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Antimicrobial and Environmental activity of biogenic CS-GO nanoparticles on Uropathogens

Fatima Hamza Alzubaidy[1] and Nawfal Hussein Aldujaili [2]

[1,2]Department of biology, faculty of science, Kufa University, Iraq

Abstract. Bioproduction options for nanoparticles are becoming a highly significant subject, with environmental and economic benefits over physical and chemical processes. The purpose of the current study is to detect the antimicrobial activity of chitosan-Graphene oxide nanoparticles that include The Minimum Inhibitory Concentration (MIC) is a method of determining antibacterial activity, Antibiofilm Activity by Tissue culture plate method, Antioxidant activity, and finally the hemolysis activity of mixing nanoparticles CS- GO that synthesis biologically. the result of Antibacterial activity showed the ID50 for inhibition is at a concentration (1 mg/ml), while the Antibiofilm activity result shows the high concentration of CS-GO nanoparticles (16 mg/ml) showed the higher activity in inhibition of biofilm formation while the less concentration (0.6mg/ml) appeared very low inhibition activity on biofilm formation. The antioxidant activity of nanoparticles showed the high scavenging ability at a concentration (600 mg/ml) as (54.06%) while the less concentration (100mg/ml) appeared scavenger ability as (29.42%) also the hemolysis activity on CS-GO nanoparticles on blood showed different ability at different concentration, the preferred concentration (1mg/ml) show no hemolysis on blood.

Keywords: Nanobiotechnology, Urinary tract infection, Chitosan-graphene oxide nanoparticles, MIC, Antibiofilm, Antioxidant, Hemolysis activity.

1. Introduction

Nanobiotechnology integrates biological concepts with physical and chemical processes to create nanoparticles with specialized functions at a cheaper cost than physical or chemical methods. Based on basic features such as size, distribution, and shape, nanoparticles have new or changed properties. The number of novels uses for NPs and nanomaterials is steadily rising [1]. Nanoparticles can be hollow or solid, and they can be formed of a variety of materials in a variety of layers, each with its function. A core functioning layer, a protective layer, and an outside layer that permits interaction with the biological environment are generally present [2]. The core functional layer usually has somebeneficial magnetic or optical activity, with fluorescence being the most frequent. The functional layer is protected from chemical damage caused by water, air, or cell componentsby the protective layer. as well as any harmful characteristics of the functional layer's chemicals. Nanoparticles can be identified using the outer layer [3].

In the production of NPS, Redox membrane proteins are used by bacteria and fungus for surface synthesis, and extracellular enzymes are used for extracellular synthesis [4]. Synthesizing NPS utilizing natural mechanisms such as carbohydrates, microbial enzymes, vitamins, polysaccharides, biodegradable polymers, microorganisms, and biological systemsis one approach to achieve this aim. The manufacture of NPs by bacteria is one method witha lot of promise [5]. The development of a controlled and scalable technique for thebiosynthesis of monodispersed and extremely stable NPS has been the focus of recent research. As a result, in green nanotechnology, Various bacterial species have been used to investigate alternative methods of NP production. [6]. Deacetylation of chitin, a protein foundpredominantly in crab exoskeletons, produces chitosan nanoparticles, a cationic biopolymer.
[7] Because of its biocompatibility, nontoxicity, mucoadhesion, biodegradability, and antibacterial activity, as well as its natural origin and inexpensive cost, this biopolymer hasbeen widely investigated for possible uses in several disciplines [8].

