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Investigation of Nephroprotective Potential of Cassia Absus L. Seeds Against Gentamicin-Induced Nephrotoxicity in Experimental Animals

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Abstract

Over the past couple of decades nanotechnology had helped advancements in all the fields like biomedical, biological and chemical sciences. Due to the development in the methods to synthesize nanoparticles in different shapes and sizes, nanotechnology is ruling in the field like medicine and agriculture. Silver nanoparticle has various important chemical and physical properties and because of this reason it is being studied widely all over the world to improve its application. These days so many methods are there to synthesize nanoparticle depending upon the usage, requirement and the availability. Nanoparticles synthesized by different- different methods have their own advantages and applications. Researches on these nanoparticles are always being carried upon to develop a technology with maximum benefits and minimal risk to environmental and human health as excess of the silver nanoparticles are cytotoxic, and unregulated discharge in the environment may have effects on both aquatic and terrestrial biota. This article discusses multiple methods for the synthesis of nanoparticles, advantages of different shapes and sizes and lastly the application of nanotechnology in Forensic science.

Keywords: Cassia absus, Creatinine, Gentamicin, Nephroprotective, Nephrotoxicity

Introduction

Because nanoparticles have a size below the diffraction limit of visible light, conventional optical microscopy is not useful. These are the particles defined as having a dimensional size between 10nm- 100nm.The characterization of nanomaterial is generally based on two methods: microscopic imaging and spectroscopic analysis. The imaging methods involve different types of microscopies such as electron microscopy, ion microscopy, and atomic force microscopy. Although microscopic methods have paved the way for scientists to see these objects at the nanoscale, most of the chemical, structural, and optical properties cannot be characterized simply by imaging. To overcome these problems, spectroscopic methods for characterization of nanoparticles have gained importance. This has opened new doors for exploring nanomaterial that mainly provides information supporting elemental and structural analysis.

The green synthesis of nanoparticles can also minimize the environmental challenges, scarcity of energy resources, and dependency on chemically derived nanoparticles. Green synthesis of nanoparticles is safer and easy to handle. Some of the commonly used sources for the manufacturing of green nanoparticles are plants, seaweed, algae, fungi, bacteria, etc. One of major advantages of gold/ silver nanoparticles synthesized from plant extract is that the process can be carried out at ambient temperature, atmospheric pressure, and performed in water, which represents an economical method for the synthesis of nanoparticles [1].

When smaller components are arranged together to form more complex aggregates this is referred to as the bottom-up approach. This method requires either a chemical or physical force that operates at the nanoscale to assemble smaller units into larger structures. However, the top-down approach leads to the formation of smaller nanoscale particles that are derived from larger ones.

Incredible properties of nanomaterials strongly depend on size and, shape of NPs, their interactions with stabilizers and surrounding media and also on their preparation method. So, controlled synthesis of nanocrystals is a key challenge to reach their (nanoparticles) better applied characteristics (different shapes)

Materials and Methods

Synthesis of nanoparticle

As the name suggests, synthesis means how the nanoparticles are going to prepare for any purpose. In the respective research synthesis of nanoparticles, extraction of drugs and detection of drugs using nanoparticles is being focused. There are so many methods or preparation techniques have been reported which can be used for the synthesis of nanoparticles like laser ablation, chemical reduction photochemical methods, ion sputtering, sol gel gamma irradiation, microwave processing and biological synthetic methods [2].

Physical methods

Evaporation- condensation and laser ablation are the most important physicals methods used for the synthesis of silver nano particles. As compared to chemical methods, physical methods have advantages because of the absence of solvent contamination in the prepared thin films and uniformity is also there in the synthesis of nanoparticles. But there is a disadvantage of physical method also, at the time of using a tube furnace at atmospheric pressure, for example space occupied by tube is very large, to achieve thermal stability it requires a lot of time and consumes a great amount of energy also.

