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4.24 Cytotoxic effect of Aspergillus sp., mediated synthesized AgNPs on A549 cells stained with DAPI (a) A549 cells control (b) A549 cells treated with 500µM/ml AgNPs (c) A549 cells treated with 225µM/ml Ag NPs.

5.1 Mice lungs of control (A), NDEA-100 ppm (B) and NDEA-150 ppm (C) with remarkable increase in size. A number of white mosaic patches are present throughout the lung surface (arrows). DNA damaging/ Genotoxic effect of NDEA and PB on lung during carcinogenesis of control (D), NDEA-100 ppm (E) and NDEA-150 ppm (F) groups of animals. Animals were provided with NDEA along with PB for 11 weeks.

5.2 Histological study of lung tissue in control and experimental groups of mice. (A) (20x) Hematoxylin and eosin stained section of lung from control animals revealed normal architecture and bronchiole is seen in the center, (B) (20x) Hematoxylin and eosin stained section of lung from NDEA (100 ppm) and PB induced carcinoma bearing animal showing neoplastic cells with
reactive atypia displaying nuclear enlargement, conspicuous nucleoli and neutrophils within the lumina and (C) (20x) Hematoxylin and eosin stained section of lung from NDEA (150 ppm) and PB induced carcinoma bearing animal showing loss of architecture with neoplastic cells. The bronchiole is surrounded by lymphomononuclear cell infiltrate showing reactive atypia of bronchiolar epithelium.

5.3

(A) DUSP-1 or MKP-1 structure modeled using SWISS MODEL WORKSPACE showing catalytic center at the middle, (B) Catalytic center of Isomeric enzyme DUSP-1 showing interaction with several molecules of NDEA. The ligand “NDEA” is shown in ‘stick’ representation and (C) Orientation of one NDEA molecule showing the best binding to Catalytic center of DUSP-1. The ligand “NDEA” is shown in ‘stick’ representation.
Abbreviations

NPs Nanoparticles
α-NADPH Nicotinamide Adenine Dinucleotide Phosphate
AgNPs Silver Nanoparticles
GNPs Gold Nanoparticles
GNPs-HSA Gold Nanoparticles Encapsulated With Human Serum Albumin
GNPs-HSA-Snd Gold Nanoparticles Encapsulated With Human Serum Albumin And Conjugated With Secnidazole
Snd Secnidazole
Brom-GNPs Bromelain Encapsulated Gold Nanoparticles
BSA Bovine Serum Albumin
HSA Human Serum Albumin
AgNO₃ Silver Nitrate
Nd(NO₃)₂ Neodymium Nitrate
AuCl₄⁻ Auric Chloride
EDC 1-ethyl-3-(3-imethylaminopropyl)-carbodiimide
EDTA Ethylene Diamine Tetra Acetic Acid
FTIR Fourier Transform Infrared Raman Spectroscopy
HEPES ethanesulfonic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>KDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>Km</td>
<td>Michaelis-Menten Constant</td>
</tr>
<tr>
<td>MES</td>
<td>2 (N-Morpholino) ethanesulfonic Acid</td>
</tr>
<tr>
<td>NIR</td>
<td>Near-Infrared Region</td>
</tr>
<tr>
<td>QDs</td>
<td>Quantum Dots</td>
</tr>
<tr>
<td>SAED</td>
<td>Single Area Electron Diffraction</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulfate</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SMAD</td>
<td>Spray Pyrolysis and Solvated Metal Atom Dispersion</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscope</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>Ultraviolet/Visible Spectroscopy</td>
</tr>
<tr>
<td>Vmax</td>
<td>Maximum Velocity</td>
</tr>
<tr>
<td>XPS</td>
<td>X-Ray Photoelectron Spectroscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>X-Ray Diffraction</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface Plasmon Resonance</td>
</tr>
<tr>
<td>°C</td>
<td>Degree centigrade</td>
</tr>
<tr>
<td>nm</td>
<td>Nano meter</td>
</tr>
<tr>
<td>mV</td>
<td>Mili volt</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reverse Phase- High Performance Chromatography</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
</tbody>
</table>
| MTT          | 3-(4,5-dimethylthiazol-2-yl)-2,5- }
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>diphenyl-tetrazolium bromide</td>
<td>Inhibitory Concentration fifty Percent</td>
</tr>
<tr>
<td>DAPI</td>
<td>4', 6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>NDEA</td>
<td>N-nitrosodiethylamine</td>
</tr>
<tr>
<td>Ppm</td>
<td>Part per Million</td>
</tr>
<tr>
<td>PB</td>
<td>Phenobarbitone</td>
</tr>
<tr>
<td>DUSP-1</td>
<td>Dual Specificity Phosphatase-1</td>
</tr>
<tr>
<td>kcal/mol</td>
<td>Kilo calorie per moles</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Lethal Dose fifty percent</td>
</tr>
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Abstract

