et al, 2016) could facilitate size and morphology control of metal nanoparticles due to the presence of an numerous of biomolecules possessing bio-reduction and bio-stabilization ability (Narayanan and Park, 2014). Specifically, many plants have been used for silver nanoparticles synthesis, such as stem bark of Callicarpa maingayi (Shameli et al, 2012), Terminalia cuneata (Edison et al, 2026), Illicium verum (star anise) (Luna et al, 2016) and pod extract of Acacia nilotica. (Jebakumar et al, 2013) Aloe vera extracts have been used for the synthesis of stable AgNPs in several previous articles investigating their antibacterial, antifungal, and mosquitocidal activity (Chandran et al, 2006; Medda et al, 2014; Dinesh et al, 2015; Zhang et al, 2010). Aloe vera extracts have substances that lead to steric repulsion between individuals preventing nanoparticles from aggregation (Dang et al, 2014). Using Aloe vera as surfactant prevents nuclei aggregation by decreasing the total surface energy because it contains a multitude of chemical constituents, such as amino acids, enzymes, minerals, vitamins (A, C and E), anthraquinones, lignin, monosaccharide, polysaccharides, salicylic acid, saponins, sterols, and minerals (calcium, phosphorous, potassium, iron, sodium, magnesium, manganese, copper, chromium and zinc) (Nandal et al, 2012). Population growth worldwide has led to increased demand for drinking water in recent years. However, water resources have suffered from pollution due to industrial sectors discharging various kinds of pollutants and harmful substances. Many kinds of pollutants such as herbicides, pesticides, dyes, plastics, oil derivatives and heavy metals (Alshawi et al, 2022; Mohammed et al, 2021; Ali et al, 2021; Ismail et al, 2022) have been found in water effluents, which is the main reason behind the development of health issues among people. Mercury and its compounds are among the most harmful species reported and adversely affect human health (Pomal et al, 2021). Mercury affects the central nervous system, liver, and kidney. Further, it can disturb the immune system due to impaired hearing, vision, paralysis and emotional instability. Many analytical techniques are employed for detecting mercury ions such as inductively coupled plasma atomic emission and mass spectroscopy, cold vapor atomic absorption spectroscopy, cold vapor atomic fluorescence spectroscopy and high-performance liquid chromatography with UV-Vis detection (Suvarapu and Baek, 2017). However, these techniques suffer from several limitations for instance time consumption, harmful solvents, equipment costs and complex procedures (He et al, 2023). Therefore, developing new approaches to determining mercury ions becomes crucial and an urgent need. Mercury (Hg) is a heavy metal that is released into the earth's atmosphere from natural sources such as volcanic action and human related sources such as coal combustion, waste combustion and smelting. Power plant flue gases are the largest source of Hg in the USA. In the atmosphere, chemical reactions convert Hg to divalent form ($Hg2^{+}$) that can bind to particulate matter. Both $Hg2^{+}$ and particulate bound Hg ingress from the atmosphere into water sources, where a small portion of the Hg can be converted into methylmercury by microorganisms and chemical processes. In water bodies, Hg accumulates in aquatic animals such as fish and others. The most common exposure of humans to mercury is through the consumption of marine and freshwater fish (Guallar et al, 2002). Historically, Hg has been used for medical purposes, such as in dental amalgam and in vaccines and flu shots as a preservative between 1930-1999 in the USAas well as in industrial products such as skin-lightening creams, antiseptic facial products, mercury-containing laxatives, diuretics, teething powders, and in latex paint. Mercury may also be ingested through high fructose corn syrup (Kostyniak, 1998). Mercury is harmless in insoluble form, but vapor or soluble forms such as inorganic mercury or methylmercury can be extremely toxic to humans. Most human mercury exposure occurs through inhalation of elemental mercury vapor released from dental amalgam and through the consumption of fish contaminated with methyl mercury (Lim et al, 2010). Once inhaled, elemental mercury vapor is rapidly accumulated into erythrocytes and undergoes oxidation to the mercuric ion (Hg) by catalase. Orally absorbed methylmercury is preferentially distributed into erythrocytes (~90%) and slowly turns into Hg through demethylation in the spleen and liver. It has been demonstrated that 6-15% of total mercury in the blood of populations consuming high-fish diets exists in the form of inorganic mercury (Berglund et al, 2005; Rodrigues et al, 2010). Because elemental mercury or methylmercury ultimately turns into $Hg2^+$ in the body (Zalups, 2000), mercuric salt has been commonly used to investigate the toxicity of mercury. After being ingested, Hg is absorbed into the bloodstream. Hg has been shown to affect protein stability, mineral loss and to result in enzyme denaturation.Silver nanoparticles (AgNPs)are widely used for therapeutic interventions and diagnosis in medical practices and have gained increasing popularity as drug carriers, nano-probes, bio-imaging and labeling agents (Bian et al, 2019). Along with increasing application of AgNPs in nano-medicine. However, concerns are also escalating over its potential toxicity against human health (Bian et al, 2019). Potential adverse effects of AgNPs against human health have been first illuminated at a concentration range of 25 ~ 500 µg/mL for 10 ~72 hrs duration in vitro, with respect to its cytotoxicity on human cell lines, reactive oxygen species (ROS) generation, oxidative stress, cell- cycle arrest, pro-apoptotic effects and genotoxicity. AgNPs readily enters the systemic circulation. Accordingly, much attention has been paid to the potential cardiovascular toxicity of AgNPs due to an easy access of AgNPs to the tissues of cardiovascular system like blood cells, heart, and blood vessels. Previously, we demonstrated that AgNPs can activate platelets, which ultimately contributed to increased thrombosis. Thrombosis and embolism are serious life-threatening complications of various diseases with hypercoagulable states that include cancer (Bian et al, 2019), nursing home confinement, surgery, trauma and heavy metal intoxication. In addition to platelets, red blood cells (RBCs), a major cellular component of blood can participate in venous thrombosis through facilitating coagulation cascade and clot formation by providing a site for the assembly of prothrombinase and tenase complexes (Bian et al, 2019). Externalization of phosphatidylserine (PS) an anionic phospholipid to the outer leaflet of lipid bilayer is key in this process (Bian et al, 2019).