Silver nanoparticles could be synthesized by using laser ablation method in solutions. PYATENKO, SHIMOKAWA and coworkers produced silver nanoparticles by irradiating an Ag target with a second harmonic of wavelength 532 nm in pure water. In all the earlier research on this technique in the liquid phase, the energy of the laser beam was comparatively low but, in their experiment, a maximum possible energy of laser pulse was used. In their experimental setup a beam from Nd: YAG laser was focused by a lens of focal length of 25

Camphorwood	55-80 nm	Quasispherical
Pipli	46 nm	Spherical
Hibiscus[33]	13 nm	Spherical

Table2: The formation of silver nanoparticles was confirmed by the change in color of the metal salt solution after the addition of the plant extract.

Shakeel Ahmed and team in their study reported the synthesis of nanoparticles using Azadirachta Indica plant aqueous leaf extract.. Typically, when the synthesis takes place through plant extract mediated bio reduction, it involves aqueous extract of the plant in the aqueous solution of suitable metal precursor generally in the form of salt. The extract of plant was prepared by cutting the fresh leaves of neem plant, boiling the leaves in distilled water for 30 minutes, and then filtering the extract with Whatman filter paper and being stored at cool temperature of around 4o C. Most preferable concentration of metal salt is millimoles. Synthesis of silver nanoparticles was also reported to be done by the aqueous extract of medicinal plant named Balsamina and Lantana camara.. Both of these plants are used for wound dressing in Indonesia. The formation of silver nanoparticle was confirmed by the color change in the solution. The solution into which balsamina leaves were added the color changes from colorless to brownish yellow whereas the solution into which lantana camara plant extract was added the color change take place from colorless to grayish brown. It also concluded that the presence of several polyphenolic compounds in the plant extract like flavonoids and terpenoids helped the reduction of Ag ions and also stabilized them [4].

Other studies also reported the preparation of silver nanoparticles with following biological agents-

- Coriandrum Sativam
- Apple extract
- Clitoria Ternatea
- Solanum Nigrum
- Aloe vera extract
- Plant extract of Shikakai and Reetha

Synthesis from Algae: As plants, algae are also present in the environment with a lot of abundance. Other than plant extract or chemicals, algae can also be used as a reducing agent for the reduction of silver ions in the solution. There are so many reports which has reported the synthesis of metallic nanoparticles. The different types of algae which can be used to synthesize silver nanoparticles like- Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana and Tetraselmis gracilis, gracilaria corticate and other algae like Chlorella Vulgaris, Spirulina platensis, Sargassum wightii, Kappaphycus alvarezii etc.

An experiment done by Laura et al., synthesis of both gold and silver nanoparticles took place. The algae used for the research was Chondrus Crispus and spirogyra insignis. The color of the solution was changed to brownish that confirmed the formation of nanoparticles. The research was done to evaluate the effect of pH on the size and the rate of formation of nanoparticles. And as result the efficiency of formation of silver nanoparticles at acidic pH was found to be very low so the characterization of nanoparticles formed ranging from pH 6 to 10 was done. SPR band appeared at 460 nm. But the U.V band spectra showed another band at higher wavelength because of the agglomeration.

The synthesis of silver nanoparticles by algae can be done two methods namely by normal marine micro algae or by microwave irradiated marine micro algae reported by Devina et al.. The algae species used in the research were Isochrysis galbana, Tetraselmis gracilis, Chlorella salina, Chaetoceros calcitrans. For the purpose, the mid exponential phase of algal growth was collected and then added to the silver nitrate solution. The brownish black colour of the solution confirmed the formation of silver nanoparticles with the plasmon resonance peak at 432 nm. One thing was most importantly observed that the reaction could only took place in light but not in the dark [4]. Some of the researches have also mentioned the synthesis of silver nanoparticles from polysaccharides extracted from marine macro algae. Some of the algae be it green or red, micro or macro which are being used to synthesize metallic nanoparticles are mentioned below. Sargassum wightii grevillea- a brown algae with the help of which silver nanoparticles can produced of size 8-27 nm.