The major objective of the present thesis was to synthesize different inorganic (Au, Ag, Nd etc.) nanoparticles (NPs) using biological systems and investigate their biomedical applications especially against cancer and infectious diseases. Therefore, following aspects have been covered in this thesis: 1) Isolation of bacteria/fungi from the campus of integral university responsible for the synthesis of inorganic nanoparticles. 2) Molecular identification and characterization of isolated bacteria/fungi by using 16s rRNA/rDNA or 18s rRNA/rDNA. 3) Synthesis and characterization of biosynthetic nanoparticles by UV-VIS spectroscopy, XRD, EDAX, SEM, TEM and Flurometry. 4) Enzymatic synthesis of gold and silver nanoparticles and their bio conjugation with several drugs. 5) Cytotoxic effect of above mentioned bioconjugated nanoparticles in normal and cancer cell lines (in vitro) to check the bioavailability and change in the potency of the drug. 6) Cytotoxicity and kinetics of above mentioned naked nanoparticles and bioconjugated nanoparticles in SD rats.

Chapter 1 is a brief introduction to the research work presented in this thesis. It begins with a bird’s eye view of the field of nanoscience and nanotechnology and their significance to different fields of science, covering from its history to the latest development. This chapter proceeds to describe different physical, chemical and biological routes of nanoparticles synthesis. Further different characterization, drug conjugation and functionalization techniques were discussed. It also gives an account of different properties and applications of nanoparticles with particular emphasis to biological applications.

Chapter 2 deals with the synthesis of silver and neodymium nanoparticles by using fungi and bacteria, which were isolated from the campus of integral university, Lucknow on the basis of their ability to withstand high concentrations of respective salts in the growth media. Further, this quality of bacteria/fungi was exploited to reduce metal salts into respective nanoparticles. The molecular identification of bacteria and fungi was done by using 16S and 18S rRNA/rDNA techniques, respectively. Their partial 16S and 18S rRNA/rDNA gene sequences got their accession numbers KC967214, KC967215 and KC967216 for Myroides odoratimimus, Acinetobacter lwoffii, Bacillus sp. respectively and KF913250 and KF913249 for Aspergillus sp. and Aspergillus fumigates respectively, from NCBI,
GenBank. All the isolated and identified bacterial strains (*Myroides odoratimimus, Acinetobacter lwoffii, Bacillus sp.*) and fungal strains (*Aspergillus sp, Aspergillus fumigates*) were used for the synthesis of silver and neodymium nanoparticles. The formation of the AgNPs and NdNPs was monitored by UV-Vis spectroscopy using Shimadzu (Model No-UV 1800) double beam UV-Vis spectrophotometer. The surface plasmon resonance of AgNPs and NdNPs exhibited an emission band at 416-418nm and 285-287nm, respectively. Furthermore TEM, SEM and DLS characterization of both types of NPs showed them to be spherical and monodispersed with sizes ranging from 9-15 nm. The stability of both the types of nanoparticles were confirmed by zeta potential. The AgNPs and NdNPs synthesized by using biological method in the present study correlates well with the one synthesized by physical and chemical methods.

Chapter 3 deals with the enzyme mediated synthesis of gold nanoparticles (GNPs) using enzymes *viz.* trypsin, bromelain and nitrate reductase. *In vitro* synthesis of GNPs was due to reduction of AuCl₄⁻ ions to GNPs by these enzymes. Eventually, formation of GNPs was confirmed by UV-Vis spectroscopy, DLS, SEM and TEM. It is well known that Surface Plasmon Resonance (SPR) of GNPs exhibit ruby red color and an emission band at 520-533nm which was observed for as synthesized GNPs. The sizes of these nanoparticles were found to be in the range of 7-24nm, which were further authenticated by the DLS, SEM and TEM.

Further, a novel method was developed for the synthesis of GNPs of different sizes using bromelain as a reducing as well as a capping agent, at varying concentrations of bromelain and at different temperatures of reactions. The best GNPs among the produced one were found to be synthesized at 40°C temperature by using 0.33mg/ml concentration of bromelain. Moreover, the formation of GNPs was identified by their characteristic ruby red color and respective SPR. GNPs produced at 0.33 mg/ml concentration of bromelain showed characteristic ruby red color with fundamental surface plasmon resonance at 522nm, whereas the hydrodynamic diameter by DLS for same nanoparticles was estimated to be 58.65 nm. The topographical studies were performed under SEM and TEM by using GATAN digital micrograph software. The particles were found to be monodispersed and spherical in shape with a size range of 8.59-12.92 nm. The stability of nanoemulsion was estimated by zeta potential which was found to be -16.6mV. However, size of GNPs
at 0.66 mg/ml, 1.66 mg/ml and 3.33 mg/ml concentration of bromelain were found to be in a range of 11.27-24.68 nm, 22.26-32.26 and 30.42-42.14 nm, respectively by TEM. Zeta potential of GNPs synthesized by 0.66 mg/ml, 1.66 mg/ml and 3.33 mg/ml concentration of bromelain were found to be -9.83 mV, -9.67 mV and -6.47 mV, respectively.

