List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>The relationship between the percentages of surface atoms vs particle size.</td>
<td>1</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Structural disparities of nanoparticles ranging from 0D, 1D, 2D &amp; 3D.</td>
<td>2</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Crystal structure of ZnO.</td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Graphene, the parent of all graphitic form.</td>
<td>10</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Schematic diagram depicting the growth scheme of ZnO nano rod by the hydrothermal process.</td>
<td>43</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Schematic diagram depicting the reduction of MTT by mitochondrial dehydrogenase enzyme and form formazan crystals.</td>
<td>48</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Schematic diagram depicting the enzymatic conversion of the tetrazolium salt [iodonitrotetrazolium (INT)] in to purplie cloured formazan.</td>
<td>49</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Schematic diagram of depicting the mechanism of the oxidation of DCFH-DA.</td>
<td>50</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Schematic diagram depicting oxidation of MitoSox Red in to 2-hydroxy-(triphenyl phosphonium) hexylethidium.</td>
<td>51</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Schematic diagram depicting the mechanism of the ΔΨm dependent accumulation of JC-1 in to healthy mitochondria.</td>
<td>52</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>Schematic diagram depicting the mechanism of Annexin V/PI staining.</td>
<td>53</td>
</tr>
<tr>
<td>Figure 2.8</td>
<td>Schematic diagram depicting the different stages in cell cycle.</td>
<td>54</td>
</tr>
<tr>
<td>Figure 2.9</td>
<td>Schematic diagram representing the Alamar blue assay principle.</td>
<td>57</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Schematic diagram depicting the ZnO interaction with biological systems.</td>
<td>74</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Representative SEM images of spherical ZnO NCs.</td>
<td>75</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>SEM images depicting the rod shaped ZnO nanocrystals.</td>
<td>76</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>(a) HR TEM and (b) XRD pattern of ~ 5 nm sized ZnO NCs, (b-inset)</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Optical photograph of 5 nm sized ZnO colloidal NCs emitting bright yellow fluorescence under UV excitation, (c) SEM image and (d) XRD pattern of ~ 200 nm sized ZnO.</td>
<td></td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>(a) Schematic diagram depicting the surface chemical modifications in ZnO NCs, (b) FTIR spectra of bare ZnO, bare silica, silica capped ZnO, starch, starch coated ZnO, and PEGylated ZnO.</td>
<td>78</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>E.coli grown on LB agar plates after incubation with different particle sizes of ZnO for two representative concentrations.</td>
<td>79</td>
</tr>
<tr>
<td>Figure 3.7</td>
<td>Number of (a) E.coli colonies grown on LB agar plates at different particle sizes of ZnO.</td>
<td>80</td>
</tr>
<tr>
<td>Figure 3.8</td>
<td>SEM images of E.coli (a) before and (b) after ZnO treatment.</td>
<td>81</td>
</tr>
<tr>
<td>Figure 3.9</td>
<td>Number of (a) S.aureus colonies grown on LB agar plates at different particle sizes of ZnO.</td>
<td>81</td>
</tr>
</tbody>
</table>
Figure 3.10 Viability of cells incubated with different concentrations of ZnO NCs (0 - 500 µM) for 12 and 24 h determined by MTT assay………………………………83

Figure 3.11 Effect of size-scale (5 nm or 200 nm) on the percentage viability of primary HUVECs and cancer (KB) cells treated with 0-500 µM ZnO NCs……………………………………………………………………83

Figure 3.12 Cell viability analysis of HUVEC cells treated with bare, PEGylated, SiO₂ capped and starch capped ZnO NCs, for 24 h …………………………………84

Figure 3.13 Cell viability analysis of KB cells treated with bare, PEGylated, SiO₂ capped and starch capped ZnO NCs, for 24 h …………………………………84

Figure 3.14 Dose dependent LDH leakage study after 12 and 24 h of incubation of ZnO NCs with cells……………………………………………………………85

