Copper Nanoparticles as Antibacterial Agents

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Abstract

Although antibiotics can treat most bacterial infections, development of microbial resistance restricts the advantages of the antibacterial agents in controlling infectious diseases. This is a major challenge that poses a serious threat prompting the search for alternative strategies to treat bacterial infections. Nanotechnology as an emerging field has been extensively used to overcome microbial resistance due to specific properties of nanoparticles such as increased drug uptake and high surface area to volume ratio. The metallic nanoparticles in nanoscale have demonstrated antibacterial activity against various bacterial species, including Gram-positive and Gram-negative bacteria, and fungi. Recently, copper nanoparticles have been widely investigated for use in fighting microbial infections. This article tries to briefly summarize the current studies related to the antibacterial properties of the copper nanoparticles. The reviewed papers reveal that the copper nanoparticles possess potent antimicrobial activities and can be used for controlling and treating different infectious diseases in the future.

Keywords: Metallic nanoparticles; Copper; Antibacterial activity; Infection; Microbial resistance

Introduction

Antibacterial agents are compounds that kill bacteria or slow down their growth without being generally toxic to the surrounding tissue. The word “antibiotics” was first introduced in 1941 by Selman Waksman to refer to antimicrobial agents produced by many microorganisms [1]. The antibacterial agents have been used in many fields, such as, textile industry, water disinfection, food packaging, and medicine [2]. Presently, the over-use of antibiotics has led to an increased occurrence of antibiotic resistant genes in various bacterial species. Many of the recognized antimicrobials have shown resistance by one species of microorganism or another [3]. Hence, a great deal of research has been performed to deal with this problem. “Nanoparticles” (NPs) has been defined by Encyclopedia of Pharmaceutical Technology as solid colloidal particles ranging in size from one to 1000 nm (one micron) [4]. Indeed, NPs exhibit a range of potentially useful applications for pharmaceutical purposes: They have the ability of targeting the drug into the site of action and consequently reducing the side effects, and increasing the drug uptake. Moreover, NPs are capable of interacting with mucosal surfaces and escaping endolysosomal compartments [5,6]. Kinetic profiles of drug release can be also modified by NPs [7]. Based on the Ostwald–Freundlich equation, the saturation solubility enhances with decreasing particle size below approximately one μm. Thus, NPs demonstrate enhanced saturation solubility and increased surface area which cause a further increase in dissolution rate according to the Noyes–Whitney equation. In contrast, the solubility of particles with normal size (above one micron) is a compound-specific constant and depends only on the temperature and the solvent [8]. The emergence of nanotechnology in the last decades has elicited immense interest in evaluating the antimicrobial activity of nano-scale metals. The use of metallic NPs results in decreased concentration as well as increased antibacterial and antifungal activity [9]. However, despite the unique set of properties of metallic NPs, there are environmental and human safety concerns regarding the use of metallic NPs, for example, release of silver causes environmental pollution [3]. The antimicrobial effects of different metallic NPs such as Alumina [10-12], silver [13,14], iron [15-19], gold [20-22], magnesium [23-25], titanium [26,27], and zinc oxide [28,29] have been widely investigated. In spite of the tremendous efforts undertaken regarding the use of metallic NPs with antibacterial effects, at present, we are far away from ideal metallic NPs with efficient activity. As a novelty discussion paper, this review will summarize the major findings for copper particles in nano-scale as antimicrobial agents.

The Privileges of NPs as Antibacterial Agents

The nano-sized particles received a great deal of attention due to their potential in biomedical and pharmaceutical applications. The particles in nano-scale are able to easily interact with bacterial membranes [30]. Based on the studies using various microscopy approaches including atomic force microscopy, transmission electron microscopy and laser confocal microscopy, after using nanoparticles, the integrity of the bacterial cell membranes changed noticeably causing bacterial cell death [31]. The advance in the preparation of metallic NPs has led to the development of a new class of antimicrobial materials. Highly ionic metallic NPs are of particular interest due to their extremely high surface areas and numerous reactive surface sites with unusual crystal morphologies [32].

