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Research Article

USE OF GREEN NANOTECHNOLOGY FOR THE PRODUCTION OF MEDICINE AND TO CHECK ITS BIOAVAILABILITY AND THERAPEUTIC APPLICATIONS IN THE DEVELOPMENT OF NEW GENERATION DIAGNOSTICS AND TREATMENT

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ABSTRACT

Green synthesized particles have shown to possess properties such as antimicrobial, antioxidant and cytotoxicity to tumor (cancer) cells. The Gold Nanoparticles (AuNPs) and Silver Nanoparticles (AgNPs) are important metallic nanoparticles of primary focus. AuNPs have good bioconjugation properties which contribute to its attachment affinity to the in vivo cells. This property of AuNPs has helped in the production of bio stable drugs that do not easily disintegrate in the biological fluids or physiological pH change. AgNPs have a good cell membrane penetration power which has helped in the synthesis of drugs with high antimicrobial efficacy. The problem of antimicrobial agent resistance by the pathogenic microorganism can be overcome to certain extent with the use of green synthesized AgNPs. The importance of AuNPs and AgNPs has been described further in this review paper. The AuNPS and AgNPs have been produced using plants, fruits, leaves extracts as well as some species specific bioresources as the stabilizers. Nano-coupled enzymes have shown to have good bioavailability properties. Nanocarriers have considerably helped in prolonging the acting period of the therapeutic and antioxidant enzymes thus elevating the efficacy of working on the target cells. Carbon based nanorods have helped to improve the manufacturing conditions of the biomaterials so that they are more biocompatible with the in vivo conditions and cells.

Keywords: Biocompatibility, Bioconjugation, Carbon nanorods, Cancer theranostics, Drug delivery, Gold and Silver nanoparticles, Green synthesis nanotechnology, Natural stabilizers, Nano-coupled enzymes

INTRODUCTION

Nanotechnology refers to the use of nanoparticles that are at par with the size of the atom and can be measured on the atomic scale for its measurements. After more than 20 years of basic nanoscience research and more than fifteen years of focused R&D, applications of nanotechnology are delivering in both expected and unexpected ways on nanotechnology's promise to benefit society.Nanotechnology is helping to considerably improve, even revolutionize many technologies, homeland security, medicine, energy, food safety and environmental sciences etc. Nanoparticles used in nanotechnology range from 1-100 nm in size. Due to its smaller size it possesses an ability of tissue and cellular penetration and thus can help in the efficient drug delivery to the target cells followed by performing its required activity. Although the nanoparticles synthesized by chemical methods are biocompatible, use of eco-friendly (green method) is simple, economically cheap, convenient and environmentally safe. Metallic nanoparticles are used in the synthesis of drugs as they don't easily dissociate and reach the target cell with comparatively less effect on non-target cells. Generally the Silver Nanoparticles (AgNPs) and Gold Nanoparticles (AuNPs) are used in the application of nanotechnology in new age medicine. AgNPs possess unique properties which find innumerable applications such as antimicrobial, anticancer, catalytic and wound-healing activities. AuNPs have a wide range of applications in chemical catalysis, electronic material design, and development of diagnostic and therapeutic nanomedicine products. Since use of chemically synthesized nanoparticles can be detrimental to non-target cells; nanoparticles synthesized from biological sources (plants, fruits and spices extract) gives us an edge over it, proving non-toxic to healthy cells. The AuNPs produced from the use of bioresources rendered more stability to the nanoparticles compared to its production by the chemical methods. Thus green nanotechnological processes not only provide stability but also can be produced under mild conditions without any interference of any extreme chemicals. AgNPs are also produced from some specific species plants, fruits and marine

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organisms with respect to their special properties. Plant Species *Indoneesiella echioides, Beta vulgaris L*., *Eclipta Alba*, *Chenopodium murale* leaf and Marine organisms like seagrass, Fruit species like *Anacardium occidentale*, *Nothapodytes nimmoniana*. Nanotechnology is not only limited to the use of metals but also some enzymes called nano enzymes. They are introduced in vivo to increase the delivery efficiency of immunoprotective enzymes to the target cells.

The forte of protein therapeutics are of high specificity and they increase efficacy like the enzymes which gives an exponential increase in the rate of Food and Drug Administration (FDA) approvals. The goal of vascular oxidative stress containment is yet to be achieved with the help of the optimal delivery of therapeutic enzymes. Some reactive oxygen species (ROS) produced by the vascular cells are implicated in some pathological conditions like acute lung injury, myocardial infarction, ischemia-reperfusion, inflammation and other maladies. The more potent antioxidant enzymes have no utility in the treatment of vascular oxidative stress due to nonfunctioning of critically important factors like poor stability and inadequate delivery to Endothelial cell lining of the vascular lumen. At the acidic milieu typical of lysosomes and ischemic pathological foci that is (pH 4-6) the catalase is active. The ROS are detrimental to the nervous tissues and its ability to cross the blood brain barrier (BBB) can prove to be fatal if its accumulation concentration is not reduced or brought under the limit. One of the main aspects of nanotechnology is the use of nano conjugates or nano derived particles. It plays an important role in the application spectra to improve the efficaciousness of the present available medications. They are the nanoenzymes and nanocarriers used in the therapeutic enzyme administration process. The other application is the carbon based nanorods and its role in the production of reliable biomaterials. This literaturereview is done on the consideration of some research previously carried out from the span of 2008 -2019 and some research papers of this time period are considered as the reference for writing this review.