Figure 3.15 Optical micrographs of (a) HUVECs and (b) KB cells treated with 0, 200 and 300 µM of 5 nm ZnO NCs for 24 h……………………………………………………………86

Figure 3.16 Confocal microscopy study of cytoskeletal F-actin arrangement of (c) HUVECs and (d) KB cells treated with 200 µM of 5 nm ZnO NCs for 24 h…………………………………………………………………87

Figure 3.17 Flow cytogram and confocal images portraying the level of reactive oxygen species (DCFH-DA assay) produced in HUVECs (a & c) and KB (b & d) cells treated with 0, 200 and 300 µM of 5 nm ZnO NCs for 24 h……………………………………………………………………88

Figure 3.18 Flow cytogram and confocal images showing the level of mitochondrial superoxide (MitoSox Red assay) produced in HUVECs (a & c) and KB cells (b & d) treated with 0, 100, 200 and 300 µM of 5nm ZnO NCs for 24 h……………………………………………………………………89

Figure 3.19 Flow cytogram and confocal images depicting the changes in (a)mitochondrial membrane potential (ΔΨm) of HUVECs (a & c) and KB cells (b & d) treated with 0, 200 and 300 µM of 5 nm ZnO NCs for 24h…………………………………………………………………90

Figure 3.20 Flow cytogram and confocal images representing apoptosis assay based on Annexin V-FITC and PI staining of cells. (a) HUVECs and (b) KB cells were treated with 0, 200 µM concentration of 5 nm ZnO for 12 and 24 h……………………………………………………………………91

Figure 3.21 Effect of ZnO NCs on cell cycle: Histogram representing cell cycle analysis of (a) HUVECs and (b) KB cells treated with 0 - 300 µM of 5 nm ZnO NCs for 24h……………………………………………………………………92

Figure 3.22 Flow cytogram and confocal microscopic analysis showing concentration of freeZn²⁺ ions in the intracellular regions of HUVECs (a & c) and KB (b & d) treated with 0-300 µM of 5 nm ZnO NCs for 24 h……………………………………………………………………93

Figure 3.23 Flow cytogram indicating the cytosolic pH level of (a) HUVECs and (b) KB cells Spectrofluorimetric data of LysoSensor Yellow/Blue dextran assay detecting the lysosomal acidity difference between HUVECs and KB cells…………………………………………………………………………………94

Figure 3.24 Schematic diagram depicting the preferential anti-cancer mechanisms of ZnO NCs. It is shown that ZnO nanocrystals rapidly dissolve in acidic tumor microenvironment leading to Zn²⁺ mediated multiple stress mechanism against cancer cells…………………………………………………………………………………95
Figure 4.1  Physicochemical characterization of as prepared graphene: (a) AFM image (b) layer thickness measured using AFM height image and (c) HR-TEM image (d) Raman spectrum 

Figure 4.2  FESEM and XPS pattern of pristine and carboxyl functionalized graphene

Figure 4.3  Contact angle measurement of pristine graphene (p-G)

Figure 4.4  Contact angle measurement of pristine graphene (f-G)

Figure 4.5  Confocal and flow cytometry data depicting differential uptake of p-G and f-G in Vero cells

Figure 4.6  Cell viability analysis in p-G and f-G treated Vero cells

Figure 4.7  LDH leakage analysis in p-G and f-G treated Vero cells

Figure 4.8  Flow cytogram showing apoptosis assay based on Annexin V-FITC and PI staining of cells. Vero cells were treated with 0 to 300 µg mL⁻¹ concentration of p-G (upper panel) and f-G (lower panel) at 37 °C for 24 h

Figure 4.9  Detection of ROS level in cells treated both p-G and f-G

Figure 4.10  SEM images of graphene treated macrophage cell line RAW 264.7: (a) control (b) p-G 75 µg/ml and (c) f-G 75 µg/ml (d) EDX spectrum of f-G treated cells