Materials and methods

Synthesis and Characterization of Nanoparticles: Aloe vera based leaf extract- (Preparation of leaf extract) 15g of inner leaf juice of Aloe vera leaves was heated at 80°c for two hours and then dried. It was then use for aqueous extract, using a ratio of 0.1:3, dry material to solvent. The resulting extracts use in all synthesis after being filtered by gravity (Vélez et al, 2018).

Preparation of Aloe vera based silver nanoparticles: 101.92 mg of $AgNO3$ was added in 50ml of distilled water. Add 50ml of this solution to 30ml of aqueous Aloe vera extract. The whole reaction was carried out in presence of air and constant & neutral pH. Stir mixture at temperature of 57°C during 3 hours and then heat 2°C/min to reach 80°C holding for 2 hours until obtaining solution with small suspended particles that could be removed by simple filtration (0.45µm).

Neem based leaf extract - (Preparation of leaf extract) Collected fresh Neem leaves and rinse thoroughly with distilled water to remove dirt. Spread rinsed leaves on a clean surface in well-ventilated area away from direct sunlight. Allowed the leaves to dry naturally in the shade until they become crispy. The dried leaves are grinded into a fine powder using mortar and pestle.

Measure 5g of Tulsi powder and transfer it to a clean glass beaker. Add 100ml of distilled water to the beaker containing the Tulsi leaf powder. Place the beaker a hot plate or stirrer and heat the mixture at 60° to 80°C temperature for 30 minutes. Then, cool the mixture to reach room temperature. Filtered the extract using a filter paper to separate the liquid extract from any solid residue.

Preparation of Neem leaves based silver nanoparticles: Prepared a silver nitrate solution by dissolving ad 1mM (16.987 mg) silver nitrate in 100ml D/W. Place it at magnetic stirrer, set temp at 60-70C. Add leaf extract drop wise. (nearly 10ml leaf extract) until it turns yellowish-brown color. Keep stirring continuously for 1-2 hours, to ensure complete reduction of silver ions & formation of silver nanoparticles. After the reaction allow AgNPs to settle at room temperature. The obtained AgNPs are purified & separated from others by centrifuge at 6000 rpm for 20 min in D/W (Repeat 3 times). After centrifugation the pellet was washed and dried and prepared powder of it. Dried silver nanoparticles were used for characterization. UV-Vis spectroscopy measures the absorption of light by nanoparticles. Silver nanoparticles exhibit a characteristic absorption peak in the UV- Vis spectrum, known as the surface plasmon resonance (SPR) peak. The SPR peak position and intensity provide information about the size and concentration of the nanoparticles.

