Chapter – 5

Antibacterial Activity of ZnO and Cu Doped ZnO Nanostructures
5.1. Introduction

Nanoparticles (NPs) have unique properties in physical appearance of the matter and chemical interactions due to its small sizes (< 100 nm) rather than their bulk equivalents. They show high antimicrobial properties especially the ZnO due to their high surface to volume ratio. Recent studies have shown that ZnO nanoparticles have toxicity to bacterial cells but minimum effect on human cells. ZnO and CuO nanoparticles are used in industries for smart modifications to plastics, cosmetics etc [1]. The doped and undoped ZnO nanoparticles have been studied for antimicrobial activity with human pathogenic bacteria, mainly *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) [2-7]. CuO, ZnO and Ag nanoparticles attract bacterial membrane, short exposure of *E. coli* cells to Ag nanoparticles affects the outer membrane and collapses the plasma membrane potential and also decrease adenosine triphosphate (ATP) [8]. When *E. coli* cells are exposed to nano-ZnO, losses in membrane integrity are caused [5]. Toxicity of nanoparticles of CuO and ZnO are connected with cell membrane damage [9]. The nanoparticles action may be due to their release of free ions. Nano-ZnO are effective for inhibiting gram-positive and gram-negative bacteria, they show high activity against spores [10-13]. Smaller ZnO NPs (<< 100 nm), show better antibacterial activity [14, 15]. The activity depends on the concentrations and surface area of the particles. The higher the concentration and larger the surface area, the better is the activity [17]. Temperature is also a factor of the activity, higher the temperature treatment, lower the activity [12]. A few studies have reported that the primary cause of the antibacterial function may be from the disruption of cell membrane activity [17].

In this chapter, antibacterial activities of ZnO NSs (SZOPVP0 and ZOpH1) and Cu doped ZnO NS (SZOCu3) of as-prepared samples have been studied against *Staphylococcus aureus* (*S. aureus*, Gram-positive bacteria), *Escherichia coli* (*E. coli*, Gram-negative bacteria, ATCC 25922 (antibiotic susceptible))) and *Klebsiella pneumoniae* (*K. pneumoniae*, Gram-negative bacteria) using well-diffusion
method. Toxicity analyses of the samples were performed using healthy young adult female rats.

5.2. Experimental

5.2.1. Preparation of inoculums and culture media

The test organisms were grown in 5 ml Luria Bertani Broth at 37°C and 0.5 McFarland’ turbidity standard was used during antibacterial susceptibility test. Mueller Hinton Agar medium was the choice of media used in the antibacterial tests. All the culture media was purchased from HiMedia Pvt. Ltd., Mumbai, India.

5.2.2. Antibacterial assay

The antibiogram experiment was done by well-diffusion method on Mueller Hinton agar medium using the test compounds, namely, SZOPVP₀, SZOpH₁ and SZOCu₃ and bacterial isolates used are Staphylococcus aureus (S. aureus, Gram-positive bacteria, no ATCC strains), Escherichia coli [E. coli, Gram-negative bacteria, ATCC 700603 (antibiotic susceptible)] and Klebsiella pneumoniae [K. pneumoniae, Gram-negative bacteria, ATCC 700603 (antibiotic resistance)].

To examine the antibacterial activity of ZnO and Cu doped ZnO NSs, 20 mg/ml of test compounds were dissolved in distilled water and subjected to vigorous vortex mixing before preparing the assay. After 18 to 24 h incubation, the plates were examined and diameters of the zone of clearance measured i.e. zone of inhibition (ZOI) [18].

5.2.3. Toxicity Analysis ZnO and Cu doped ZnO NSs

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines, 3rd October 2008. Experiments were performed using healthy young adult female rats, nulliparous, non-pregnant and weighing 25-30 g. The animals were randomly divided into 4 groups (three test compounds and one control). They were identified by the markings using
picric acid. One rat was unmarked and the others were marked on head, body, tail, for proper observation.

The animals were housed in polypropylene cages with sawdust litter in a temperature controlled environment ($23\pm2^\circ\text{C}$). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24 h period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The rats were fed with standard laboratory animal food pellets and water.

The test substance was administered in a single dose by gavage using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water). Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 40 mg/ml (single dose). After the administration of test substance, food for the mice was withheld for 2 h. The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, and daily thereafter, for a total of 2 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes.