Figure 4.11  Confocal Raman spectral mapping of graphene treated macrophage cells

Figure 4.12  Fluorescence confocal microscopy images of (a) p-G (75 µg/ml) and (b) f-G (75 µg/ml) treated macrophage cell line RAW 264.7

Figure 4.13  Differential interference contrast (DIC) imaging showing energy dependent endocytosis of graphene by RAW 264.7 cells

Figure 4.14  Effect of p-G and f-G on cell viability. RAW 264.7 cells incubated with different concentrations (0-75 µg/ml) of p-G or f-G for 48 h and Alamar blue assay was performed

Figure 4.15  Effect of p-G and f-G on plasma membrane integrity. RAW 264.7 cells incubated with different concentrations (0-75 µg/ml) of p-G or f-G for 48 h and plasma membrane integrity was studied using LDH leakage assay

Figure 4.16  Assessment of intracellular ROS. Confocal microscopy and flow cytogram showing the expression of reactive oxygen species (DCF-DA assay) in RAW 264.7 cells

Figure 4.17  Apoptosis assay by Annexin V/Propidium iodide staining. Confocal images and flow cytogram showing Annexin V/PI stained RAW 264.7 cells treated with: 0, 75µg/ml p-G and 75 µg/ml f-G samples

Figure 4.18  Viability of HUVEC cells incubated with different concentrations of both p-G and f-G (0 – 200 µg/ml) for 24 h

Figure 4.19  Dose dependent LDH leakage study after 24 h of incubation of both graphene with HUVEC cells
Figure 4.20  Flow cytogram portraying the level of reactive oxygen species (DCFH-DA) produced in HUVECs cells treated 0.5 and 50 µg/ml of both p-G and f-G for 24 h. .......................................................... 124

Figure 4.21  Effect of graphene treatment on the oxidative degradation of lipids in HUVEC cells.......................................................... 125

Figure 4.22  Detection of glutathione oxidation level of in HUVEC cells............. 126

Figure 4.23  Flow cytometric analysis of the intracellular Ca²⁺ level of HUVEC. . 127

Figure 4.24  Flow cytogram depicting the changes in (a) mitochondrial membrane potential of HUVEC cells treated with 0, 5 and 50 µg/ml of both p-G and f-G for 24 h.......................................................... 128

Figure 4.25  Flow cytogram representing apoptosis assay based on Annexin V-FITC and PI staining of cells. HUVECs cells were treated with 0, 5 and 50 µg/ml concentration of both p-G and f-G 24 h.............................. 129

Figure 4.26  Genomic microarray analysis depicting the up regulation/ down regulation of gene related to Nuclear division, Mitosis, Cell division, Regulation of cell cycle, Spindle organization.......................... 131

Figure 4.27  Genomic microarray analysis depicting the up regulation/ down regulation of DNA repair related genes........................................ 132

Figure 4.28  Genomic microarray analysis depicting the up regulation/ down regulation of Protein DNA complex assembly, Chromatin assembly, Chromosome Organization related genes........................................ 132

Figure 4.29  Genomic microarray analysis depicting the up regulation/ down regulation of mitotic sister chromatid segregation related genes......... 133

Figure 4.30  Comet analysis shows the dose dependent DNA damages in HUVECs.......................................................... 134

Figure 4.31  Assessment of hemolytic activity. (a) Hemolysis analysis of different concentrations (0-100 µg/ml) of both graphene systems treated whole blood.......................................................... 135

Figure 4.32  Analysis of platelet activation and aggregation. Flow cytometry based platelet activation analysis showing the expression of activated platelet marker CD62p and resting platelet marker CD42b.......................... 137

Figure 4.33  Platelet count analysis of whole blood treated with different concentrations (0-75 µg/ml) of p-G and f-G showing normal platelet count.......................................................... 138