GO is a nanomaterial that has been utilized in a variety of applications for more than150 years [9]. It is the forerunner of graphene, a two-dimensional carbon allotrope that is one of the best in the world. Electrical conductivity [10], thermal properties [11], transparency [12] and mechanical strength [13]. Chitosan is a polysaccharide made up of glucosamine (2-amino- 2-deoxy-D-glucose) and N-acetyl glucosamine (2-acetamido-2-deoxy-D-glucose) units connected by (1-4) links. Chitosan has a cationic composition due to the presence of amino groups in its structure, which confers biological activity in low pH environments, particularly through association with Proteins, polysaccharides, and phospholipids are all negatively charged substances present in bacterial cells [14]. Research on chitosan modification is growing due to biomedical and pharmaceutical uses, intending to improve its already well- established antibacterial capabilities against *Staphylococcus aureus* and *Escherichia coli*. Because the biopolymer's bactericidal effectiveness has previously been proven, they are themost commonly seen microorganisms in scientific literature [15]. Increased free radical generation can overwhelm resistance enzymes like catalase, peroxidase, and superoxide dismutase, causing severe biological consequences (e.g., death) by oxidizing membrane lipids, cell proteins, enzymes, and DNA, affecting cellular respiration [16]. Lower-weight

chitosan offers a number of advantages versus high-weight chitinoids when it comes to removing free radicals [17].Chitosan has been proven to prevent Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli* from forming biofilms.

Graphene has been used in medicine in recent years, notably for DNA sequencing [18],biosensor creation, and cell differentiation and proliferation [19]. Its functional derivative, GO, offers unique properties that make it more suited for biological uses. Its ability to disperse in a range of solvents distinguishes it, making it easier to handle [20]. GO is also usedto deliver anticancer drugs to biological cells [21], as well as aptamers for ATP probing in mouse epithelial cells and gene transfer [22]. The antibacterial activity of GO was investigatedby looking for an inhibitory zone around the GO disc, which showed bacterial toxicity against Staphylococcus aureus and Escherichia coli [23].

2. Material and procedure

2.1. Antioxidant Activity of CS-GO nanoparticles

Antioxidant activity of CS-GO Nanoparticles The DPPH technique was used to test the extract's ability to scavenge free radicals. The DPPH solution (0.006 percent w/v) was made in 95 percent methanol. Discoloration was detected at 517 after 30 minutes in the darkincubation, using freshly produced DPPH solution in test tubes, and mixed NPs (100, 200, 300, 400, 500, and 600 mg/ml) were added to each test tube until the total volume was 2 ml(UV visible spectrophotometer). Measurements were carried out in at least three different ways.

A DPPH solution with the same volume (without NPs) was used as a control, and 95 percentmethanol was utilized as a blank. The following equation was used to compute the percentage of DPPH free radical scavenging:

DPPH scavenging impact (%) = (Ao –A1) / Ao X 100

where Ao is the absorbance in the absence of mixed nanoparticles and A1 is the absorbancein the presence of mixed nanoparticles. The test chemicals' actual absorption reduction was compared to the positive controls [24].

2.2. Antibiofilm Activity of SC-GO NPs by Tissue Culture Plate Method

The gold standard test for identifying the formation of biofilms was TCPM [25]. In 10mL of trypticase soy broth with 1% glucose, a loopful of freshly cultivated isolates was injected. After that, the infected broth was maintained in the incubator for 24 hours at 37°C.Bacterial suspensions were further diluted in a new medium at a ratio of 1:100. A sterile polystyrene tissue culture plate with 96 flat bottom wells was used to pour the prepared bacterial suspension into individual wells. The tissue culture plate also contained control organisms. Sterility must be ensured, and non-specific binding must be discovered . the onlysterile broth was utilized. After 24 hours of incubation at 37°C, the plate was gently tappedto remove

the contents of the wells, followed by washing with 200 l of phosphate buffer saline. To eliminate any free germs in the wells, The washing operation was carried out fourtimes in total. Sodium acetate (2%) was added to the wells and left for 30 minutes to repair the biofilms formed by bacteria adhering to them. Crystal violet was used to stain the

biofilms that had been repaired (0.1 percent). The wells were carefully cleaned withdeionized water after 30 minutes to eliminate any remaining discoloration.

The optical density (OD) of pigmented bacterial biofilms were assessed using a micro- ELISA reader after they had dried (at 570 nm wavelength). The test was repeated three times,with the average of the three OD findings. Optical densities revealed bacterial adherence tothe wells and biofilm formation. The OD values were computed, and biofilm development was classed as strong, moderate, or non/weak, as described in previous studies. (Table 1) [26] [27].