5.3. Results and Discussion
5.3.1. Determination of Zone of Inhibition (ZOI)

The antibacterial activities of ZnO and Cu doped ZnO NSs (SZOPVP0, SZOpH1 and SZOCu3) have been studied against namely \textit{Staphylococcus aureus} (\textit{S. aureus}, no ATCC strains), \textit{Escherichia coli} (\textit{E. coli}) and \textit{Klebsiella pneumoniae} (\textit{K. pneumoniae}).

Figures 5.1-5.3 show the well-diffusion tests of as-prepared NSs at concentration of 20 mg/ml dispersed in distilled water. The presence of ZOI clearly indicates the antibacterial effect of as-prepared samples. The results are summarized in Table 5.1.
Figure 5.1 Antibacterial activity of samples (SZOPVP₀, SZOpH₁ and SZOCu₃) showing against *Staphylococcus aureus* (*S. aureus*).

Figure 5.2 Antibacterial activity of samples (SZOPVP₀, SZOpH₁ and SZOCu₃) showing against *Escherichia coli* (*E. coli*).
Figure 5.3 Antibacterial activity of samples (SZOPVP₀, SZOpH₁ and SZOCu₃) showing against *Klebsiella pneumoniae* (*K. pneumoniae*).

Table 5.1 Antibacterial activity of ZnO and Cu doped ZnO NSs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Particle size (TEM) (nm)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>SZOPVP₀ (no PVP)</td>
<td>47.92</td>
<td>11</td>
</tr>
<tr>
<td>SZOpH₁ (pH = 7.5)</td>
<td>19.53</td>
<td>15</td>
</tr>
<tr>
<td>SZOCu₃ (Cu 7.5% mol)</td>
<td>18.10</td>
<td>18</td>
</tr>
</tbody>
</table>

It is evident from the Table 5.1 that smaller particles are more effective. Cu doped ZnO sample (SZOCu₃) showed more activity against bacteria (ZOI) than undoped ZnO samples (SZOPVP₀ and SZOpH₁) and also it is seen that the antibacterial effects of as-prepared samples are more effective (ZOI) to Gram-negative bacteria (*E. coli* and *K. pneumoniae*). We have studied size dependent antibacterial effect of as-prepared samples. The particle sizes of the test compounds were determined in the previous chapter (Chapter III). This study reveals that smaller particles have more antibacterial effect to Gram-negative bacteria strains than Gram-positive bacteria strain. This may be due to the large...
surface to volume ratio that can accumulate on the bacteria, killing it faster than large particle. In addition, the antibacterial effect of ZnO NPs may be due to the destruction effect of ZnO NPs on the bacterial cells and increased production of active oxygen such as hydrogen peroxide \((H_2O_2)\) which leads to the penetration of particles into the cell membrane of bacteria leads to the formation of injuries and the death of bacterium was occurred [19, 20].

### 5.3.2. Signs Recorded During Acute Toxicity Studies

**Table 5.2** Toxicity studies using rat as model organism.

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>Model 1 ZOPVP(_0)</th>
<th>Model 2 ZOpH(_1)</th>
<th>Model 3 ZO(_{Cu_3})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Alertness</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Grooming</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Touch responce</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Pain responce</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Tremors</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Convulsion</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Righting reflex</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Gripping strength</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Pinna reflex</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Corneal reflex</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Writhing</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Pupils</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Urination</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>Salivation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Skin colour</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

**Key:** N- normal; A – Absent
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Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, and autonomic and central nervous systems, and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h. The observations are presented in Table 5.2.

The above data presented in Table 5.2 show that the test compounds (SZOPVP₀, SZOpH₁ and SZOCu₃) did not have any potential toxic effect in the animal model. This suggests the possibility of exploring these compounds further to check antibiotic resistance isolates.

5.4. Conclusion

1. Antibacterial activities of ZnO and Cu doped ZnO NSs (SZOPVP₀, SZOpH₁ and SZOCu₃) have been studied against *S. aureus* (Gram-positive bacteria), *E. coli* (Gram-negative bacteria) and *K. pneumoniae* (Gram-negative bacteria) using well-diffusion method and the toxicity analyses of the samples were performed using healthy young adult female rats.

2. The presence of ZOI clearly indicates the antibacterial effect of as-prepared samples. Cu doped ZnO sample (SZOCu₃) showed more activity against bacteria (ZOI) than undoped ZnO samples (SZOPVP₀ and SZOpH₁). This clearly indicates that smaller particles are more effective, which assures more interactions with the bacteria.

3. Toxicity analyses of the as-prepared samples (SZOPVP₀, SZOpH₁ and SZOCu₃) do not have any potential toxic effect in the animal model.
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5.5. References


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