- Caulerpa racemose- green macro algae, 10nm size
- Chaetomorpha linun- green macro algae, 3-44 nm size
- Gelidium amansii- red macro algae, 27-54nm size

Synthesis from fungi: Due to the production of proteins in large quantities, high yields, easy handling and very less toxicity, fungi became very useful for the synthesis of silver nanoparticles. The synthesis through algae gives an advantage that the biomolecules derived from the fungus improve the stability and confer the biological activity as well. Fungi also provide high tolerance to metals. Fungus provides advantages over bacterial systems because production of biomass is good and also no additional step is required to extract the filtrate. Also, fungal strains are chosen over bacterial one because their tolerance is better and metal bioaccumulation property is also good. When the fungi are used to synthesize the silver nanoparticles, the process involves culturing the fungus on agar, followed by the transfer to the liquid medium, the biomass produced is then transferred to the water to facilitate the release of compounds which will be helpful in the synthesis. Then after the filtration, biomass is discarded and the solution of $AgNO_3$ is added to the filtrate. Then the mixture is incubated at a temperature range of about 30o C to 60o C. This step should be done in dark for about 24 hours [5].

In a study reported by Hulikere et al synthesis of nanoparticles by marine endophyte fungi (Cladosporium cladosporioides) was reported. Marine endophytic fungi contain myriads of bioactive molecules, and surely have a capability of producing secondary metabolites like flavonoids, alkaloids, peptides polyphenols, quinones etc. the addition of aqueous extract of endophytic fugus to the solution of metallic salt the change of color from colorless to dark brown confirmed the formation of nanoparticles. The color change takes place due to the surface plasmon resonance of silver nanoparticles.

Entomopathogenic fungus can also be used to synthesize the silver nanoparticles as these provide advantages being non- pathogenic and insecticidal. However, studies who discuss about the synthesis of silver nanoparticles using entomopathogenic are lacking. Shruti Tyagi, Pankaj Kumar Tyagi and coworkers in their study synthesized nanoparticles using Beauveria bassiana an entomopathogenic fungi along with their antimicrobial potential. And after that the antimicrobial activity was compared with that of antibiotics and AgNPs-antibiotic combination as well. The reduction Ag+1 to Ago caused the change in the color of the solution thereby confirming the formation of nanoparticles. The maximum absorbance at 450 nm was observed.

There are so many fungus species which are being used for the purpose to synthesis of mostly spherical silver nanoparticles. For example

- Fusarium oxysporum
- Verticillium
- Aspergillus fumigatus
- Penicillium fellutanum
- Aspergillus flavus
- Fusarium semitectum
- Alternaria alternata
- Rhizopus stolonifera
- Phanerochaete chrysosporium

Synthesis through bacteria: Other than fungi and algae, bacteria have also been used for the production of silver nanoparticles. There are so many species of bacteria used for the same purpose to produce different shapes of nanoparticles. For example- Bacillus licheniformis, B. subtilis, Pseudomonas stutzeri AG259, Klebsiella pneumonia, E. coli, Enterobacter cloacae etc.

Shapes and characterization of silver nanoparticles: The properties of nanoparticles are significantly depending upon their shapes and sizes. Preparation method and stabilizer are also responsible. In various studies both non- spherical (planar) and 3 dimensional are being discussed.

Cubic shape cubic shaped nanoparticles are being synthesized by ethylene glycol as reducing agent where PVP can be used as stabilizer at a temperature of 161oC.. When the temperature was reduced or increased the formed silver nano particles shape was not cubic. Suggested that the shape was strongly dependent on the temperature. In other study silver cubes can also be synthesized by using ethylene glycol, PVP and HCl at a temperature of 140o C. A faster method was also suggested by Siekkinen et al.