MATERIALS AND METHODS

Synthesis of silver nanoparticles (AgNP) using natural stabilizers

Plant extracts of capsicum, ginger and garlic were used in the dried powdered form as the natural source of biosynthesis for the AgNPs. Silver nitrate $AgNO_3$ (>99%) as the metal source for the synthesis. Bacterial source of Escherichia coli, Staphylococcus aureus and fungal source of Candida albicans were used for the analysis of the antimicrobial activity of the synthesized AgNPs. Deionized water (DI) was used for all the experimental purposes in order to balance the ionic ratio. Capsicum (cap), ginger (gin) and garlic (gar) extracts were prepared using the required amount of powder in DI water at the 55°C for 15 mins and stored. This aqueous extract was used as the reducing agent and standardized with the $AgNO_3$ at particular concentration, time and temperature. Individual [cap,gin,gar] as well as the combine mixture [gar-gin,cap- gin,cap-gar] was used for the preparation of the AgNPs. The extracts were uses in a ratio of 1:1 and heated at 55°C for 25 mins and vigorously stirred. The formation of AgNPs was indicated by the colour change in solution from yellowish solution to yellowish-brown colloidal dispersion. Characterization of the synthesized AgNPs was done by the X-ray spectroscopy (SEM-EDX) for the presence of metallic silver, Dynamic Light Scattering (DLS) for the size and distribution, UV Vis Spectrophotometry for absorbance spectrum was seen in the range of 190 to 800 and Fourier Transform Infrared Spectroscopy to classify molecules surrounding the nanoparticles. The analysis of the *invitro* activity was done using the well diffusion assays in which the pathogens [*Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*] were inoculated in the Mueller Hinton Broth at 37ºC for 24 hours and the antibacterial activity of synthesized AgNPs was agar diffusion assays wherein the colony forming units (CFU)per ml of microbial suspension 105 per ml were inoculated on the Mueller Hinton Agar, kept for drying, punched wells in the agar and the nanoparticle sample solution(nss) was poured in the agar wells. The activity of the nss was determined on the basis of the size Zone of inhibition (ZOI). Absence of ZOI indicates no antimicrobial activity (Reda et al. 2019).

Synthesis of silvernanoparticles (AgNP) from special bioresources (exotic)

Collection of samples was done from the Palk Bay by collecting at a depth of 2 m, which was found to be *Syringodium isoetifolium*. After the collection cleaning, drying was performed followed by powdering and storage. Extract was prepared by adding 1 g of powder in 100 ml of distilled water followed by boiling and filtration. AgNPs biosynthesis was done by taking 5 ml of the prepared extract and was mixed with 95 ml of 1 mM $AgNO_3$ and heated at 450 C (Otunola et al. 2018). Synthesis was indicated by colour change from pale green to reddish brown. Using the heating and incubation method the effect of various concentrations of $AgNO_3$ in biosynthesis was studied. Kinetics and pH effect were characterized using UV-vis spectroscopy (Kumar et al. 2019). AgNPs characterization was done in aspects of optical property, crystalline nature, size, purity of NPs, Morphology, size distribution and stability and presence of possible functional group responsible for capping and reduction using UV-vis spectroscopy, X-ray diffraction Field emission scanning electron microscopy, energy dispersive X-ray spectroscopy, high resolution transmission electron microscopy, zeta potential analysis and Fourier transform infrared spectroscopy respectively (Mahendran et al. 2016). Bioinformatics tools for hypothetical approach were used in protein sequencing of *Syringodium isoetifolium* and retrieved from NCBI. Using Molegro virtual docker face centered cubic silver crystal was docked with protein and interaction were observed in PyMOLv1.3r1-edu and interaction site confirmed using FINDSITE-metal online server. Antibacterial activity was determined using Mueller Hinton agar plates 13 human pathogenic bacterial strains were seeded along with different concentrations of AgNPs. The

plates were incubated overnight and the zone of inhibition in mm was measured. The MBC and MIC of AgNPs against test pathogens was determined by observing turbidity and loopful inoculum was streaked on sterile nutrient agar and incubating at 37°C for 24 Hrs. Haemolytic assay was used to evaluate cytotoxicity. 100ml diluent suspension of erythrocytes/PBS (stock) mixed with 100 ml of varying concentration of AgNPs. Taking PBS as negative controland 0.1% Triton X100 as positive control. After this incubation was done at room temperature for 1 hour. The suspension was centrifuged and poured into 96 well microtiter plates and absorbance at 540 nm was recorded using ELISA reader then the haemolysis % was calculated. Atremia Cytotoxicity Assay was used for testing the cytotoxicity of bioactive compounds. In a 24 well plate containing 1 ml of sterilized sea water and various concentrations of $AgNO_3$ solution, 10 healthy larvae of A.salina were placed. Taking negative control as sterile sea water without AgNPs, maintained under light source at 25°C for 24 hrs. The number surviving larvae and percentage mortality was calculated after incubation and through probit analysis 50% lethal concentration LC50 was determined (Ahilaa et al. 2016).