Scanning Electron Microscopy (SEM): SEM is another imaging technique that provides surface morphology information of nanoparticles. It is particularly useful for analyzing larger nanoparticles and obtaining 3D images. Silver nanoparticles on blood contaminated by mercury toxin. Mercury chloride dissolved 0.2gm of Mercury Chloride ($HgCl_2$) in 10ml of distilled water. Mixed a small percentage of mercury chloride solution with blood.

Test: Mixed a small percentage of synthesized silver nanoparticles with blood and added the mercury chloride solution to the blood. Performed staining and observed the structures of the blood cells under light microscope and analyzed the structures and checked the nanoparticle efficacy. Binding of bio-coated RBCs with silver nanoparticles, bio-coating of RBCs with PEG: Prepared PEG buffer by mixing 100ml of PEG in 50ml distilled water. Taken blood samples of different blood group; A+, B+ and O+. Centrifuged blood samples at 10000rpm for 5 min. Discarded supernatant. Combined RBCs and PEG buffer solution in equal proportion (1:1). Incubated overnight at 4°C. Next day centrifuged RBCs with low speed to pellet them out. Performed blood staining to ensure there is no destruction of RBCs after bio-coating. Binding of bio-coated RBCs with silver nanoparticles: Added silver nanoparticles solution in bio-coated RBCs. Incubated RBCs overnight at 4°C. Next day performed blood staining of all the different blood groups and observed the structures of the blood cells under light microscope and analyzed the structures and checked the nanoparticle efficacy.

RESULTS

1. Aloe vera based silver nanoparticles: The aloe vera extract which are extracted and dried are mixed with AgNO3 for 3hrs for proper breakdown of metabolites and giving maximum exposure to mixing with silver nitrate solution, the resultant product formed was then separated using gravity filtration. A brownish orange solution was formed which confirms the result of nanoparticles was formed. (Fig.1). Neem leaves based silver nanoparticles: The neem leaves extracts were mixed with AgNO3 for 3-6hrs until greenish slurry was formed. This slurry was then dried under a hot air oven for 5 min at 70-80°C. The dried extract was scraped out and stored in a reagent bottle. The dried extracts so formed was black coloured powder in nature (Fig. 2).

Figure 1: Aloe vera based synthesized nanoparticles: A brownish orange solution was formed which confirms the result of nanoparticles is formed

Figure 2: Neem leaves based silver nanoparticles: The dried extract was scraped out and stored in a reagent bottle.

2. Characterization of the nanoparticles: UV-Spectrophotometry:- Silver nanoparticles exhibit a characteristic absorption peak in the UV- Vis spectrum, known as the surface plasmon resonance (SPR) peak. The SPR peak position and intensity provide information about the size and concentration of the nanoparticles. Peaks were visualized under Ultra violet wavelength (190-900nm) at ranging around 300 370nm. 2.2 Scanning electron microscopy: - The nanoparticles formed was visualized under scanning electron microscope at 3000x the magnification. Nanoparticles were visualized majorly of Rod Shaped, and having size ranging up to 2-5 nanoscales. (Fig. 3) 2.3 Action on blood: - Impact of silver nanoparticles on blood contaminated by mercury toxin: - For comparative study on blood, healthy blood cells were stained and visualized under microscope. Staining was performed using Giemsa stain and spherical shaped RBCs were observed along with other cells such as neutrophils and eosinophils (Fig. 4).

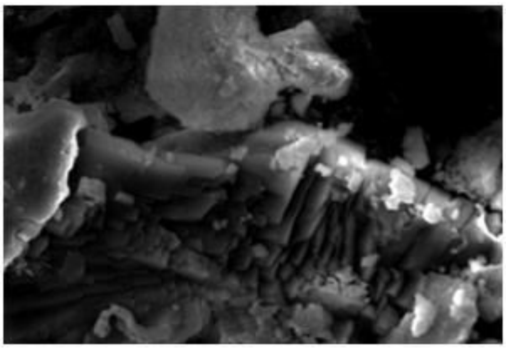

Figure 3: Scanning electron microscope: Nanoparticles were visualized Rod Shaped , and having size ranging up to 2-5 nanoscales.