Rod shape generally thermal, photochemical and electro-based template method are used to produce rod shaped nanoparticle. Orendoff et al synthesized silver nanorods using NaBH4 as reducing agent in the presence of citrate and allowed the continued growth of seeds into nanorods in the presence of ascorbic acid and CTAB. He was able to synthesize nanorods with longitudinal and transverse plasmon peaks. Potassium tartaric acid as a reducing agent in the presence of PVP was used by Gu et al. for the synthesis of nanoparticles in their study. The diameter and length of the nanorods was controlled by changing the ratio of silver nitrate and PVP. Wire shape to achieve nano wires a physical template is must needed like carbon nanotube or zeolites reported by Liu et al in their study. So, for the synthesis of particularly nanowires they used AgBr, $AgNO_3$ and gelatinous template and synthesized nanorods of 80nm diameter and 9 micrometer length. On the other hand, Jana et al used rod like micelle template for the synthesis of the same. There are so many other studies exist reporting the formation of nanowires using different-different chemicals (reducing and capping agents), different molar ratios of the reagents and using different physical templates as well. Bars and triangular (pyramid) shaped Wiley et al firstly reported the synthesis of pyramid- shaped silver nanoparticles using polyol method, in which ethylene glycol was the solvent and reducing agent applying NaBr. In the next research Wiley et al reported the synthesis of silver nano bars by only changing the concentration of NaBr. The method involves two solutions, first containing 48mg AgNO3 and 3ml Ethylene glycol and the second one containing 48 mg PVP and 0.068 mg NaBr in 3ml of EG using a 2-channel syringe pump added dropwise to 5 ml of EG which reached 1550Cin the oil bath. Process was completed after 1 hour and was characterized by SEM. Kai et.al synthesized triangular shaped nanoparticle by chemical reduction method which was done via suitable method that was quick and easy [1].

Discussion

Spherical and flower-shaped synthesis of flower shaped nanoparticle takes place by the reduction of silver nitrate with the help of ascorbic acid in the presence of PVP whereas in the other study reported, for the purpose of the synthesis of flower shaped silver nanoparticle, wet-chemical method in the presence of CTAB is reducing agent was used. The synthesis of spherical shaped nanoparticle, very convenient method using chemical reduction was reported by Kai et al. his study reported the synthesis of spherical shaped silver nanoparticle using silver nitrate, PVP and sodium borohydride as reducing, and stabilizing agents resulting in the formation of monodisperse silver nanoparticles.

Importance of nanotechnology in forensic science: So as DNA profiling has the greater influence on biotechnology in the past 50 years, when it came into existence, in the same way nanotechnology being an emerging field has also a greater influence on the modern technology. Nanoparticle has a great importance in several field of Forensic Science like fingerprints, security documents, paints, inks and in many more. It is also proven to be the most powerful tool. In the context of recent technical advancements, Nanotechnology is one such prodigal product. As nanotechnology can be used in various field of Forensic Science the growing demand of this nanotechnology has pushed most of the scientist to go in the efficient strategic objectives and sound skills in the field of nanotechnology. Introduction of nanotechnology in particularly forensic science has and will change the process of investigations as well. The use of nanotechnology has contributed a lot in forensic science by make the investigation process faster, more accurate, more effective, more sensitive and easier to apply. Latest nanotechnology-based techniques can help to analyze the evidence on the crime scene itself that will not only save the time of analysis but also leads to the reduction in chance of error. The respective field in which Nanotechnology plays a vital role in in forensic science are as follows

Nanoparticles in Fingerprints: Fingerprints are the marks which are left behind on any surface porous or non-porous by sweat residues which generally have to ability to identify a person. Out of the three types of fingerprints (latent, patent plastic) mostly latent print are found on any crime scene. These latent prints are not visible to the naked eye and also need chemical treatment to get visible on the surface. So, to make these types of prints visible, powder method is generally used. This powder mainly comprised of carbon black, aluminum flake for the development of fingerprint.. This finger print powder will stick to the residues which was left by the finger itself resulting in the formation of characteristics patterns which everyone identifies as a fingerprint. The ideal powder will only stick to the pattern and do not adhere to the background giving a clear appearance of the fingerprint pattern. Many common materials used to develop these fingerprint pattern on any surface, adhere with the background also and this adherence power contributes towards its drawback. Since they adhere to both print and the background it results in making a clear identification significantly harder to achieve. To overcome this drawback nanotechnology comes into action. As a very useful method for the fingerprint visualization on paper was discovered in the early 1980's focusing on the attachment of negatively charged silver