Synthesis of Silver Nanoparticles (AgNP) Plant extracts stabilizers

Biogenic synthesis and spectroscopic characterization for the *invitro* assessment of the AgNPs with respect to its antioxidant, antimicrobial and cytotoxicity was carried out using the leaf extract of *Indoneesiella echioides*. Preparation of plant extract, synthesis of Ag nanoparticles ,characterization of silver nanoparticles using UVVis Spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR), *invitro* antioxidant assay, agar diffusion assay, assessment of *invitro* cytotoxicity and cytomorphological alterations was done (Kuppurangan et al. 2016).

The methods and materials used for the C. murale leaves as stabilizers for the synthesis of the AgNPs was similar to the above mentioned methods with the minor difference that they were mixed with water for hydrodistillation and the analysis was carried out using a varian gas chromatography (Abdel-Aziz et al. 2014).

Synthesis of Gold Nanoparticles (AuNPs) using Tea extract

$NaAuC_{l4}$ (Alfa-Aesar) and Tea were taken from organic sources, Transmission electron Microscope (TEM) images were obtained. TEM samples were prepared by placing 5 μL of gold nanoparticle solution on the 300 mesh carbon coated copper grid and allowed the solution to sit for five minutes; removing excess solution carefully. The average size and size distribution of gold nanoparticles synthesized were determined. Same process was repeated using Gum Arabic. Also, using both of the different organic sources, the procedure was repeated with elevated temperature of 40°C. The absorption measurements were done using Varian Cary 50 UV-Visspectrophotometers with 1 mL of gold nanoparticle solution in disposable cuvettes of 10 mm path length. In vitro stabilities of the four different tea-mediated gold nanoparticles (TAuNPs) were tested. TEM measurements of all different gold nano constructs were robust in nature under *invitro* conditions. For the cytotoxicity evaluation, MTT assay was done. All experiments were performed 3 times in quadruplets and gold untreated controls were considered as 100% viable (Nune et al. 2009).

Bioavailability assessment of nanoparticles

SOD1 (Cu/Zn superoxide dismutase) and catalase are two enzymes that work to generate neuroprotection in patients having CNS disorders. The reactive oxygen species (ROS) that causes cellular damage and cell death at the neuronal level are curbed away by the SOD1 and catalase enzymes. But these enzymes cannot reach the target neurons due to their disintegration and elimination from the circulation process. Their inactivation is caused due to protease present in the blood streams. So for the stability and efficient delivery of these enzymes to cross the blood brain barrier they are coupled with nanoparticles to overcome the disintegration *invitro* to some extent → Nano Enzymes are prepared mixing block polymers (pLL-PEG or PEI-PEG) along with the enzyme SOD1 and catalase individual or both. This is done by using the phosphate buffered saline and HEPES buffered saline at specific pH. These complexes were crosslinked using GA/NaBH4, BS3 or EDC/S-NHS.

Monoenzymes and Bi-enzymes were formed. The crosslinking of enzymes was determined using the Polyacrylamide gel shift assay. 125I-labeled SOD1 was prepared using IodoBEADS. Cytotoxicity of the nanoenzymes was determined with the help of CellTier96 Aqueous Cell Proliferation Assay (MTS). The cell viability was estimated using the absorptivity of each sample. For the in vivo studies the nano enzymes in the saline solution was administered intravenously via the tail vein in mice. With the use of DLS, AFM, testing cellular accumulation, TCA precipitation and capillary depletion and statistical analysis the experiment was performed (Klyachko et al. 2012).

The determination of antioxidant activity was performed and the free radical scavenging ability on 2, 2diphenyl-2-picrylhydrazyl (DPPH) was determined to assess the scavenging ability on DPPH beta -carotene was carried out by bleaching assay. All these experiments were performed in triplicates. *Invitro* cytotoxicity of the AgNPs was evaluated against HeLa cells at different concentrations. The plant extract alone was tested for anticancer activity, and was found that the viability of cancer cells decreased with an increase in the concentration It shows potency in inhibiting cancer cells.