Further, GNPs were also synthesized at different temperature by keeping concentration of bromelain (0.33 mg/ml) constant because at this concentration of bromelain, best sized nanoparticles were produced. The so produced nanoparticles were found to be monodispersed and spherical in shape when analysed under SEM. The sizes of nanoparticles synthesized at 50 °C, 60 °C and 70 °C temperature were found in the range of 22.6-31.1 nm, 36.2-39.5 nm and 50.8-62.1 nm, respectively under SEM. The stability of these nanoparticles were analyzed by measuring zeta potential and it was found to be −9.56 mV, -6.83 mV and -6.12 mV, at 50 °C, 60 °C and 70 °C temperature, respectively.

Furthermore, Human Serum Albumin encapsulated gold nanoparticles (GNPs-HSA) were synthesized by using different concentrations (viz- 0.33 mg/ml, 0.66 mg/ml and 1.66 mg/ml) of trypsin and their synthesis was confirmed by their characteristic SPR absorption bands which were appeared at 522 nm, 527 nm and 539 nm, respectively. The pronounced red shifting of the plasmon has been associated with increased nanoparticle size. Thus, there might be a dose dependent increase in size which was confirmed by TEM analysis. Here, TEM analysis for GNPs synthesized at 0.33 mg/ml, 0.66 mg/ml and 1.66 mg/ml concentration of trypsin, revealed particle size in the range of 8±2 nm, 10±3 nm and 17±3.5 nm respectively. However, the hydrodynamic diameter by DLS was found to be 40±2 nm, 75.65±4 nm and 110±5 nm for 0.33 mg/ml, 0.66 mg/ml and 1.66 mg/ml concentration of bromelain, respectively. Further, SEM micrograph image of all the samples indicated that particles were spherical in shape and monodisperse.

Furthermore, the conjugation of GNPs was done with an anti-bacterial drug secnidazole. This time GNPs were synthesized by nitrate reductase (as a reducing agent) and Human Serum Albumin (HSA) (as a capping protein). Again, synthesis of GNPs-HSA was authenticated by UV-Vis spectroscopy, DLS, SEM and TEM. Also the binding of secnidazole with GNPs-HSA was confirmed and authenticated by SPR absorption band (UV-Vis spectroscopy) which appeared at 525 nm, with a significant
broadening and a slight decrease in intensity compared to the Plasmon band for GNPs-HSA at 527 nm. The absorption spectrum of Human Serum Albumin encapsulated gold nanoparticles conjugated with secnidazole (GNPs-HSA-Snd) revealed two peaks at 320 nm and 525 nm corresponding to secnidazole aromatic nitrite transitions and GNPs-HSA respectively, suggesting its binding to GNPs-HSA. TEM micrographs of GNPs–HSA-Snd revealed the size of these nanoparticles to be 7 ± 2 nm, whereas DLS confirmed their size (18 ± 3nm) in terms of hydrodynamic diameter due to the interaction and binding of solvent to the surface of nanoparticles.

Eventually, the GNPs-HSA and GNPs-HSA-Snd were found to be negatively charged with a zeta potential of -13.3 mV and -7.97 mV respectively. The quantitative estimation of secnidazole conjugated with GNPs-HSA was determined by RP-HPLC chromatography by using the C-18 column. The amount of bioconjugated secnidazole was found to be 70% indicating efficient binding of secnidazole with GNPs-HSA.

Chapter 4 acknowledges the antibacterial activity of AgNPs, NdNPs and GNPs-HSA-Snd (synthesized in chapter 2 and 3) against different pathogenic bacteria. The bacterial strains *Myroides odoratimimus* (Accession No. KC967214), *Acinetobacter lwoffii* (Accession No. KC967215), *Bacillus sp.* (Accession No. KC967216) and fungal strains *Aspergillus fumigatus* (Accession No. KF913250) and *Aspergillus sp.* (Accession No. KF913249) mediated synthesized AgNPs and NdNPs showed substantial antibacterial potential. The best MIC was observed for *Aspergillus sp.* mediated synthesized AgNPs against *Escherichia coli* (NCIM 2065) and it was found to be 20µM/ml. Similarly, for NdNPs, synthesized by *Aspergillus fumigates* showed best antibacterial activity against *Micrococcus luteus* (NCTC 10240) and it was found to be 11.49µM/ml