Antibacterial Properties of Copper

Copper is a readily available metal and one of the essential trace elements in most living organisms. Copper particles in nano-scale have many applications in industry, including their use in gas sensors, high
temperature superconductors, solar cells and wood preservatives [33]. This metal has been also used as potential antimicrobial agent since ancient times. Copper-containing compounds such as CuSO\(_4\) and Cu(OH)\(_2\) are used as the traditional inorganic antibacterial agents [34]. Also, aqueous copper solutions, complex copper species or copper-containing polymers are used as antifungal compounds [34]. Moreover, the control of legionella in hospital water distribution systems via the copper and silver ionization method is one of the most common applications of this metal in the modern healthcare setting [35]. Copper ions have demonstrated antimicrobial activity against a wide range of microorganisms, such as Staphylococcus aureus, Salmonella enteric, Campylobacter jejuni, Escherichia coli, and Listeria monocytogenes [36]. Currently, copper has been registered as the first and only metal with antimicrobial properties by the American Environmental Protection Agency (EPA) [37]. This material kills 99.9% of most pathogens within 2 h contact [38]. Also, in some cases, this metal possesses properties better relative to the other expensive metals with antimicrobial activity, such as, silver and gold [3]. For instance, the Cu NPs showed higher antibacterial effect relative to the silver NPs against E. coli and Bacillus subtilis (B. subtilis) [39,40]. The copper surfaces could be used to kill bacteria, yeasts, and viruses which are known as “contact killing” (contact-mediated killing). Contact killing by copper was reported to occur at a rate no less than seven to eight logs per hour, and in general, subsequent to the extended incubation. No live microorganisms were recovered from the copper surfaces. This leads to the idea of using copper as a self-sanitizing material [41].

Copper Bacterial Resistance

As mentioned in previous sections, copper has been utilized as an antibacterial agent in medicine. The most common copper-resistant bacteria were isolated from animals, plants and humans-associated bacteria. Different mechanisms are involved in copper homeostasis in various bacteria. For example, in E. coli, two chromosomally encoded systems, including cue and cus are responsible for copper resistance. The CueP, a periplasmic protein, is an extra component of the cue system and possesses a critical role in copper resistance. The plasmid-borne copper resistance system is also responsible for survival of some bacteria, such as E. coli in copper-rich environments. Other Gram-negative bacteria, such as Versinia pestis, Versinia pseudotuberculosis, Versinia enterococolitica, Citrobacterkoseri, and Erwiniacarotovora possess CueP-like proteins [42].

The Toxicity Mechanisms of Cu NPs against Bacteria

One of the most known NPs’ toxicity mechanisms is the interaction between the bacterial cell membrane and NPs, which leads to the disruption of the bacterial membrane integrity and finally results in the death of the microorganism. It has been shown that several factors, including temperature, pH, concentration of bacteria and NPs, as well as aer autocatalysis can promote the toxicity mechanism of Cu NPs [2,43]. Cu particles in nano-scale have been shown to have antibacterial effects on the bacterial cell functions in multiple ways, including adhesion to Gram negative bacterial cell wall due to electrostatic interaction (Figure 1), having effect on protein structure in the cell membrane, denaturation of the intracellular proteins, and interaction with phosphorus- sulphur-containing compounds like DNA [34]. Also, in one comprehensive study, mechanisms of antibacterial activity of Cu NPs were investigated using E. coli as a biological tool [44]. The results showed that the treatment of E. coli cells by Cu-NPs at the minimum bactericidal concentration (MBC) resulted in 2.5 times overproduction of the cellular reactive oxygen species (ROSs). Also, the NP-mediated increase in ROS level led to noticeable lipid peroxidation, protein oxidation, DNA degradation and finally cell killing.