nanoparticles to the positively charged fingerprint residue resulting in the formation of clear image. The pH of this around 14. It is also observed that instead of silver, gold colloid can also be used to for the same purpose and at low pH it is highly negative and will easily attach to the positively charge fingerprint residue. Both of these particles (silver and gold) become nucleating sites for the Silver Physical Development. When it comes on reliability, many of the researchers prefer going through the colloidal gold approach for the generation of nucleating sites for Silver Physical Development. Gold colloidal is more reliable because they are more stable. With the use of gold nanoparticles targeting amino acids on the non-porous surface by researchers which will allow better analysis of latent fingerprints. Multi Metal Deposition was also reported to be useful in the visualization of latent fingerprints. For the detection of latent print late Dr. Menzel used photoluminescent CdS semiconductor nanocrystals capped with dioctyl sulfosuccinate. To apply nanocrystals dyes on articles pre-fumed with cyanoacrylate ester and without pre-fuming sticky side of the electrical tape was his main concept. He was the first who used quantum dots for the latent fingerprint visualization. A study by Samet band et el. Contributed towards the several steps forward in the application of nanotechnology for the latent print development. Two methods were developed. In the first one- in the petroleum ether, gold colloid was functionalized with long chain of hydrophobic molecules. These nanoparticles readily enter the lipid fraction of the latent fingerprint residue and act as nucleating sites for Silver Physical Developer and proved to be worked well in both non-porous and porous surface. This method works by a different principle than that of Multi Metal Deposition. In the second method Q-dots capped with ZnS was functionalized with the long chain of hydrophobic molecules like octadecane amine. And again, the development was achieved by the first method i.e. *via* hydrophobic interaction. ZnO-SiO_2 nano powder was also developed using the conventional heating method in which fingerprints usually developed by dusting and Small Particle Reagent (SPR). To solve gunshot/firing cases Mike worked and developed a new approach using a nanoscale developer in combination with an X-Ray source which can be useful for the visualization of latent print. This technique will work even if the has been washed or rubbed. CdSe/ZnS nanoparticles can also be used to enhance the latent fingerprint directly without the requirement of any additional development method. On the multi-colored or patterned backgrounds, hybrid nano powder can improve the visualization. Other nano powders are also being developed which are used in conjugation with SALDI-TOF_2-MS which will be helpful in the development and detection of fingerprints. Nano-Fingerprint Residue Visualization: Microscopy: The body creams and sunscreen lotions can be detected by using residual Titanium dioxide or Zinc oxide nanoparticles and while sweat can be detected due to its inorganic components [3].

Nanotechnology in detection of Explosives: So, as we know, terrorist activities are on edge and its spread has assumed an alarming proportion across the globe these days so the detection of explosive has become a very serious and important issue. This thing leads to the new research on new and advanced technologies for the protection of human life and property. In the Post Blast situation, detection of explosive generally involves the collection of vapors of the explosive material used for the blast and particulate samples and analyzing them using a sensitive sensor system. So, for this purpose nano sensors generally have the ability to fulfill all the requirements for an effective for the trace detection of explosives. Due to the tunable nature and unique properties of nanomaterial like carbon nanowires, nanotubes and other nanostructures, portable/ hand-held system which are most sensitive at molecular level could well be possible. The concept of "electronic nose" has been developed in 1980's aiming to imitate the sensing capabilities of human nose using electronic sensors and pattern recognition technology. These electric noses can detect extremely small quantities of airborne chemicals. Nanomaterials in Explosive Detection System consists of carbon nanotubes, nanowires and other nano structures having surface area and very unique set of optical, mechanical and electrical properties which make very useful in detection of molecules with high-sensitivity. A setup usually comprises of an array of nano sized sensors all together connected with the circuit. It works by changing the electrical signal in a unique way when each sensor unit reacts to the adsorption of analyte molecule such as trace explosive. The combination of responses from the whole array will produce a finger like complicated measurement. Nanomechanical sensors is considered as an alternative approach to chemical adsorption by using a nanomechanical response of cantilevers. Cantilever beam operate on the change in the resonance frequency generally due to mass adsorption. One advantage of this cantilevers system is that they can be made selective to a particular explosive substance. These are the more mature technology than sensors based on nanowires and nanotubes. A new technology has been developed by researchers for the detection of TNT (2,4,6, trinitro toluene) the integrates MIPs (molecularly imprinted polymers) with that of SERS. Procedure involves the deposition of xerogels of micron thickness derived from thin-films of sol-gel, on the SERS active surface as the sensing layer. After this the xerogels were molecularly imprinted for TNT using non-covalent interactions with the polymer matrix. When the TNT comes in contact of the polymer matrix, it results in the formation of unique SERS bands. Like this other sensor are also been developed which can detect even a single molecule of TNT by giving single molecule resolution. In this technique the carbon nanotubes are coated with protein fragments Bombolitins (found in bee venom). Different nanotube-peptide combination reacts differently to different nitro aromatic compounds so by using different nanotubes coated with different types of bombolitins will help in identifying unique 'fingerprints' to detect different types of explosives Pandya et al. in his study revealed the use of turmeric extracted curcumin- nanoparticle based detection of TNT up to 1nM level in the aqueous solution. And Chu et al. proposed the detection of TNT based on amine-terminated nanoparticles.