It was observed that GNPs-HSA-Snd showed greater potency against *Klebsiella pneumoniae* (NCIM 2957) and *Bacillus cereus* (NCIM 2156) than pure drug and it was found to be 12.2 and 14.11 times respectively. The antimicrobial activity of the nanoparticles is due to the penetration of NPs into the bacterial cells and production of ROS, damage of cell membrane, and release of cell contents for AgNPs. It is suggested that the shape and size of AgNPs and release of silver ions from the nanoparticles, might have contributed to the bactericidal properties.
Furthermore, *in-vitro* cytotoxicity of AgNPs, NdNPs, pure bromelain and bromelain encapsulated GNPs (brom-GNPs) were tested against the selected human cancer cell lines, such as MCF-7, MDA-MB 231, A549, HeLa and normal cell line 3T3-L1. These NPs were found highly effective against prescribed cell lines. The dose-response activities were checked by MTT assay and IC$^{50}$ was calculated. *Aspergillus sp.* mediated synthesized AgNPs showed maximum activity against lung cancer cells (A549) and IC$^{50}$ for the same was found to be 133 µM/ml. Similarly, *Aspergillus fumigatus* mediated synthesized NdNPs showed substantially greater activity against HeLa cancer cell line with IC$^{50}$ 428 µM/ml. The change in potency of bromelain after capping with GNPs and pure bromelain were studied by MTT assay against 3T3-L1, MCF-7 and MDA-MB 231. IC$^{50}$ for brom-GNPs were found to be 24.54 µg/ml, 12.12 µg/ml and 7.70 µg/ml against 3T3-L1, MCF-7 and MDA-MB 231, respectively, and IC$^{50}$ for pure bromelain were found to be 10.7 µg/ml, 14 µg/ml, and 8.71 µg/ml against 3T3-L1, MCF-7 and MDA-MB 231, respectively. The biosynthesized AgNPs induce apoptosis in the selected human cancer cells as shown by the ultra-structural changes observed in the form of cell shrinkage, formation of membrane blebs and apoptotic nuclei which was analysed by the ROS (reactive oxygen species) and DAPI (4',6-diamidino-2-phenylindole) nucleic acid staining method.

Moreover, studies are needed to elucidate the toxicity and the mechanism involved with antimicrobial and anticancer activity of the synthesized AgNPs, NdNPs and GNPs.

Chapter-5 deals with the development of an animal model of primary lung cancer for investigating the mechanism of diseases and for the development of therapeutic strategies. A reproducible mice model for lung carcinoma was successfully developed by using oral dosage of N-nitrosodiethylamine (NDEA) followed by, again, oral dosage of phenobarbitone (PB). After 15 days of oral administration of NDEA (100ppm to 150ppm), the tumor was promoted with 500ppm dosage of Phenobarbitone (PB) along with set concentration of NDEA for another 15 days. Tumors were detected in 58.33% of mice provided with 100 ppm NDEA along with 500 ppm of PB. While, 75.00% of mice were observed to developed tumor provided with 150 ppm NDEA along with 500 ppm of PB in drinking water. Characteristic features of these lung tumors, were: (i) Appearance of
tumors within a short period of time, (ii) dose-related progression of the lung carcinogenesis (iii) Targeted lung carcinogenesis with no effect on other organs and (iv) No specific strain of mice was required (as in the present study lung cancer is induced in Swiss albino mice). Further, the mechanism of NDEA induced lung carcinogenesis was investigated by studying the inhibitory effect of NDEA on Dual specificity phosphatase-1 (DUSP-1) using molecular docking. Free energy of binding for ‘NDEA- DUSP1catalytic domain-interaction’ was found to be -3.99 kcal/mol.

Further, *in vivo* toxicity of bromelain mediated GNPs was studied. For the study, brom-GNPs were given intraperotinary in the mice and they were observed for 24-48hrs. The numbers of dead mice were counted and probit values were calculated. The probit values were ploted against log/doses and then the dose corresponding to probit 5, i.e, 50% was found to be log LD50=1.41 which corresponds to LD50=25.7mg/kg body weight.

The present study has limitations in giving information about the association of different oncogene products with early stages of carcinogenesis. However, it can be safely stated that this study provides a technique to develop a lung cancer model using Swiss albino mice.

Hence, this thesis is the complete study of synthesis of biogenic inorganic nanoparticles, their characterization and bioconjugation with drug(s) and subsequently, their effect on pathogenic bacteria and cancer cell lines. Apart from *in vitro* studies, an *in vivo* study to check their acute toxicity was also conducted. This thesis proposes bromelain encapsulated gold nanoparticles as a safe therapeutic agent and a vehicle for drug delivery system.