Synthesis of Cu-based NPs

Cu-based NPs can be synthesized using five major techniques: chemical treatment, thermal treatment, electrochemical synthesis, photochemical methods, and sonochemical techniques. The “chemical treatment” has been used as the most popular method among them and some of the more modern techniques have utilized this method for synthesis of Cu-based NPs [45]. Lately, green synthesis of environmentally friendly NPs without toxic waste products during the preparation has been introduced. In this kind of preparation technique, safe biotechnological tools are used as an alternative to conventional physical and chemical synthesis and named green nanobiotechnology. In this method, NPs are prepared using biological routes such as bacteria, fungi, plants and enzymes or their byproducts, such as proteins [46].

The Copper Particles in Nano-scale as Antibacterial Agents

In 2008, Ruparella et al. investigated antimicrobial properties of silver and Cu NPs on E. coli, B. subtilis and S. aureus [39]. Results of minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and disk diffusion test revealed that the Cu NPs were more efficient compared to the silver particles against B. subtilis which is suggested to be due to more affinity of the Cu NPs to surface amines and carboxyl groups of B. subtilis. In contrast, silver NPs demonstrated more antimicrobial effect against E. coli and S. aureus relative to Cu NPs.

Another application of copper oxide (CuO) NPs for antimicrobial applications was introduced by Ren et al. [47]. The metal oxide NPs were prepared using thermal plasma (Tesima TM) technology, which allows the continuous gas phase production of bulk nano-powders. The prepared CuO NPs in suspension were active against a range of bacterial pathogens, including S. aureus, meticillin-resistant S. aureus, Staphylococcus epidermidis, E. coli, and Pseudomonas aeruginosa (P. aeruginosa) with MBCs ranging from 100 mg/ml to 5000 mg/ml.

The feasibility of the use of Cu NPs as antibacterial agents against E. coli was examined by Raffi and coworkers in 2010 [34]. The
antibacterial activity was assessed in liquid as well as solid growth media. On solid media, the antibacterial characterization of the prepared NPs was measured by colony forming unit (CFU). In liquid media, the antibacterial behaviour of Cu NPs was studied by determination of the optical density (OD) of the different concentrations of Cu NPs at a fixed wavelength. The growth inhibition results obtained from both studies were in good agreement. Moreover, the scanning electron microscopy (SEM) of bacterial cells treated with Cu NPs demonstrated the formation of cavities and pits in the cell walls and changes in cell morphology from normal rod-shaped to irregular appearance. The results also exhibited that the antibacterial efficacy of Cu NPs depended on the concentration of the NPs; low concentrations just led to a delay in the lag phase, showing the micro-nutritional role of copper for bacteria. In contrast, at higher concentrations, they showed bacterial growth inhibition.

In the same year, another research group assessed the toxicity of aggregated zero valent Cu NPs (ZVCN) against E. coli using a centrifuged mixture design experiment [48]. Five environmental parameters including, temperature, pH, aeration rate, NP concentration, and bacteria concentration which were presumed to have major effects on the toxicity of the NPs against the bacteria, were assessed. According to their study, the interactive effects of the tested parameters as well as the primary effects of them are efficient on the toxicity of Cu NPs.

On the whole, ZVCN will have highest toxicity on nanoparticles under acidic conditions and higher temperature, high aeration and high concentration of NPs and bacteria. When any one of the independent variable is changed, the toxicity of NPs changes significantly.

In an effort to improve the antimicrobial properties of Cu NPs, Mohan et al. utilized carbon nanotubes [49]. The prepared Cu NPs were grafted on the surface of multiwall carbon nanotubes (MWCNT). According to their research, carbon nanotubes increased the surface area of Cu NPs, and therefore the number of colonies of E. coli reduced in the Cu-MWCNT system compared to the pure Cu NPs and MWCNT. The antimicrobial efficiency (% kill) of Cu-MWCNT were found to be 75% ± 0.8 while pure Cu NPs showed a low-percent kill against E. coli (52% ± 1.8). The possible mechanism of the bactericidal effect of Cu-MWCNT is the release of Cu ions from Cu-MWCNT and their entrance to the bacterial cells and the subsequent disrupts of biochemical processes. Taken together, Cu-MWCNT can be used as a biocidal composite in biomedical devices and antibacterial system.