Analysis of efficiency of Nanocarrier

Reagents selected for this experiment were Methoxy Poly (ethylene glycol) (mPEG), Poly (lactic-co-glycolic acid) (PLGA), Bovine liver catalase, labeled goat anti-mouse antibodies, N-succinimidly-biotin and protease cocktail derived from the Streptomyces griseus. Protein Iodination of all the proteins is done using radiolabeled with Na125I using the Idogen. This is then followed by removing the

unbound iodine using the gel permeation chromatography. Copolymer synthesis was performed using 2 different methods: Protonnuclear magnetic resonance spectroscopy (1H-NMR) and gel permeation chromatography (GPC). mPEGPLA:- Lactide is recrystallized twice in anhydrous ether and mixed with mPEG and raised to 140°C for 2 hours in N_2 atmosphere and polymerization for 6 hours is done by adding 2-ethyl-hexanoate. The polymer obtained is later dissolved in dichloromethane (DCM) and precipitated twice in cold diethyl ether. Biotin PEG-PLGA Polymer of carboxylate end group and PEG-diamine was freeze dried overnight and conjugated under N_2 atmosphere at Rt for 18 hours. Dicyclohexylurea the resulting precipitate later was precipitated twice in anhydrous ether. FTIR was later usedto verify biotin conjugation at 1630 cm^{-1}. Later 2 types of PNC synthesis is carried out unloaded solid core PNC and catalase loaded PNC. For solid core PNC- PEG-PLGA with or without Biotin-PEG-PLGA was dissolved in acetone. Later HCl is used to neutralize it and PEG concentration was determined by a colorimetric assay based on the PEG-Barium Iodide complex. For PLA concentrations enzymatic assay is used. For protein loaded PNC- Nano carrier synthesis based on the double emulsion and freeze thaw strategy was performed. 2 homogenizations are done to obtain PNC which are finally suspended in 1 ml PBS and stored at 40°C prior to use. Antibody-streptavidin conjugate preparation is done using a hetero bifunctional cross linker, SMCC on the streptavidin (SA) molecule. Binding of the anti- PECAM/SA or IgG/SA conjugates to PNC is also performed where PNC diameter was obtained by DLS. Determination of the substrate permeabilities in the PLGA was done using 2 chamber diffusion apparatus and were performed in triplicate for 2 independently cast polymer films. Proteolytic degradation of enzymeloaded PNC performed to obtain the scattering (absorbance) by using a microplate reader. Cell culture used was the pooled human umbilical vein endothelial cells which were seeded into a gelatinized 12 mm coverslips in 24 well plates. In the cell culture endothelial targeting of the anti-PECAM/PNC/catalase is done using radiotracing and fluorescent microscopy. Antioxidant protection studies in the cell culture and

Injecting of anti PECAM/PNC/Catalase in mice along with the isolation of perfused lungs of mouse and the 2-photon imaging of H_2O_2 in the intact lungs of mouse was also performed (Sivapragasama et al. 2014).

Nanozymes for determining the enzymatic activity

Chemicals, enzymes and SBP materials are obtained first and then the sample pretreatment process begins. Isolation of the human digestive resistant carbohydrates is done and the product is obtained in a form of supernatant. Preparation of micro-scale materials is done later where the product is freeze dried using liquid nitrogen. Determination of the non-sugar fraction of sugar beet pulp where the total dietary fiber was calculated as follows

TDF=(weight of residue-protein-ash blank)/weight of the pellet. Chemical dissolution, enzymatic digestion tests followed thermal analysis using Differential scanning calorimetry (DSC).

In the end SEM is used to observe using JOEL by coating with Au-Pd. The micro-scale materials were also subjected to chemical dissolution and enzymatic digestion (Dziubla et al. 2008).

Carbon nanotubes for biocompatibility

Amorphous hydrogenated carbon thin films were deposited on c-Si (crystalline silicon) (100) Radio Frequency reactive magnetron sputtering in a high vacuum chamber with 20% H_2 as the reactive gas. Ultrasonic cleaning in Tetrachloroethylene, acetone, and methanol at 600°C. Human platelet-rich plasma (PRP) was prepared after the centrifugation of whole blood at 800 rpm, at room temperature (RT). Using venipuncture blood was obtained from the healthy donors. The a-C:H thin films later are cleaned by N_2 air flow for removing dirt and contaminants. The EFM mode, based on the same principles as AFM, gives electric properties on a sample surface by measuring the electrostatic force between the surface and a biased AFM cantilever that applies a voltage between the tip and the sample. The EFM measurements are based on a double-pass technique: the surface relief is traced in the region where the short-range van der Waals forces are dominant, whereas during the second pass, the biased cantilever is raised to a certain height. The signal changes will change the electrostatic forces and this gives rise to following signals:-

1) Amplitude of oscillations of a cantilever (MAG),2) Phase shift (PHASE), 3) Product sin of phase shift (MAG*SIN), 4) Product of cos of phase shift (MAG*COS) (Karagkiozaki et al. 2008)(Table 1).