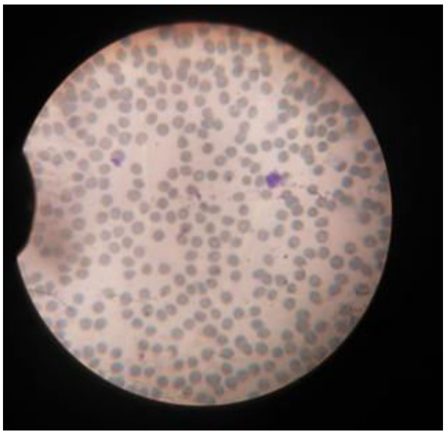

Figure 4: Healthy blood cells were stained and visualized under microscope. Staining was performed using Giemsa stain and spherical shaped RBCs were observed along with other cells such as neutrophils and eosinophils

Action of mercury on silver nanoparticle coated blood cells: - Used 3 different blood groups ie :- A+, B+ and O+ Control result: - Mixed a small percentage of mercury chloride solution with blood, the result were negative which indicated total hemolysis of RBC cells . (Table 1) (Fig. 5) Test result: - Mixed a small percentage of synthesized silver nanoparticles with blood and added the mercury chloride solution to the blood. the result was negative which indicated total hemolysis of RBC cells. (Table 2) (Fig. 6) 2.4 Binding of bio coated RBCs with silver nanoparticles: - It is observed that when the RBCs were exposed to nanoparticles, cell hemolysis occurred at very high rate. This was due to the extreme small size of the nanoparticles which led to these particles to enter and cross the cell membrane, leading to cell membrane rupture and causing hemolysis (Fig.7,8 and 9). This hemolytic behavior was countered by creating a bio coat over the RBCs using bio coating agents i.e. PEG (Poly-Ethylene-Glycol). PEG coating was performed and then the cells were exposed to silver nanoparticles and observed under microscope. The results were observed (Table 3). This table indicates that out of three selected blood groups only B+ and O+ didn't show hemolysis and A+ blood group shown partial hemolysis. It was observed that though there binding of nanoparticles over the PEG bio coat but in case of A+ blood grp the nanoparticles crossed the membranes of RBCs and entered the cell leading to osmotic shock and cell death. However B+ and O+ were alive which results in the binding of the nanoparticles over the surface of RBCs. This shows that the nanoparticles were prevented from entering the membrane walls of the cells. (Fig. 10, 11 and 12)

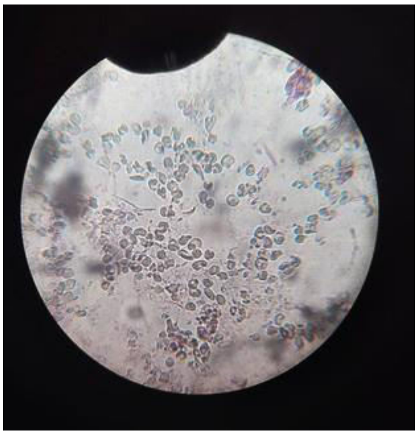

Figure 5: Control result (Hemolysis) The result were negative which indicated total hemolysis of RBC cells.

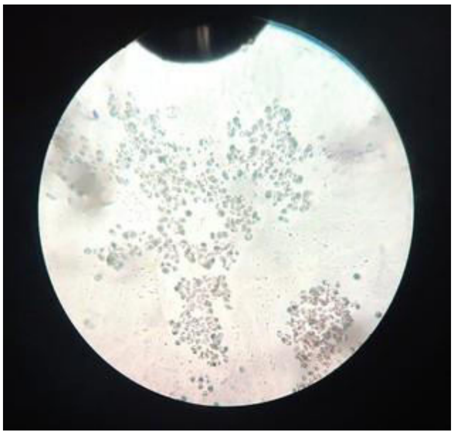

Figure 6: Test result (Hemolysis) The result were negative which indicated total haemolysis of RBC cells.

Table 1: Control Analysis

Sr. No	Test Blood Group	Shape analysis (without Nanoparticles)	Result
1	A+	Hemolysis Total cell death	Negative
2	B+	More than Partial hemolysis	Negative
3	O+	Hemolysis Total cell Death	Negative

Table2: Test Analysis

Sr. No	Test Blood Group	Shape analysis (with Nanoparticles)	Result
1	A+	Hemolysis Total cell death	Negative
2	B+	More than Partial hemolysis	Negative
3	O+	Hemolysis Total cell Death	Negative

Through the above tabular data formed we can conclude that red blood cells have undergone extreme oxidative stress in both the presence and absence of nanoparticles. Thus, without the help of any external bio coats, the oxidative action of mercury on the cell membranes could not be prevented under other circumstances.

