Antibacterial Activity of Graphene Oxide Nanosheet Against *Salmonella* Associated Food Contamination

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ABSTRACT

Salmonella is a Gram-negative bacterium and the causative agent of typhoid and salmonellosis. Fever, headache, nausea, vomiting, abdominal pain and diarrhea, are the common symptoms of salmonella infections. Graphene Oxide Nanosheet (GO Nanosheet) has been used as antimicrobial agents against many bacterial pathogens. However, the efficiency of GO Nanosheet to prevent Salmonella infections is poorly understood. Therefore, in this study, we used the chicken muscle attachments model to determine the antibacterial activity and binding capacity of GO Nanosheet against Salmonella typhi and Salmonella paratyphi. Results indicated that GO Nanosheet were significantly effective in blocking Salmonella attachment to chicken muscle. This study suggests an alternative strategy for reduction of Salmonella contamination in fresh and frozen food products.

Keywords: Salmonella, Graphene Oxide Nanosheet, Food contamination, Attachment model.

Introduction

Salmonella is the second common foodborne pathogen that causes gastroenteritis by consumption of *Salmonella* contaminated food or water [1]. Poultry, egg products, pork, companion animals, and ready to-eat food products are the most common source of *salmonella* [2, 3]. Foodborne pathogen infects millions of people annually leading to sever economic loose and sometimes death. Symptoms of *salmonellosis* include gastroenteritis, bloody diarrhea, abdominal cramps, myalgia, fever, headache, nausea and vomiting. However, these symptoms can be much complicated in pregnant women, children, and the elderly with a weekend immune system [4]. Antibiotics are considered very important and well known treatments of such bacterial infections. However, the wide spread use of antibiotics accelerated the emergence of multidrug resistant pathogens [5]. The treatment with antibiotics is become really challenging to pharmaceutical and medical professionals to prevent such multidrug resistant pathogens, which creates a serious problem in increasing the death rate due to bacterial infections [6]. Therefore, the world needs new treatment strategy to prevent or minimize the high rate of such bacterial diseases.

Recently, the emergence of nanoparticles and their antibacterial properties created tremendous change in prevention of bacterial infections. Nanoparticles are considered very

important new strategy to solve the problem of multidrug resistant pathogens by many ways. The small size of nanoparticles enables them to penetrate the bacterial cell wall and interfere with the bacterial biochemical pathways and destroying their organelles, which eventually lead to the bacterial death [7]. Also, it can bind to bacterial cell wall, which ultimately lead to bacterial cell disruption [8]. Another way of the nanoparticle effects on bacteria is can hinders the synthesis of bacterial nucleic acid by inhibiting the bacterial enzymatic activity [9]. Nanoparticles also can generate reactive oxygen species (ROS), which have a very important role in distraction the bacterial cell membrane [10].

Graphene oxide (GO) is one of the widely used nanoparticle that has a multidisciplinary applications, such as antibacterial properties [11-13]. GO is a versatile carbon nanomaterial that has a distinctive chemical and physical properties, such as mechanical stiffness, high surface-to-volume ratio, and electronic transmission characteristics [14, 15]. However, the effect of GO Nanosheet on bacterial cell attachments has not been characterized yet. In the current study, the effects of GO Nanosheet on *salmonella* associated chicken contamination were investigated. This strategy may provide an alternative method to reduce *salmonella* contamination in fresh and frozen chicken products.

Materials and Methods

Bacterial strains and growth media

In this study, we used *Salmonella typhi* and *Salmonella paratyphi* isolated from diarrhea patients and diagnosed by Al-Hashmia Hospital in Babylon Governorate. Bacteria were cultured in brain heart infusion (BHI) agar or broth (Difco, Sparks, MD) and incubated at 37 °C for 24 h throughout the study.

Salmonella muscle attachment model

In this experiment, chicken muscles were used to conduct the attachment model. All Chicken experiments were performed in the postgraduate laboratory at Al-Qasim Green University. Chicken attachment model was optimized using *Salmonella* chicken skin attachment model [16, 17]. Briefly, inspected specific-pathogen-free (SPF) chicken muscles were obtained from the Babylon hatchery and cut into uniform, 6 mm circular sections by biopsy punch. The uniform muscle samples were placed in 1.5 ml of sterile centrifuge tubes. *S. paratyphi* and *S. typhi* were grown to mid-log phase (OD_{600} 0.6 ± 0.8) and diluted 10,000 times in phosphate-buffered saline (PBS). By serial dilution and plate colony count the bacterial concentrations were determined. 1 ml of diluted bacterial strains (~$1x10^3$ CFU) was added to each muscle sample. The attachment experiment was conducted at room temperature for 30 min to allow bacteria to adhere to the muscle. After this period, the chicken samples were washed gently two times with 1 ml PBS by inverting the tubes up and down ten times then chicken samples were also washed a third time on a shaker for 30 min at room temperature to remove all unattached bacteria. After washing, muscle samples were homogenized in 250 µl PBS by a hand held tissue homogenizer, then 750 µl PBS was added to the homogenate. Bacterial numbers were determined by serial dilution and plate colony count. The experiments for each bacterial strain included four replicates and each experiment was repeated ten times.