Table 1: Instrumentations and detection methodology.

Characterization of the nanoparticles	Purpose
Scanning electron microscopy-energy- dispersive X-ray spectroscopy (SEM- EDX)	To detect the presence of elemental silver
Size and Zeta-Potential Analysis	Size and size distribution analysis of the AgNPs
UV-Vis Spectrophotometry	The absorbance spectrum of different samples was recorded
Fourier-Transform Infrared Spectroscopy	Classification of the biomolecules present within the plant extracts surrounding the nanoparticles.
Invitro Antimicrobial Activity of AgNPs	Done using Mueller Hinton Broth (MHB) cultures Cytotoxicity test by Dulbecco's modified eagle's medium (DMEM) Antioxidant activity by DPPH assay
Statistical Analysis	One-way analysis of variance (ANOVA)
High-resolution transmission electron microscopy (HRTEM) analysis	Determination of surface morphology and size of silver nanoparticles
Agar diffusion assay	To check the antimicrobial activity

RESULTS

In the current situation microbial resistance to antimicrobial agents is one of the major concerns in the new generation of drug research, discovery and administration. So the antimicrobial activity of the AgNPs has been under the radar for the production of efficient drugs to increase its potential activity towards the reduction and eradicate the growth of the pathogenic microorganisms. Green synthesis of nanoparticles(NPs) is done with the help of the extracts of plant, fruits or leaves. NPs synthesized via green methods prove to be more economical, less number of steps and does not require purification or culturing.

The standard method of analysis of the microbial growth in response to any antimicrobial agent is via the inspection of the Zone of Inhibition (ZOI). In the current method antimicrobial activity of the AgNPs produced from capping of Capsicum (cap), ginger (gin) , garlic (gar) and gar-gin, cap-gin, cap-gar mixture were checked on the pathogenicity of Escherichia coli, Staphylococcus aureus, Candida albicans. The formation of the AgNPs was determined by the change in colour of the reaction solution from yellowish solution to yellowishbrown colloidal dispersion. The formation of more stable AgNPs was seen using the individual or mixture of plant extracts compared to use of chemical stabilizers. The flavonoids and phenolic compounds present in the ginger and garlic extract have excellently contributed to the reduction process during AgNPs formation. The FTIR of the NPs showed the wave number in the range of 4000-600 cm^{-1}. The *invitro* antimicrobial activity of the of NPs coupled with the extracts of cap,gar,gin and mixture of gar-gin,cap-gin, cap-gar was screened against activity of Escherichia coli, Staphylococcus aureus, Candida albicans. This was done using the agar diffusion assay. The activity of the AgNPs was determined by the size of the ZOI. Larger the ZOI more is the antimicrobial activity of the AgNPs. The cap gar AgNPs showed maximum antimicrobial activity towards Escherichia coli (ZOI=33±0.26 mm). The mixture of the extracts with AgNPs showed more activity compared to individual plant extracts. Gram positive bacteria were more affected compared to the gram negative bacteria as they have an outer layer that the gram positive bacteria lack. The collaborative antibacterial effects of the AgNPs and the natural compounds such as flavonoids, allicin, allyl cysteine etc. in the plant extracts have shown to have a higher bactericidal effect. AgNPs also showed high antifungal activity against fungal species such as the Candida albicans and some others also. The AgNPs target the pathogenic microbes by attacking its cell membrane (Table 2).

Table 2: Anti-microbial activity of silver nanoparticles.

Nanoparticles capped	**Zone of inhibition (in mm)**		
Plant extracts	***E. coli***	***S. aureus***	***C. albicans***
Cap	22 ± 0.12	27 ± 0.41	12 ± 0.36
Gar	22 ± 0.44	24 ± 0.16	11 ± 0.19
Gin	21 ± 0.53	26 ± 0.32	11 ± 0.48
Cap-Gar	30 ± 0.26	24 ± 0.48	25 ± 0.20
Gin-Cap	23 ± 0.09	33 ± 0.29	22 ± 0.53
Gar-Gin	20 ± 0.05	30 ± 0.36	23 ± 0.33

UV-vis spectroscopy confirmed that the formation and size of AgNPs is dependent on the concentration of $AgNO_3$. The stability of NPs depends on $AgNO_3$ concentration and also on reducing and capping agents. Using minimal concentration of reducing agent and $AgNO_3$ swift synthesis was possible. Stability and efficient reduction was possible at alkaline pH, being a marine plant. Crystal structure is in fcc arrangement and is in nano regime. Through FTIR it was observed that amino acids acted as reducing and capping agents. FE-SEM illustrated that AgNPs are polydisperse and in the 10-50 nm range. It was found that with the increase in size of NP cluster, the protein metal interaction decreases. The AgNPs were potent for inhibiting pathogenic bacterial growth because of properties like catalytic activity and chemical stability .The AgNPs at bactericidal concentration were found to be non-toxic thus hemocompatible. According to Artemia cytotoxicity assay the AgNPs exhibited cytotoxicity at very low concentration and thus are eco-friendly.