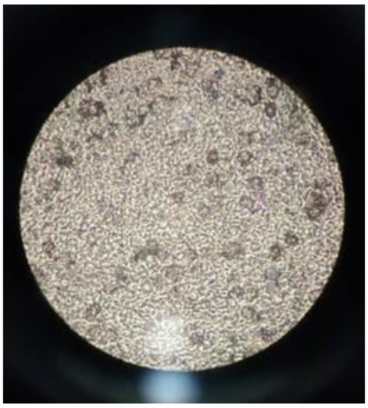

Figure 8: A+ Blood grp (Negative) [Non-biocoated]

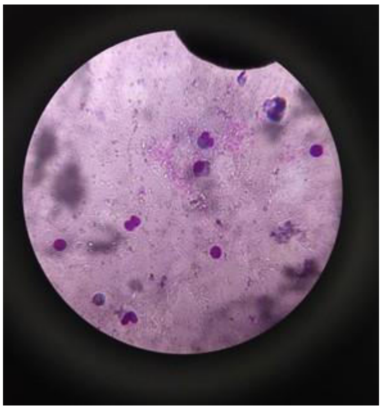

Figure 8: B+ Blood group (Negative [Non-biocoated]

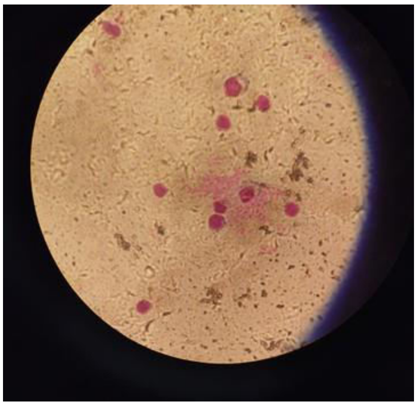

Figure 9: O+ Blood group (Negative) [Non-biocoated]

(The above test result resembles the action of silver-nanoparticles over non bio coated RBCs of blood group A+, B+ and O+)
A+ Blood group (Negative) [Non-biocoated]
B+ Blood group (Negative) [Non-biocoated]
O+ Blood group (Negative) [Non-biocoated]

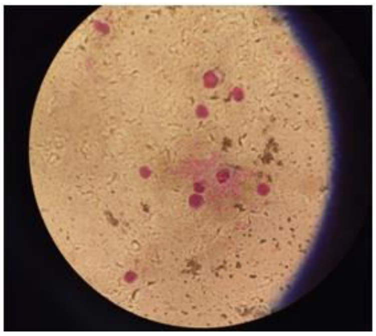

Figure 10: A+ Blood group (Negative) [Biocoated]

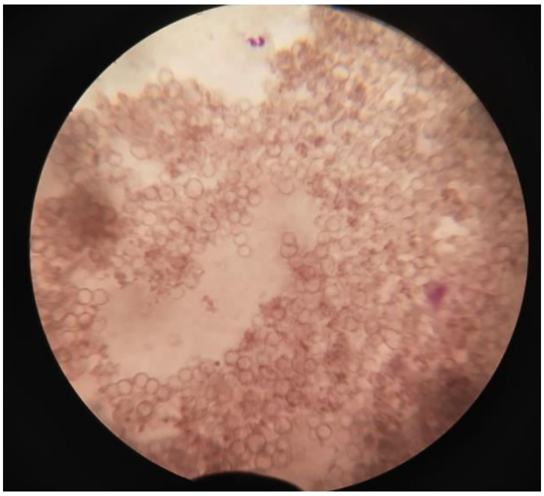

Figure: B+ Blood group (Positive) [Biocoated]

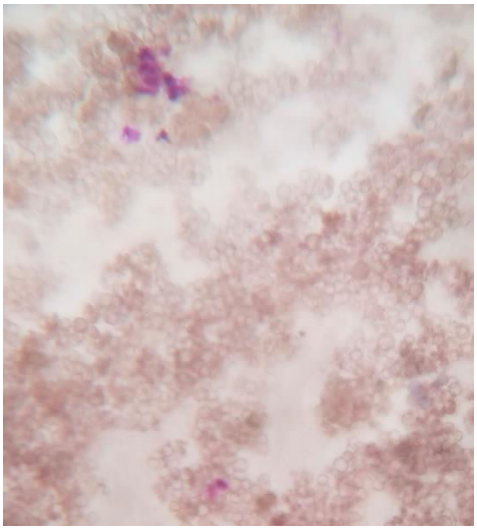

Figure 12: O+ Blood group (Positive) [Biocoated]