Nanoparticles in Questioned Documents: In the area of questioned document one such technique is AFM is which allows the visualization of ink and its examination crossing in documents to determine the sequence of strokes of the ink and the 3-D morphology. Ink analysis in the questioned document is the very important part. So, nano-sized have been introduced in the inks [8]. Various types of nanoparticles are been developed that can be useful in the new generation of anti-counterfeiting inks. Duplicating or counterfeiting of valuable is a serious problem so to prevent this security features, barcodes, holograms are being used generally on the document. Nanotechnology may offer a solution to this menace. Recently researchers at Tezpur University have developed a light emitting nano-composite based ink that will be invisible or barely detectable under visible light but will glow under UV light due to this ability the ink shows the potential to be used as an anti-counterfeiting material on paper and plastic. Pawan Kumar et al. in his study reported the described the unique combination of all the possible kinds of security ink formulations which was based on lanthanide doped luminescent nanomaterials, plasmonic nanomaterials, semiconductor and carbon-

based quantum dots as well as metal organic frameworks to make them possibly be useful in anti-counterfeiting applications.

Nanoparticles in DNA Analysis and Typing: As murder cases, rape cases and other crimes are increasing with each passing day so DNA for its analysis purpose is also becoming important and necessary. DNA analysis can be done by hairs, fibers, blood stains, saliva and semen resulting in punishing the perpetrator and put him behind the bars. DNA analysis is very expensive and time taking so to better and fast analysis, nanotechnology can be one of the most important techniques. Recent advanced devices used for DNA analysis are microfluidic devices with the advantages of shorter examination time, low risk of contamination, and can directly applicable to the crime scene. Various magnetic nanoparticles are also being developed for the extraction of DNA from biological sources. Also, other advancement in this technology includes formation of carbon nanotubes on which DNA can be analyzed using AFM and analyzing the DNA sequencing with the help of developing nanotechnology-based tools which can be used to read DNA sequence directly in a molecule. Other most important technology is microfluidic chip technology has proved to be very useful and effective in medical applications. At present the most widespread applications of micro-fluidic system is post-PCR quantization. These systems are currently used by the various Forensic Laboratories because it takes less than 2 minutes per sample for the analysis. Due to their small size use of these devices at the crime scene is suggested. As for DNA typing good PCR extraction is very important for which magnetic nanoparticle, and copper nanoparticles are being used. For the isolation of DNA, the magnetite nanoparticles with carboxylic compounds are used as adsorbents for PCR amplification. So, when PCR technique combines with nanotechnology it can prove to be a very beneficial for investigators to examine every fragment of specimen [9,10].

Conclusion

In the field of toxicology, nanoparticles are most effectively used for the detection of different toxic materials, drugs from numerous forensic evidences like hair, blood, saliva, vitreous humor, remains of body skeleton and even from the person's fingerprint. For the enhancement of detection limit gold, silver and titanium nanoparticles are generally being used. To speed up the process of toxicological analysis and for screening method of forensic toxicological drug screening a nano sensor is developed which may be used as an immediate spot test and as a main substitute for on the field test which is low-cost, active and stable.

Nanotechnology plays a very important role in detection of illicit drug because it comprising of unique physio-chemical properties and various advantages like selectivity, sensitivity, cost effectiveness, affordability and automation. Some other researches have also reported method for detection of trace amounts of clonazepam using silver nanoparticles in the presence of melamine.

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