The characterization of AgNPs produced using *Indoneesiella echioides* as the natural stabilizer using the FTIR which showed the presence of different functional groups. Peaks were observed at approx. 3420.94, 2270.25, 1709.83, 1160.21, 1369, 1227.19, 1071.53 and 540.2 cm^{-1}. The XRD analysis showed the nanoparticles crystallization at the size of 19 nm.The antioxidant activity of the synthesized AgNPs were determined using the DPPH assay and its highest percentage of inhibition was seen at 69%. Its antimicrobial activity was assessed using the well diffusion method. Candida albican, Aeromonas hydrophila, Staphylococcus aureus, Rhodococcus rhodochrous and Pseudomonas aeruginosa were tested for their potential resistance towards the AgNPs. The ZOI test at 3 different concentrations indicated that AgNPs do have an excellent antimicrobial activity once the silver ions penetrate the cell membrane of the pathogenic microorganisms. The anticancer activity of these AgNPs were checked on the A549 and HBL-100 cells using the MTT reduction assay. The IC50 of the cancer cell was found at the concentration of 30 µg/mL of the AgNPs of the A549 cells. The HBL100 cells showed cytotoxic effect at the concentration of 60 µg/mL. This proves the antimicrobial, antioxidant and cytotoxicity towards the cancer cell lines of the AgNPs (Table 3).

Table 3: Antimicrobial activity of Silver Nanoparticles at different concentration by agar well diffusion method.

Microorganism	**Zone of inhibition (mm)**		
	100 µg/mL	**150 µg/m**	**200 µg/m**
Candida albicans	32	34	35
Aeromonas hydrophila	31	34	36
Staphylococcus aureus	30	32	34
Rhodococcus rhodochrous	30	31	32
Pseudomonas aeruginosa	28	30	31

The phytochemicals that are present in the tea help in the synthesis of gold nanoparticles that are robust in nature and hence they stabilize the AuNPs against agglomeration. Due to the organic stabilizers present in the tea, there wasn't a need to use chemicals in these experiments. The Plasmon resonance wavelength , λmax of T-AuNPs is ~535 nm as observed. It is important to remove the harmful, unreacted chemicals and by-products when we make the use of chemicals in our experiment because they can cause the reduction of biogenic chemical functionalities present on peptide backbones, thereby eliminating the biospeficity of biomolecules. The use of thiol-containing organic compounds makes the nanoparticles highly stable. TEM, Differential Centrifugal Sedimentation (DCS, Disc Centrifuge, CPS Instruments) and Dynamic light scattering (DLS) were used independently determine the physico-chemical properties such as size, shape and morphology of AuNPs produced.

The particle shape was found spherical when observed using TEM and the size ranged from 15-45 nm. The time required for nanoparticles to traverse a sucrose density gradient created in a disc centrifuge is determined by DSC technique which measures the size of the AuNPs. The hydrodynamic diameter of T-AuNPs suggests that tea phytochemicals such as catechins, theaflavins and thearubigins are capped on the nanoparticles, which are in the range of 105-165 nm. Zeta Potential (ζ) i.e. the measurement of the charge on AuNPS gives us information on thestability of nanoparticle dispersion. Thus also indicating therepulsive forces present, predicting the stability of the nanoparticulate dispersion. Role of Tea Phytochemicals→ The water soluble phytochemicals present in the organic tea leaves are Catechins, Theaflavins and Thearubigins, which are oligomers of catechins. Commercially available families of catechins such as Catechin, Epicatechin, Epicatechin gallate, Catechin gallate, Epigallocatechin, and Epigallocatechin gallate were used for performing a couple of experiments. They proved that catechin and epigallocatechin gallate (EGCG) not only work as reducing agents but also as stabilizing agents. But epigallocatechin and epicatechin can only serve as reducing agents and require an external stabilizing agent such as Gum Arabic. 80% Theaflavins are present in commercially available organic tea leaves which were used in the experiments. The dual nature of Theaflavin as stabilizing as well as reducing agent is proved. When Gum Arabic (GA) is used with Tea extract maybe acting as a biochemical medium to drive the reactions to completion while simultaneously producing wellshaped and robust gold nanoparticles. At 40°C, the nanoparticles get arbitrarily segregated and their size varies from 15-30 nm. The *invitro* studies show excellent stability of T-AuNPs in biological fluids at physiological pHs.