DISCUSSION

The green synthesis of silver nanoparticles (AgNPs) using plant extracts has demonstrated its potential as an environmentally friendly and efficient method. In this study, the use of Neem and Aloe Vera leaves for AgNPs synthesis was explored. The characterization of these Nanoparticles through UV-Vis Spectrophotometry and scanning electron microscopy confirmed their successful production and established their unique properties. The obtained nanoparticles were found to be in a state of solid as well as in liquid state. However, upon analysis the liquid form of Nanoparticles shown more efficient activity as compared to the solid nanoparticles, while the solid nanoparticles shown much better results during shape and structural analysis. One of the primary objectives of this research was to evaluate the impact of AgNPs on blood contaminated by mercury toxin. Notably, our experiments revealed that AgNPs were unable to prevent the destruction of red blood cells (RBCs) induced by mercury chloride contamination. This result underscores the severity of mercury toxicity and the urgent need for effective detoxification methods. As a response to the challenges posed by mercury-induced hemolysis, the focus of this study shifted to the binding of bio-coated RBCs with AgNPs. In these experiments, it was observed that AgNPs effectively bound to RBCs of blood groups B+ and O+, with only partial hemolysis observed in the A+ blood group. This finding points to the potential of AgNPs as a means of mitigating the effects of mercury toxicity, particularly in specific blood group scenarios. Though this experiment was conducted under few blood group type the work on expanding the research on other blood sugars are needed to be explored (Ahmed, 2020).Through other ideal research writing, we acknowledged that mercury concentration when upon reaching a certain concentration can lead to total cell hemolysis and non bio-coated RBCs with AgNPs could prevent this cell necrosis, thus efficiency of nanoparticles and bio-coating ability of RBCs are some major fields which are needed to be worked on (Houston et al, 2011).

CONCLUSION

In conclusion, the green synthesis of silver nanoparticles using plant extracts provides a promising avenue for the development of innovative nanomaterials with a range of applications. The significance of this research lies in its contribution to the understanding of mercury detoxification and the potential use of AgNPs as a therapeutic strategy. However, it is clear from our findings that while AgNPs exhibit favorable properties, they are not a complete solution for preventing mercury-induced hemolysis in all blood groups. Further research is necessary to explore more effective detoxification methods or to enhance the binding efficiency of AgNPs to RBCs.The pressing need for effective mercury detoxification methods, given the widespread contamination of water sources, underscores the importance of ongoing research in this field. This study opens the door to future investigations, including the optimization of nanomaterial binding, exploration of biomedical applications beyond detoxification, and further environmental remediation efforts. In conclusion, this research contributes to the broader fields of nanotechnology, biotechnology, and environmental science, providing valuable insights into the potential of green-synthesized AgNPs and addressing the urgent need for innovative solutions to mercury contamination and its impact on human health and the environment.

When AgNPs are used to treat mercury toxicity in red blood cells (RBCs), it can raise immunological issues. It can also produce inflammation through interactions with macrophages, which may intensify the consequences of mercury poisoning or prevent the nanoparticles from binding to RBCs. Furthermore, unique surface characteristics or coatings on nanoparticles could stimulate immunological recognition and cause inflammation. To fully analyse AgNPs immunomodulatory effects on mercury toxicity, more research is necessary. This research must include in-depth analyses of immune cells, cytokine production, and general immunological function.

FUTURE LINE OF WORK

Given that mercury contamination is a significant health concern, further research can focus on developing more effective methods for detoxifying blood or water sources contaminated with mercury. This could involve the use of advanced nanomaterials or innovative green synthesis approaches. Collaborate with experts in fields such as medicine, nanotechnology, and environmental science to tackle complex issues related to mercury contamination and the development of sustainable solutions. Investigate the potential biomedical applications of silver nanoparticles in scenarios beyond mercury detoxification. This might include their use in drug delivery systems, wound healing, or as antimicrobial agents. Continue research on the safety aspects of silver nanoparticles, especially in the context of their interaction with human blood. This can include toxicity studies and long-term effects.