Figure 4.34  (a) Prothrombin time (PT) of p-G and f-G (0-75 µg/ml) treated blood plasma samples showing no significant variation from normal range, which is depicted as shaded region in both graphs....................... 139

Figure 4.35  Activated partial thromboplastin time (aPTT) ratio of p-G and f-G (0-75 µg/ml) treated blood plasma samples showing no significant variation from normal range, which is depicted as shaded region in both graphs. 139

Figure 4.36  Pro-inflammatory cytokine expression: (a) Flow cytometric analysis showing percentage expression of pro-inflammatory cytokines from p-G and f-G treated PBMCs. Expression from PBS and LPS treated cells were taken as 0 and 100%, respectively (b) Dot plots showing relative expression of various cytokines in p-G and f-G treated PBMCs.......................................................... 140
Figure 4.37  Immunostimulation analysis. (a) Proliferation of lymphocytes on exposure to different concentration of p-G and f-G for 3 days. Optical microscope images cells treated with (b) PBS, (c) PPHA-M (positive control), (d) p-G and (e) f-G. Graphene treated cells can be seen well separated whereas PHA-M treated cells are agglutinated.

Figure 4.38  Immunosuppression analysis. Cell viability analysis on lymphocytes exposed to mixture of PHA-M + p-G or f-G. Optical microscopic images of (b) p-G and (c) f-G treated cells.

Figure 5.0  In vivo real time imaging of Swiss albino mice injected with 99mTc alone.

Figure 5.1  In vivo real time imaging of Swiss albino mice injected with 99mTc f-G.

Figure 5.2  Histological analysis of mouse heart tissue sample after the post administration of both samples for 1,8,30 and 90 day.

Figure 5.3  Schematic diagrams depicting the normal structure of lung.

Figure 5.4  Histological analysis of control (a), p-G (c,d) and f-G (e,f) treated mice lung tissue sample for 1 day.

Figure 5.5  (a) Optical image, (b) Raman spectral mapping of lung tissue sample (c) Raman spectra of graphene from mouse lung.

Figure 5.6  Histological analysis depicting that after 8 day the p-G treated lung tissue showed mild congestion (a,b). f-G treated samples shows the presence of graphene and hyperplastic epithelium (c,d,e).

Figure 5.7  (a) Optical image, (b) Raman spectral mapping of lung tissue sample (c) corresponding Raman spectra from lung tissue.

Figure 5.8  Histology of 1 month p-G samples shows hyperplasia of bronchial epithelium. f-G treated samples displaying hyperplasia and hypertrophy of bronchial epithelium.

Figure 5.9  (a) Optical image, (b) Raman spectral mapping of lung tissue sample (c) corresponding Raman spectra from lung tissue.

Figure 5.10  p-G treated 3 month lung tissue sample shows mild dilatation of bronchus and bronchioles and mild hyperplasia and hypertrophy of bronchial epithelium (a,b). f-G treated sample (c, d) shows mild dilation of bronchioles, occasional emphysema and moderate congestion.

Figure 5.11  (a) Optical image, (b) Raman spectral mapping of 8 day spleen tissue sample (c) corresponding Raman spectra from spleen tissue.

Figure 5.12  Histology analysis of control (a,b), p-G treated (b,c) and f-G treated mouse spleen sample for 1 day.

Figure 5.13  (a) Optical image, (b) Raman spectral mapping of 24 h Spleen tissue sample (c) corresponding Raman spectra from spleen tissue.

Figure 5.14  Histology analysis shows the 8 day p-G treated spleen samples with increased number of megakaryocytes (a,b). f-G treated samples shows increased number of macrophages.