The crosslinking of the enzymes was checked using electrophoresis. The oligomeric subunits of SOD1 were 16 kDa and that of catalase was 60 kDa. Retardation of protein bands was seen as the oligomeric subunit cannot migrate due to its size. Some of the complexes that passed through the gel were of higher molecular weight indicating the crosslinking and no free subunits were observed indicating there was maximum conjugation. The efficiency of cellular uptake of these crosslinked nano enzymes was done to check its ability to cross blood brain barriers using the *invitro* model of the bovine brain microvessel endothelial cells. There was a substantial increase in the cellular absorption of these nano enzymes which suggests efficiency to reach the target cell. The cytotoxicity of these nano enzymes were evaluated using the *invitro* model of CATH. neurons. Comparing the cell viability in both the non crosslinked nano enzymes and the cross linked nano enzymes there was same or little higher cell viability for the cross linked nano enzymes. So to conclude these nanoenymes are non toxic to cells at the standard concentration of these nano enzymes. The in vivo stability of SOD1 nano enzymes suggests that in the non crosslinked state it dissociates faster in the serum. But the crosslinked nano enzymes (SOD1) showed higher stability. Brain delivery was studied using the capillary depletion test to assess the efficacy of the nanoenzymes to deliver the SOD1 at the target tissue or cell. The crosslinked nano enzymes showed a 10% increase in the delivery compared to the native SOD1 or non crosslinked SOD1.

The results indicated that the formed brown solutions were incubated overnight in dark in 40°C which resulted in the biosynthesis of silver nanoparticles and showed maximum absorption at 440 nm. The antioxidant activity of the aqueous extract was evaluated using DPPH scavenging and beta carotene bleaching assays. The antimicrobial activity of C. murale leaf extract showed positive against S.aureus.

Due to the phytochemical coating in biosynthesized AgNPs, they are more active as compared to chemically synthesized AgNPs. Also they are biocompatible. The anticancer activity is due to the apoptosis mechanism by NPs. Anticancer activity results from reactive oxygen species formation (ROS) , damaging equilibrium of the cell , thesize and shape of AgNPs depends on this. Apart from therapy AgNPs can be used in diagnosis like bioimaging , MRI -imaging and biosensing due to their self-fluorescence ability. Biogenic nanoparticles are biodegradable and can be cleared by urination from the body.

Designing of the anti-PECAM/PNC/Catalase was performed which resulted in the formation of the nonaggregating coupling to the biotin-PNC. Targeting of the anti-PECAM/PNC/Catalase in the cell cultures gives the result that higher targeting capacity of the catalase-loaded anti-PECAM/PNC or the smaller solid core antiPECAN/PNC can be explained in a much more effective multivalent binding of the enzyme catalase loaded preparation, which almost carries 10 times more anti-PECAM molecules. Pulmonary targeting of the antiPECAM/PNC/Catalase circulating in the naive animals results in the reduction of circulating pool of formulation which is directly due to endothelial binding.

Anti-PECAM/PNC/catalase protects endothelial against oxidative stress where the PECAM-targeted PNC deliver encapsulated catalase which are active to the pulmonary endothelium, which resulted in an alleviation of the vascular

oxidative stress in lungs. Results indicate that the detoxifying therapeutic enzymes can be encapsulated into polymer nanocarriers, which can target the vasculature by affinity moieties coupled to their surface; and be stealth (PEG-coated) and small enough to circulate and provide interendothelial delivery it also protects enzymatic cargo from proteolysis, while allowing their activities towards diffusible toxic substrates. An advantage of this drug delivery system is that substrate diffusible PNC serves rather as a protective protein cage, hence there is no need for drug release. This paradigm may find medical utility including targeting of antioxidant enzymes. In animal studies the PECAM targeted PNC loaded with the encapsulated therapeutic enzymes have sufficient affinity to EC to accumulate in the pulmonary vasculature.

Materials which are bio-based are in high demand to be used as packaging and encapsulating materials in the nutraceutical applications. The sugar beet pulp non-sucrose carbohydrates were isolated and the acid hydrolyzed to produce micro-scale materials which give unique tubular structure. These tubular structures are referred to as 'stacks of tubules'. These are biocompatible and sustainable and must be generally recognized as safe (GRAS) for human health. DSC analysis showed that the reduced particle sizes do not affect the thermal stability and these 'stack of tubules' at the micro-scale can be incorporated for human applications.

Hemocompatibility studies of the a-C:H thin films were used in this work that the films were deposited under floating conditions and their behaviour is much better than the ones deposited under the application of a negative bias voltage. Results were derived from AFM analysis.

CONCLUSION

The main objective of nanotechnology in bioscience or medicine (pharmaceuticals) is to increase the efficiency of drug action and make it more impactful in terms of drug delivery. The motive of this literature review was to analyze the pattern of the experiments carried out in the span of the last 12 years in the field of nanotechnology and its allied uses in the pharmaceuticals or drug manufacture industries. Even though there are no doubts about the amazements that Nanoscience and Nanotechnology will bring to the world, one cannot look past the potential harmful side effects of nanoparticles when administered through oral or intravenous pathways, its environmental toxicity and non-biodegradable products. Thus green nanotechnology provides tools for the transformation of biological systems to green approaches to nanomaterial synthesis. Hence there is an immediate need to develop eco-friendly biosynthetic processes that reduce the use of toxic chemicals. Nanoparticles (NPs) which form the basis of all the nanotechnological processes can be manufactured with the use of natural stabilizers or chemical stabilizers. The natural stabilizer renders to an appreciable increase in the antimicrobial activity to the Nanoparticles due to its synergism with the naturally available compounds in the bioresources used as natural stabilizers.