REFERENCES

1. Ahmed S, Ahmad M, Swami BL, Ikram S. (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research. 7(1):17-28. doi:https://doi.org/10.1016/j.jare.2015.02.007.
2. Alshawi JMS, Mohammed MQ, Alesary HF, Ismail HK, Barton S. (2022) Voltammetric Determination of Hg2+, Zn2+, and Pb2+ Ions Using a PEDOT/NTA-Modified Electrode. ACS Omega. 7(23):20405-20419. Published 2022 Jun 1. doi:10.1021/acsomega.2c02682.
3. Ahmed Th. A. Ibrahim. (2020) Toxicological impact of green synthesized silver nanoparticles and protective role of different selenium type on Oreochromis niloticus: hematological and biochemical response. Journal of Trace Elements in Medicine and Biology,Volume 61,126507, ISSN 0946-672X
4. Ali, Ismail HK, Alesary HF, Aboul-Enein HY. (2021) A nanocomposite based on polyaniline, nickel and manganese oxides for dye removal from aqueous solutions.18(7):2031-2050. doi:https://doi.org/10.1007/s13762-020-02961- 0.
5. Berglund M, Lind B, Björnberg KA, Palm B, Einarsson Ö, Vahter M. (2005) Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environmental Health. 4(1). doi:https://doi.org/10.1186/1476-069x-4-20.
6. Bhui DK, Misra A. (2012) Synthesis of worm like silver nanoparticles in methyl cellulose polymeric matrix and its catalytic activity. Carbohydrate Polymers. 89(3): 830-835. doi:https://doi.org/10.1016/j.carbpol.2012.04.017
7. Bian Y, Kim K, Ngo T, et al. (2019) Silver nanoparticles promote procoagulant activity of red blood cells: a potential risk of thrombosis in susceptible population. Part FibreToxicol. 16(1):9. Published 2019 Feb 14. doi:10.1186/s12989- 019-0292-6.
8. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. (2006) Synthesis of Gold Nanotriangles and Silver Nanoparticles Using Aloe vera Plant Extract. Biotechnology Progress. 22(2):577-583. doi:https://doi.org/10.1021/bp0501423.
9. Dang TMD, Le TTT, Fribourg-Blanc E, Dang MC. (2011) Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2(1):015009. doi:https://doi.org/10.1088/2043-6262/2/1/015009.
10. Dinesh D, Murugan K, Madhiyazhagan P, et al. (2015) Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from Aloe vera extracts: towards an effective tool against the malaria vector Anopheles stephensi? Parasitology Research.114(4):1519-1529. doi:https://doi.org/10.1007/s00436-015-4336-z.
11. Edison TNJI, Lee YR, Sethuraman MG. (2016) Green synthesis of silver nanoparticles using Terminalia cuneata and its catalytic action in reduction of direct yellow-12 dye. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy. 161:122-129. doi:https://doi.org/10.1016/j.saa.2016.02.044.
12. Guallar E, Sanz-Gallardo MI, van't Veer P, et al. (2002) Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med. 347(22):1747-1754. doi:10.1056/NEJMoa020157.
13. He Z, Li F, Zuo P, Tian H. (2023) Principles and Applications of Resonance Energy Transfer Involving Noble Metallic Nanoparticles. Materials (Basel). 16(8):3083. Published 2023 Apr 13. doi:10.3390/ma16083083.
14. Houston, M.C. (2011), Role of Mercury Toxicity in Hypertension, Cardiovascular Disease, and Stroke. The Journal of Clinical Hypertension, 13: 621-627.
15. Ismail HK, Ali, Alesary HF, Nile BK, Barton SK. (2022) Synthesis of a poly(p-aminophenol)/starch/graphene oxide ternary nanocomposite for removal of methylene blue

dye from aqueous solution. Journal of Polymer Research. 29(5). doi:https://doi.org/10.1007/s10965-022-03013-6.