Table 1. Tissue culture plate technique for assessing biofilm development

Optical densities values	**Adherence**	**Biofilm formation**
< 0.120	Non	Non/weak
0.120- 0.240	Moderate	Moderate
> 0.240	Strong	Strong

2.3. Hemolysis Effect of CS-GO Nanocomposite

The percentage of hemolysis was used to assess the hemolytic toxicity of mixed NPs.On the blood of one healthy donor, hemolysis experiments were performed: 15 liters of nanoparticles at three concentrations (1, 5, and 10 mg/ml) were added to 285 liters of wholeblood. The positive control is triton X-100, whereas the negative control is blood only. On ashaking plate, the suspension is incubated for 24 hours at room temperature.

The suspension is centrifuged at 10000 for 5 minutes after the incubation period. X Mark (Biorad, USA) microplate scanning spectrophotometer analyzes the supernatant in a 96-wellplate at 550 nm.

H (percent) = (OD550nm sample – OD550nm Tyrode) x (OD550nm Triton X-100 1 percent

- OD550nm Tyrode)*100.

The controls (both positive and negative), respectively, produced 100% and 0% lysis [28].

2.4. Antibacterial activity of CS-GO nanocomposite

After a 24-hour incubation period at 37°C,the minimum inhibitory concentrations (MICs) were measured

visually and spectrophotometrically. The minimum inhibitory concentration (MIC) for combined NPs was determined as the lowest NP concentration thathalted growth visually or reduced growth by 80% as compared to growth in the drug-free well. The 50 percent increase is noteworthy when compared to growth in the drug-free well.The IC 50 was determined as a 50% suppression of growth in comparison to growth in the drug-free well [29].

3. Result and discussion

3.1. Antibiofilm activity of CS-GO Nanocomposite on E.coli

The results of this study show that CS-GO nanoparticles can inhibit biofilm formationin *E. coli* bacterial isolates from UTI at various concentrations. For example, the high concentration of CS-GO (16mg/ml) has very high activity in inhibiting biofilm formation, whereas the inhibition titer decreases at lower concentrations (1mg/ml), implying that the effective concentration of CS-GO is higher. According to these findings, which are in line with prior study, the antibiofilm feature of chitosan is mostly owing to its polycationic nature, which is given by the functional amino groups (NH3 +) of N-acetylglucosamine units. The positive charge of CS interacts electrostatically with negatively charged biofilm components such as EPS, proteins, and DNA, preventing the production of bacterial biofilms [30]. GO is an antibacterial carbon nanomaterial that may be employed alone or in conjunction with other substances [31].