GO Nanosheet effect on *Salmonella* growth and muscle attachment

To test the antibacterial properties of GO Nanosheet on *S. typhi* and *S. paratyphi* growth and to determine the optimum concentration to be used in attachment experiments, 0, 12.5, 25, 50,100, and 200 µg/ml solutions of GO Nanosheet in BHI broth were prepared from the stoke solution (400 µg/ml). At each concentration of GO Nanosheet, four culture tubes were inoculated with *S. paratyphi* and four culture tubes with *S. typhi* (5 concentrations of GO Nanosheet x 4 replicates = 20 cultures for each strain) and cultures were grown overnight at 37 °C. OD_{600} values were measured, and average values at each concentration were calculated. Colony numbers were calculated at each concentration by serial dilution. After determination of the dose that is not affecting the growth of *S. paratyphi* and *S.*

typhi, all GO Nanosheet were tested following the *S. paratyphi* and *S. typhi* muscle attachment model described above. The two experimental groups were muscle + *S. paratyphi* + GO Nanosheet (treatment), and muscle + *S. paratyphi* (control). This procedure was also applied to *S. typhi* as flows; two experimental groups were muscle + *S. typhi* + GO Nanosheet (treatment), and muscle + *S. typhi* (control). Experiments were included four replicates and each experiment was repeated ten times for each strain.