In another research, Theivasanthi and Alagar indicated that Cu NPs developed using electrolysis technique possessed more antibacterial effect against E. coli bacteria relative to those prepared through a chemical reduction process [50]. Use of electrical power in Cu NPs preparation led to an enhancement in the NPs' antibacterial effects. As a whole, the authors proposed the feasibility of use of this material in water purification, antibacterial packaging as well as air filtration.

A novel plastic antimicrobial agent including polypropylene with embedded Cu metal or CuO NPs was examined by Delgado et al [51]. Based on their study, the ability of composites to kill bacteria depended on the type of Cu NPs. It was shown that antimicrobial effect of CuO was more than that of the Cu metallic NPs in killing E. coli since for the CuO NPs the formation of oxide layer was not required which led to high ion release rates. Besides, in terms of CuO NPs, the metal was already in the oxidation condition which resulted in the particle dissolution, while for the Cu metal the previous formation of an oxide layer was necessary.

In 2012, Chatterjee and coworkers introduced a simple robust method for synthesis of Cu NPs via reduction of CuCl2 in the presence of gelatin as a stabilizer [52]. Treatment with the NPs made E. coli cells filamentous with average filament size varied from 7 to 20 μm relative to the normal cell size of nearly 2.5 μm. The NPs were highly effective against E. coli at a much low concentration. The antibacterial effects of produced NPs were also observed in an E. coli strain resistant to multiple antibiotics as well as Gram-positive B. subtilis and S. aureus.

Aqueous solution of starch capped copper nanoparticles with bactericidal effect against both Gram negative and Gram positive bacteria at nano-molar concentrations were produced using starch as green capping agent [53]. Based on the in vitro studies on of the 3T3L1 cells, the capped NPs exhibited cytotoxicity at much higher concentration relative to Cu ions. Based on the results, the introduced starch capped water soluble Cu NPs are promising candidates for different applications for instance in photothermal therapy or cellular imaging.

In another interesting investigation, the antibacterial effect of CuO NP was studied on Legionella pneumophila [54]. According to a whole-genome microarray, CuO NPs significantly affected the expression of genes involved in metabolism, transcription, translation, DNA replication and repair, virulence, and unknown/hypothetical proteins.

Another research group showed that the antibacterial effect of CuO NPs was dependent on the particle size and a significant increase in antibacterial activities against both Gram-positive and Gram-negative bacterial strains was achieved using the highly stable minimum-sized monodisperse CuO NPs [55].

Thikkae Padil et al. produced highly stable CuO NPs using gum karaya, as a naturally occurring polysaccharide component in plants via green technology [56]. The CuO NPs with smaller particle sizes demonstrated higher antibacterial activities. The authors noted that CuO NPs produced via a simple, mild, and environmentally friendly method may possess promising applications for instance in wound dressing, bed lining, active cotton bandages, and medical and food industries.

Das and coworkers fabricated CuO NPs by thermal decomposition methods and investigated their antioxidant and antibacterial effects [57]. Their produced NPs demonstrated free radical scavenging activity up to 85% in 1 h which is relatively higher compared to other metal oxide NPs. Furthermore, the CuO NPs exhibited proficient antibacterial activity against E. coli and P. aeruginosa. Bacterial growth significantly decreased with increasing NPs' concentration.

Usman and coworkers synthesized pure Cu NPs using chitosan polymer as a stabilizer [3]. Their findings showed that the chitosan-stabilized NPs were effective against Gram-negative microorganisms, including Salmonella choleraesuis and P. aeruginosa, as well as Gram-positive bacteria such as methicillin-resistant S. aureus and B. subtilis, and also yeast species such as Candida albicans. Moreover, the prepared NPs indicated more antimicrobial effects against Gram-negative microorganisms (such as P. aeruginosa) compared to Gram-positive bacteria. Taken together, the researchers introduced a simple and cost-effective approach for synthesis of Cu NPs which has future potential for pharmaceutical and biomedical applications.