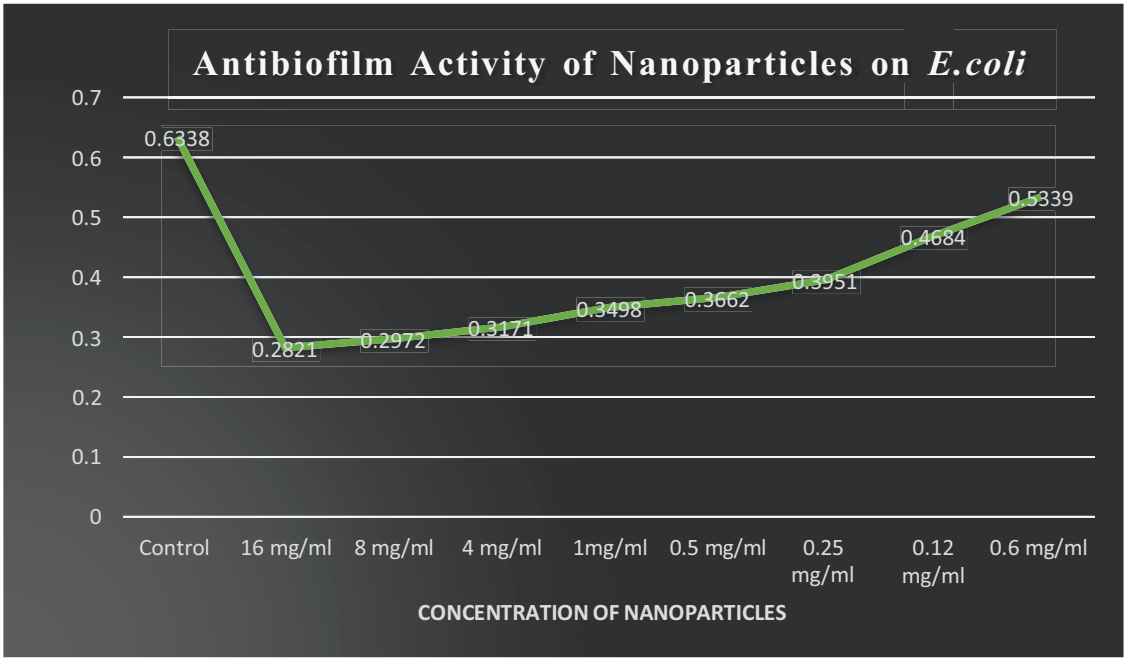

Figure 1. Antibiofilm activity of CS-GO Nanoparticles on biofilm formation in *E.coli* isolated from UTI

3.2. Antioxidant activity of CS-GO nanocomposite

According to the current study, Chitosan-Graphene oxide Nanoparticles can scavenge free radicals, and this ability increases as increase the concentration of CS-GO. As shown in Figure (2), the Scavenger ability is (29.42 percent) at 100 mg/ml, and it increases to (54.06 percent) at 600 mg/ml of CS-GO nanoparticles. The DPPH radical scavenging experiment result ascribed to [32] [33] revealed that graphene increases the antioxidative efficacy of nanoparticles at greater concentrations, but chitosan has very little influence on the activity at all doses. Electron transport [34] [35] and hydrogen donation from functional groups (-OH) linked to graphenic structure [36] are other essential factors. The reduction of DPPH, a stable free radical, is used in the DPPH assay technique. Antioxidants that scavenge DPPH radicalscan transfer hydrogen to free radicals, forming non-radical species [37].