16. Jebakumar Immanuel, Edison TN, Sethuraman MG. (2013) Electrocatalytic Reduction of Benzyl Chloride by Green Synthesized Silver Nanoparticles Using Pod Extract of Acacia nilotica. ACS Sustainable Chemistry & Engineering. 1(10):1326-1332. doi:https://doi.org/10.1021/sc4001725.
17. Jairo Lisboa Rodrigues, Simião S, Cristina V, Barbosa F. (2010) Methylmercury and inorganic mercury determination in blood by using liquid chromatography with inductively coupled plasma mass spectrometry and a fast sample preparation procedure. Talanta. 80(3):1158-1163. doi:https://doi.org/10.1016/j.talanta.2009.09.001.
18. Kostyniak PJ. (1998) Mercury as a potential hazard for the dental practitioner. N Y State Dent J. 1998;64(4):40-43.
19. Lim KM, Kim S, Noh JY, et al. (2010) Low-level mercury can enhance procoagulant activity of erythrocytes: a new contributing factor for mercury-related thrombotic disease. Environ Health Perspect. 118(7):928-935. doi:10.1289/ehp.0901473.
20. Luna CM, V.H.G. Chávez, Enrique DíazBarriga-Castro, Núñez NO, Mendoza-Reséndez R. (2014) Biosynthesis of silver fine particles and particles decorated with nanoparticles using the extract of Illicium verum (star anise) seeds. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy20 doi: https://doi.org/10.1016/j.saa.2014.12.076.
21. Medda S, Hajra A, Dey U, Bose P, Mondal NK. (2014) Biosynthesis of silver nanoparticles from Aloe vera leaf extract and antifungal activity against Rhizopus sp. and Aspergillus sp. Applied Nanoscience. 5(7):875-880. doi:https://doi.org/10.1007/s13204-014-0387-1.
22. Mohapatra B, Kuriakose S, Mohapatra S. (2015) Rapid green synthesis of silver nanoparticles and nanorods using Piper nigrum extract. Journal of Alloys and Compounds. 2015; 637:119-126. doi:https://doi.org/10.1016/j.jallcom.2015.02.206.
23. Mohammed MQ, Ismail HK, Alesary HF, Barton SK. (2021) Use of a Schiff base-modified conducting polymer electrode for electrochemical assay of Cd(II) and Pb(II) ions by square wave voltammetry. Chemical Papers. 76(2):715- 729. doi:https://doi.org/10.1007/s11696-021-01882-7.
24. Narayanan KB, Park HH. (2014) Antifungal activity of silver nanoparticles synthesized using turnip leaf extract (Brassica rapa L.) against wood rotting pathogens. European Journal of Plant Pathology.140(2):185-192. doi:https://doi.org/10.1007/s10658-014-0399-4.
25. Pomal NC, Bhatt KD, Modi KM, et al. (2021) Functionalized Silver Nanoparticles as Colorimetric and Fluorimetric Sensor for Environmentally Toxic Mercury Ions: An Overview. J Fluoresc. 31(3):635-649. doi:10.1007/s10895- 021-02699.
26. Shameli K, Bin Ahmad M, Jaffar Al-Mulla EA, et al. (2012 Green Biosynthesis of Silver Nanoparticles Using Callicarpa maingayi Stem Bark Extraction. Molecules. 17(7):8506-8517. doi:https://doi.org/10.3390/molecules17078506.
27. Suvarapu LN, Baek SO.(2017) Recent Studies on the Speciation and Determination of Mercury in Different Environmental Matrices Using Various Analytical Techniques. Int J Anal Chem. 2017:3624015. doi:10.1155/2017/3624015.
28. Urvashi Nandal, Bhardwaj R, KrishiVigyan Kendra, Pratap M. ALOE VERA: (2012) A VALUABLE WONDER PLANT FOR FOOD, MEDICINE AND COSMETIC USE – A REVIEW. Published online January 1, 2012.
29. Vélez E, Campillo G, Morales G, Hincapié C, Osorio J, Arnache O. (2018) Silver Nanoparticles Obtained by Aqueous or Ethanolic Aloe vera Extracts: An Assessment of the Antibacterial

Activity and Mercury Removal Capability. Journal of Nanomaterials. 1–7. doi:https://doi.org/10.1155/2018/7215210

30. Zalups RK. (2000) Molecular interactions with mercury in the kidney. PubMed. 2000;52(1):113–143.
31. Zhang Y, Yang D, Kong Y, Wang X, Pandoli O, Gao G. (2010) Synergetic Antibacterial Effects of Silver Nanoparticles@Aloe Vera Prepared via a Green Method. Nano Biomedicine and Engineering. 2(4). doi:https://doi.org/10.5101/nbe.v2i4.p252–257.