Figure 5.15  (a) Optical image, (b) Raman spectral mapping of 8 day spleen tissue sample (c) corresponding Raman spectra from spleen tissue.
Figure 5.17 (a) Optical image, (b) Raman spectral mapping of Spleen tissue sample (c) corresponding Raman spectra from spleen tissue……………………165

Figure 5.18 Histology analysis of 3 month sample. (a,b) p-G samples, (c,d) f-G samples…………………………………………………………………………………………………………………………165

Figure 5.19 (a) Optical image, (b) Raman spectral mapping of Spleen tissue sample (c) corresponding Raman spectra from spleen tissue………………166

Figure 5.20 Schematic diagram shows the normal structure of the liver……………………166

Figure 5.21 Histology of 1 day liver samples. (a,b) Untreated liver tissue, (c,d) p-G and (e,f) f-G post treated for 1 day……………………………………167

Figure 5.22 (a) Optical image, (b) Raman spectral mapping of liver tissue sample (c) corresponding Raman spectra from liver tissue……………168

Figure 5.23 Histology of 8 day liver samples. p-G treated samples shows the presence of Kupffer cells (a,b), f-G treated liver sample with mild portal congestion and some sinusoidal (c,d)……………………………………………………………168

Figure 5.24 (a) Optical image, (b) Raman spectral mapping of liver tissue sample (c) corresponding Raman spectra from liver tissue………………169

Figure 5.25 Histology of 1 moth liver samples. p-G liver samples with prominent cells lining sinusoids (a,b), f-G liver sample showing severe damages in hepatocytes……………………………………………………………………………………………170

Figure 5.26 (a) Optical image, (b) Raman spectral mapping of liver tissue sample (c) corresponding Raman spectra from liver tissue………………170

Figure 5.27 (a,b) p-G treated liver tissue, (b,c) f-G treated liver tissue. Both samples treated liver samples showed more degeneration in hepatocytes………170

Figure 5.28 (a) Optical image, (b) Raman spectral mapping of liver tissue sample (c) corresponding Raman spectra from liver issue……………………171

Figure 5.29 Schematic diagram shows the structure of the kidney…………………………171

Figure 5.30 Histology analysis of 1-day kidney samples. Untreated mouse kidney sections show the normal histological pattern. (c, d) p-G treated kidney sample showing degenerated glomeruli and (e, f) f-G kidney sample with regions of infarct and casts in the distal tubules…………………………………………………………………………………172

Figure 5.31 (a) Optical image, (b) Raman spectral mapping of kidney tissue sample (c) corresponding Raman spectra from kidney tissue…………………173

Figure 5.32 Histology of 8 day kidney samples. p-G treated kidney samples displayed granuloma formation in glomeruli (a,b,c). f-G treated samples shows severe degeneration of glomeruli………………………………………173

Figure 5.33 (a) Optical image, (b) Raman spectral mapping of kidney tissue sample (c) corresponding Raman spectra from kidney tissue………………174

Figure 5.34 Histology of 8 day kidney samples. (a,b) p-G treated kidney samples displayed severe congestion, degeneration and necrosis of glomeruli. (c,d) f-G treated samples shows infarction and interstitial nephritis………………174

Figure 5.35 (a) Optical image, (b) Raman spectral mapping of kidney tissue sample (c) corresponding Raman spectra from kidney tissue…………………………175

Figure 5.36 Histology of 3 moth kidney samples. (a,b) p-G and (c,d) f-G samples shows the degeneration in glomeruli……………………………………175
Figure 5.37  (a) Optical image, (b) Raman spectral mapping of kidney tissue sample (c) corresponding Raman spectra from kidney tissue……………….176

Figure 5.38  Histology analysis of mouse brain samples………………………176

Figure 5.39  Histology analysis of mouse testis samples………………………177

Figure 5.40  RT-PCR analysis of mouse serum sample after the treatment with both p-G and f-G for 1 day………………………………………………178

Figure 5.41  RT-PCR analysis of mouse serum sample after the treatment with both p-G and f-G for 8 day………………………………………………179

List of Tables

Table 1. Blood biochemistry analysis of mouse treated with both p-G and f-G for 1, 30 and 90 day………………………………………………………………179