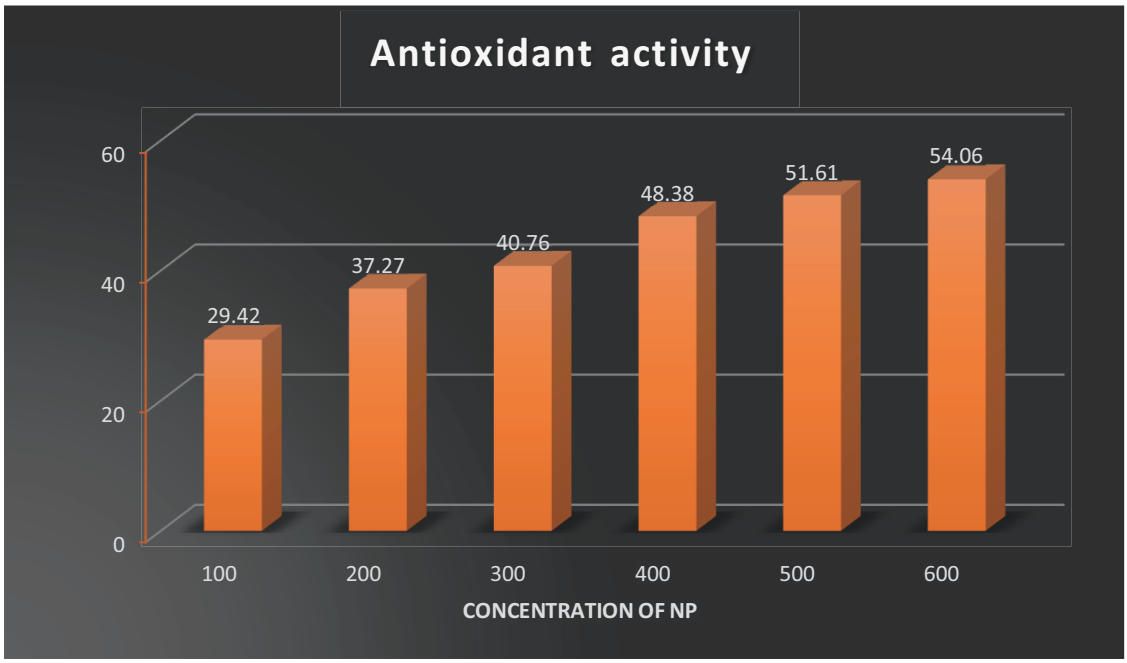

Figure 2. Antioxidant activity of CS-GO nanoparticles

3.3. Antibacterial activity of CS-GO nanocomposite by MIC

As demonstrated in Figure (3), the CS-GO nanoparticles had an ID50 of 1 mg/ml forinhibiting 80 percent of the growth in E. coli found in the urine after a urinary tract infection. According to the findings, the antibacterial activity of GO-CS nano-fibrillar was concentration-dependent. The GO-CS nano-hybrid also has antibacterial activity against *E. coli*. comparable to the broth test. These findings are consistent with earlier findings from other studies [38] [39].

When it comes to GO-CS nano-hybrids, the greatest antibacterial activity against *E. coli* was reported. It can be linked to the synergistic action that results from the combination of two of the most renowned

antibacterial compounds, CS and GO. Graphene-basedmaterials' antimicrobial properties, as well as CS, has been reported to include both chemical and physical mechanisms. As a consequence, the crossing method suggests that the antibacterial presentation of GO and CS are combined. The combined interaction of GO functional groups and CS amine groups may boost nano-hybrid absorption and penetration,resulting in lipid molecule extraction damage [40] [41] [42]. The combined presence of GO and CS has a chemical consequence may be extrapolated to result in the formation Increased antibacterial activity of hybrids due to the production of more reactive oxygen species (ROS) [43].

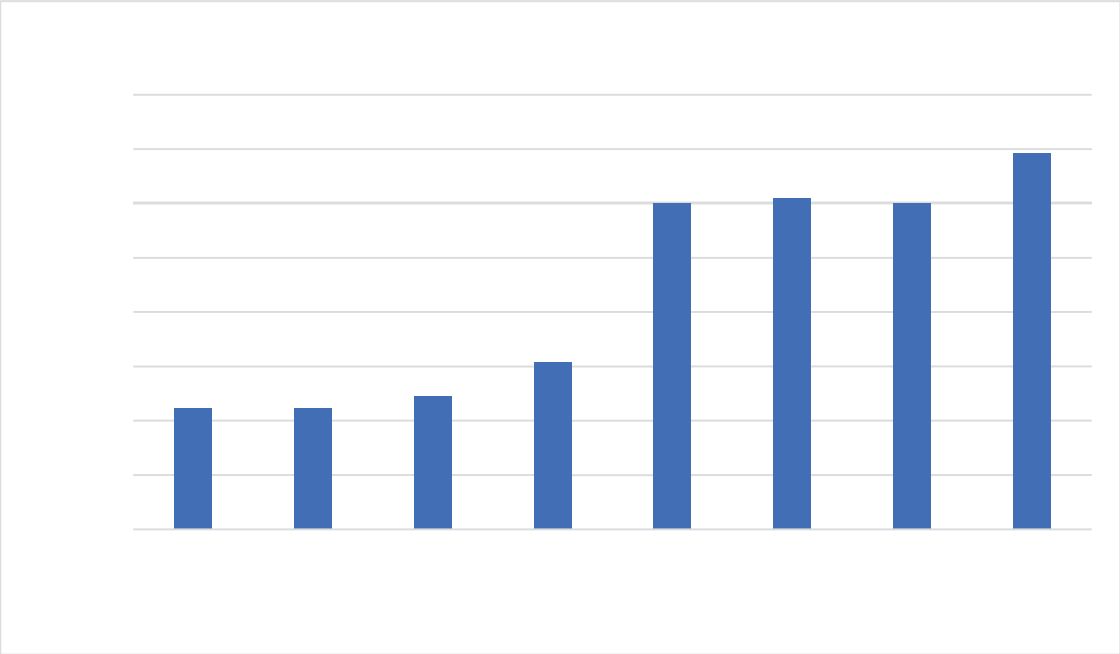

Figure 3. Minimum Inhibitory Concentration of CS-GO nanocomposite on *E. coli* isolated from UTI