Mostly metallic nanoparticles are used due to their diverse biomedical properties. The metallic nanoparticles under consideration in this literature review are the Silver Nanoparticles (AgNPs) and Gold Nanoparticles (AuNPs). The AgNPs possess antibacterial and antifungal activities and they can penetrate the cell membrane of the pathogenic microorganisms and have great bactericidal and fungicidal activity. One of the major problems faced in the drug administration field is the microbial resistance towards the antimicrobial agents. This to a great extent is being tackled by the use of the NPs or the nano coupled medicine. Plant extracts such as *Indoneesiella echioides* synthesized AgNPs imparts really good antimicrobial activity on Candida albican, Aeromonas hydrophila, Staphylococcus aureus, Rhodococcus rhodochrous and Pseudomonas aeruginosa along with this it's also known to have considerably increase the level cytotoxicity for cancer cell lines. Increase in the amount of cytotoxicity is observed by administering an increased amount of AgNPs.

AgNPs synthesized using *Anacardium occidentale* fruit extract exhibited the results where the microbial organisms showed moderate sensitivity towards it hence can be used for medicinal applications. The antimicrobial activity of AgNPs showed a good effect against the two pathogenic bacteria; Bacillus subtilis and Klebsiella pneumoniae.

The biosynthesis method performed using extract of S. isoetifolium was easy, swift and cost effective which synthesized silver nanoparticles with good stability, structure, nano size and optical property. The interaction with proteins would make it applicable in drug delivery systems. The process being eco-friendly and NPs were stable and biocompatible making it applicable in many fields such as biomedical and industrial. Due to many beneficial properties of biogenic AgNPs like self-fluorescence they can replace radiolabeled isotopes used for detecting tumors, thus reducing side effects in patients. Oral administration in healthy individuals showed no toxicity so it has the potential to be used as cancer theranostic agent. Taking in consideration the rising demand the industrial production is very much needed in future by green synthesis. Unprecedented opportunities would be awaiting by the synthesis of target-specific gold particles by making the use of phytochemicals of various plant species. The biocompatibility of AuNPs was observed *invitro* cell culture assay. When AuNPs containing doxorubicin were administered to breast cancer cells (MCF-7 and MDA- MB-231), it showed notable hindrance of breast cancer cell proliferation compared to pristine doxorubicin. Among the various inclusive processes in this area of research one of the main applications is the use of nano enzymes to make the enzymes more stable for its delivery to the target cells. The nano enzymes are enzymes labeled with Na 125I and crosslinked with block copolymers. They can efficiently move through the serum without getting dissociated and reach the site of action. They are shown to have no cytotoxicity effect as long as they are administered in the recommended amount.Thus they also help in transport of antioxidant analeptic to the nervoustissue.

Demonstration of PNC indeed provided a good path which enhances and extends the benefits of therapeutic protein such as catalase and antioxidantenzymes.

Both the capacity to target PNC to the vascular endothelium and the extent therapeutic duration by reducing its proteolytic inactivation was the result of this effect. The enzyme whose substrates were readily permeable across the polymer layer was observed to have a long term enzyme activity. Further this provided a platform for controlling substrate diffusion rates and localization of enzymatic therapies which were directed to a diverse substrate range which had control selectivity and were adjusted to fit a broad variety of treatment of oxidative stress which were evaluated to new avenues in xenobiotic detoxification. Studies prove that the sugar beet pulp has stable materials and their degree of dissociation is high which can be used for human application which targets the upper digestive system. The microscale materials were found to be thermally stable at physiological temperatures of humans. The microscale tubular structures of the sugar beet pulp for the encapsulation process were proved to be efficient. Detailed evaluations of all surface properties are needed to make a way into vexing the problems of the biomaterials failure for biosensing and biomedical application. This literature review is trying to throw some light on the various aspect covered by nanotechnological processes carried in a green way, the use of AgNPs and AuNPs along with its excellent biomedical and bioconjugation properties, how these properties have positively contributed to the field to drugs synthesis and its use with a greater progressive impact on the cancer cell lines, its bioavailability properties which makes the enzymes delivery more efficient to nerve cells, and the production of nanoparticles derived components such as the nanoenzymes, nanocarriers with its potential role in expanding the therapeutic properties of enzyme and carbon based nanorods in order to detect the surface properties of the biological cells to efficiently produce biocompatible biomaterials for further research and development. As we can see that nanotechnology forms the base for any kind of advancement in the field of therapeutics and pharmaceuticals, it's very important to keep a track of all the past and current development or research taking place here.

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