Another interesting preparation of Cu NPs was presented by Subhankari and Nayak who presented a novel biological technique using ginger (Zingiber officinale) extract [58]. Cu NPs prepared via
green synthesis approach was found to be more effective against *E. coli* relative to copper sulphate solution and pure ginger extract. The researchers noted that this green method involved cheap and non-toxic materials and could be useful in water purification, air quality management, and antibacterial packaging.

In an attempt to produce highly stable CuO NPs via a green chemistry approach, aqueous extract of *Acalypha indica* leaf was used [59]. The resultant particles were found to be effective against *E. coli, Pseudomonas fluorescens* and *Candida albicans*. Moreover, based on the MTT assay, they possessed cytotoxicity activity against MCF-7 breast cancer cell lines.

In a research carried out by Agarwala et al., antibiofilm activity of CuO and iron oxide NPs was assessed against multidrug resistant biofilm forming uropathogens [60]. CuO NPs was found to be more toxic compared to iron oxide NPs and also possess dose dependent antibiofilm properties.

Giannouisi et al produced Cu, Cu$_2$O and Cu/Cu$_2$O NPs using hydrothermal procedure as a cost-effective and eco-friendly method [61]. The Cu-based NPs led to pDNA degradation in a dose-dependent manner and extensive degradation of ds CT-DNA. Also, Cu$_2$O NPs exhibited increased antibacterial effect against the Gram-positive strains. Hence, the possible reaction pathway was investigated. The results proved ROS production and lipid peroxidation.

The use of green nanotechnology for synthesis of Cu NPs has been also investigated by Parikh and coworkers [30]. For biosynthesis, *Datura Meta* leaf extract was used with the ability of metal ion reduction in NPs. The proposed method has some advantages: It is efficient, rapid, easy, inexpensive, and ecofriendly. The antibacterial behavior of Cu NPs against *E. coli, Bacillus megaterium*, and *B. subtilis* was found to be more than that of the extract.

Other nano-structured Cu was developed and investigated by Tomasz and coworkers in 2015 [62]. The copper NPs showed a high antibacterial effect against Gram positive bacteria such as clinical methicillin resistant *S. aureus* strains. The antibacterial effects of Cu NPs were found to be even higher than that of Ag NPs. Also, the synthesized NPs demonstrated antifungal activity against Candida species. Hence, the prepared copper NPs can be used as an alternative for prevention of biofilm formation as well as reduction in bacterial or fungal adhesion at lower cost compared to use of silver.

The anti-biofilm activity of Cu NPs against *P. aeruginosa* was studied by Lewis Oscar et al. [63]. The authors reported that Cu NP treatments at 100 ng/ml led to a 94% reduction in biofilm formation and stated that their proposed NPs could be used as coating agents for controlling biofilm formation on surgical devices as well as medical implants.

In another study, the cytotoxicity of the synthesized CuO NPs in colon cancer cells was explored [64]. The researchers found that CuO NPs inhibited the cell proliferation in HT-29 human colon cancer cells via downregulation of Bcl-2 and Bcl-xL as the apoptosis regulatory proteins.

Taken together, in recent years researchers have paid much attention to Cu NPs due to their antibacterial activity against different microorganisms, (summarized in Table 1). This highlights the potential of the copper particles in nano-scale as effective antibacterial agents in biomedical and industrial applications.