3.4. Hemolysis activity of CS-GO nanocomposite

In this investigation, the hemolysis impact of nanoparticles on blood was not active atconcentrations of 1 mg/ml, but it was active at concentrations of 5,10 mg/ml, as shown in Figure (4). Yildirim et al. found that chitosan–graphene nanocomposites alter the membrane of blood cells, most likely by membrane deformation, resulting in membrane damage and hemoglobin release, regardless of surface modification. The proteins on the surface of erythrocytes can bind to the surface of chitosan–graphene films, reducing the lifetime of red blood cells dramatically [44]. According to the researchers, graphene oxide (GO) derivativesinteract with neutral, positively charged, and negatively charged lipid membranes (Liao et al.) The erythrocyte membrane can be disrupted by electrostatic interactions between GO and the positive groups of phosphatidylcholines located in the outer monolayer. Hemocompatibility may be improved via GO surface coatings. The erythrocyte membranehas previously been demonstrated to be damaged by GO and graphene sheets, resulting in adose-dependent hemolytic impact on RBCs [45].

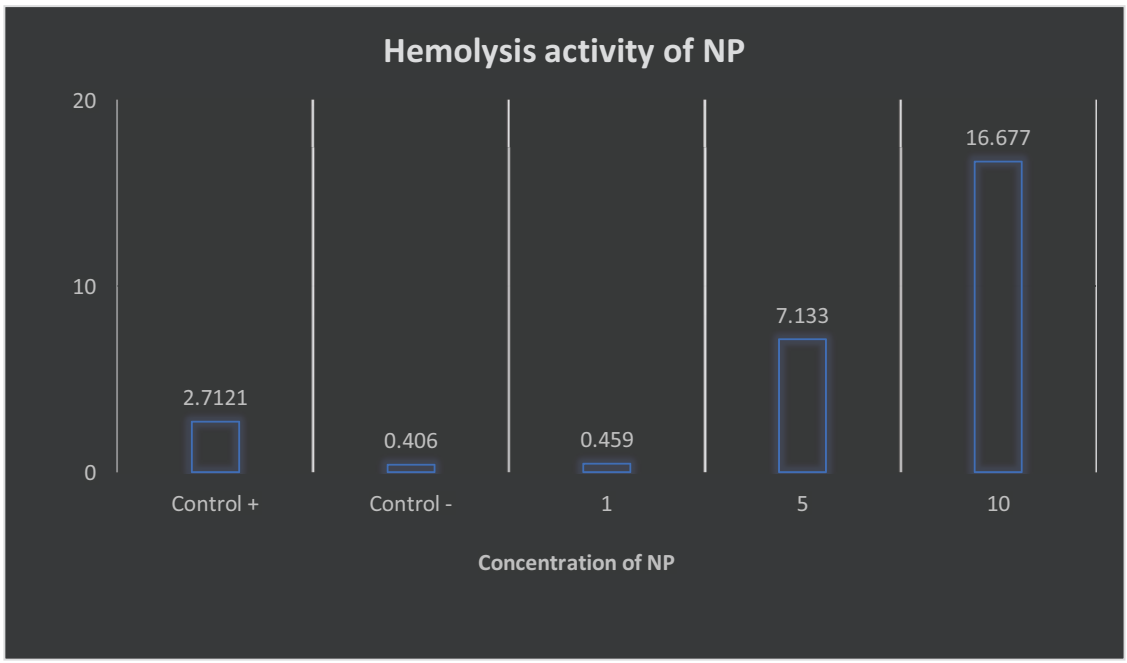

Figure 4. Hemolysis activity of CS-GO nanocomposite on healthy blood

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