<table>
<thead>
<tr>
<th>Targeted microorganism</th>
<th>Targeted microorganism</th>
<th>Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>9</td>
<td>Wet chemical synthesis</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20-95</td>
<td>Thermal plasma technology</td>
<td>[47]</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12</td>
<td>Inert gas condensation method (IGC)</td>
<td>[34]</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25</td>
<td>N/A</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>22</td>
<td>Wet chemical synthesis</td>
<td>[49]</td>
</tr>
<tr>
<td><em>Escherichia coli; Bacillus megaterium</em></td>
<td>24</td>
<td>Electrolysis method</td>
<td>[50]</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>10</td>
<td>N/A</td>
<td>[51]</td>
</tr>
<tr>
<td><em>Escherichia coli; Bacillus subtilis, Staphylococcus aureus</em></td>
<td>50-60</td>
<td>Simple reduction method</td>
<td>[52]</td>
</tr>
<tr>
<td><em>Staphylococcus aureus, Escherichia coli (DH5a) and Salmonella typhi</em></td>
<td>10</td>
<td>Microwave irradiation</td>
<td>[53]</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>40-80</td>
<td>Heating the Cu$_2$O NPs</td>
<td>[54]</td>
</tr>
<tr>
<td><em>Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus</em></td>
<td>20 ± 1.24-28.9 ± 1.22</td>
<td>Gel combustion route</td>
<td>[55]</td>
</tr>
<tr>
<td><em>Escherichia coli, Staphylococcus aureus</em></td>
<td>4.8-7.8</td>
<td>Colloid-thermal synthesis process</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Escherichia coli, Pseudomonas aeruginosa</em></td>
<td>15-30</td>
<td>Thermal decomposition method</td>
<td>[57]</td>
</tr>
<tr>
<td><em>Salmonella choleraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans</em></td>
<td>2-350</td>
<td>Through chemical means in chitosan polymer medium</td>
<td>[3]</td>
</tr>
</tbody>
</table>
in vitro investigations should be effective. It has been shown that the toxicities of bulk and nano-sized CuO were mostly affected by soluble Cu ions. The accumulation of copper in human body leads to production of the harmful radicals such as hydroxyl radical [65].

**Conclusion**

There is a growing body of scientific evidence which confirms the antibacterial properties of metallic NPs against various bacterial species, including Gram-positive and Gram-negative bacteria as well as fungi. Based on the literature, in some cases Cu NPs showed higher antibacterial effect compared to the other metallic NPs. However, the in vitro investigations should be confirmed by in vivo assays using animal models prior to Cu NPs' use in the clinical setting. Hopefully, in the not too distant future, we could probably witness using Cu NPs as effective antibacterial agents in biomedical and industrial applications to fight against pathogenic microorganisms.

**Declaration of Interest**

All authors declare that they have no conflict of interest.

**References**


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**Table 1:** The Cu NPs as antibacterial agents.

<table>
<thead>
<tr>
<th>Species/Microbial Type</th>
<th>Minimum Inhibitory Concentration (MIC) (µg/mL)</th>
<th>Method of Synthesis/Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>100-200</td>
<td>Green synthesis method</td>
</tr>
<tr>
<td>Pseudomonas fluorescens and Candida albicans</td>
<td>26-30</td>
<td>Green synthesis method</td>
</tr>
<tr>
<td>Methicillin resistant Staphylococcus aureus, Methicillin resistant Staphylococcus epidermidis, Vancomycin resistant Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas spp., Proteus mirabilis</td>
<td>25-30</td>
<td>Commercial CuO NPs were used</td>
</tr>
<tr>
<td>Gram-positive: (Bacillus subtilis, Bacillus cereus, Staphylococcus aureus) and Gram-negative: (Xanthomonas campestris, Escherichia coli)</td>
<td>10-44</td>
<td>Hydrothermal synthesis method</td>
</tr>
<tr>
<td>Escherichia coli, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus</td>
<td>6</td>
<td>Green synthesis method</td>
</tr>
<tr>
<td>Staphylococcus aureus, Candida species, Staphylococcus epidermidis</td>
<td>50</td>
<td>Reduction of copper salt with hydrazine in the aqueous SDS solution</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>55</td>
<td>One pot method</td>
</tr>
<tr>
<td>Gram-positive: (Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis) and Gram-negative: (Pseudomonas aeruginosa, Shigella sonnei, Escherichia coli)</td>
<td>20</td>
<td>Based thermal decomposition</td>
</tr>
</tbody>
</table>

**The Toxicity of Copper Nanoparticles**

In spite of commercial use of NPs, their release into the environment (e.g., soil and water) is of the most important problems which affect public health, beneficial bacteria and microbial communities [2]. The information about the hazardous effects of CuO is limited. It has been shown that the toxicities of bulk and nano-sized CuO were mostly affected by soluble Cu ions. The accumulation of copper in human body leads to production of the harmful radicals such as hydroxyl radical [65].