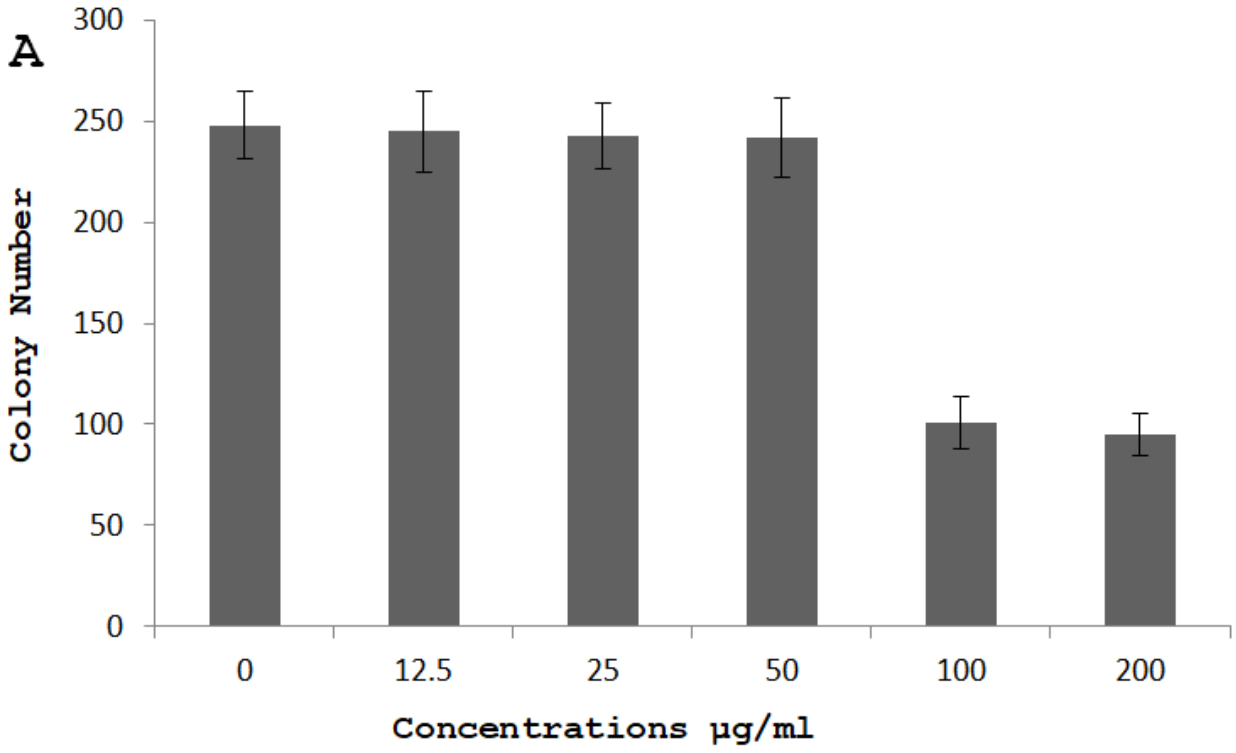
Statistical analysis

Normality of bacterial counts was checked by visual assessment of histograms using PROC UNIVARIATE in SPSS for Windows 9.3. The log10 transformation was applied for colony counts to be normally distributed, and transformed data were analyzed by Student's t test ($P < 0.05$).

Results

Effect of GO Nanosheet on *Salmonella* growth

GO Nanosheet concentrations at 12.5, 25, and 50 µg/ml had no significant effects on the growth of both *Salmonella strains*. GO Nanosheet concentrations at 100 and 200 µg/ml resulted in high growth inhibition of bacterial strains. However, the effect of GO Nanosheet on *S. typhi* growth was greater than its effect on *S. paratyphi* (Figure 1.A-Figure 2.A). Therefore, all muscle attachment experiments were conducted at 25 µg/ml concentration because it does not inhibit the growth of *salmonella* strains. When bacterial viability was checked, colony numbers were correlated with the OD readings (Figure 1.B-Figure 2.B).



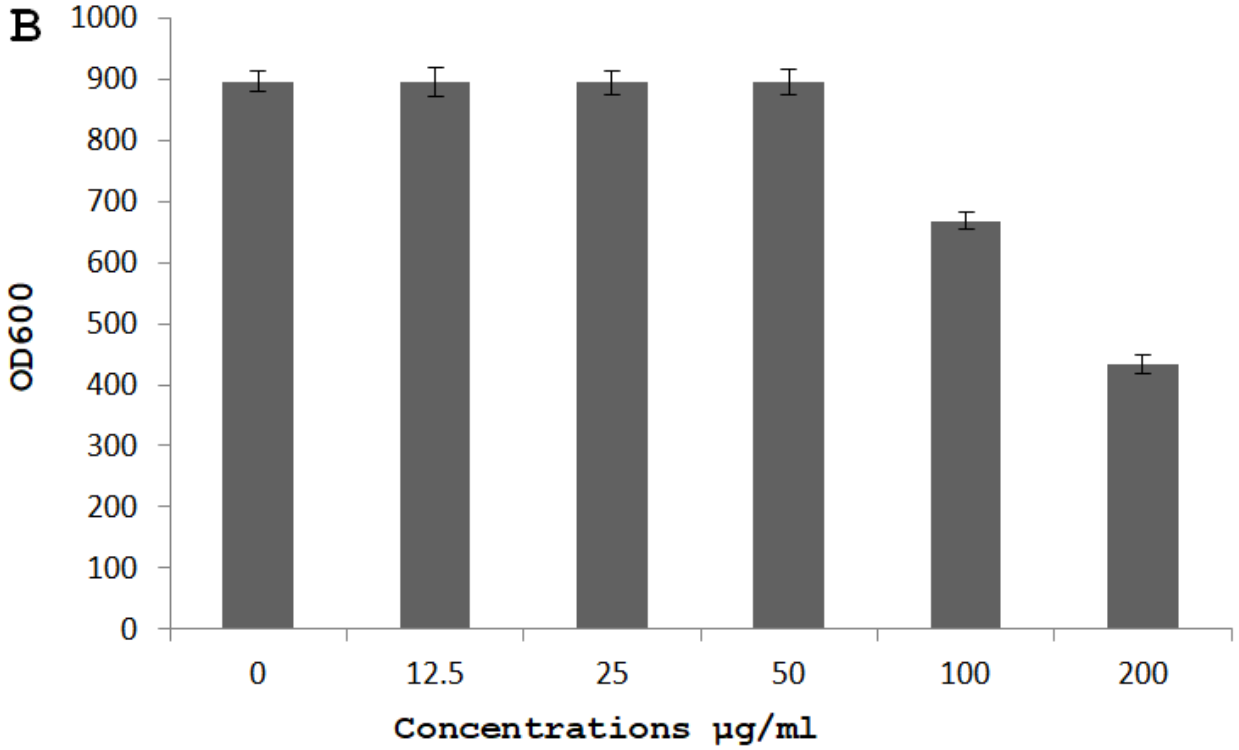
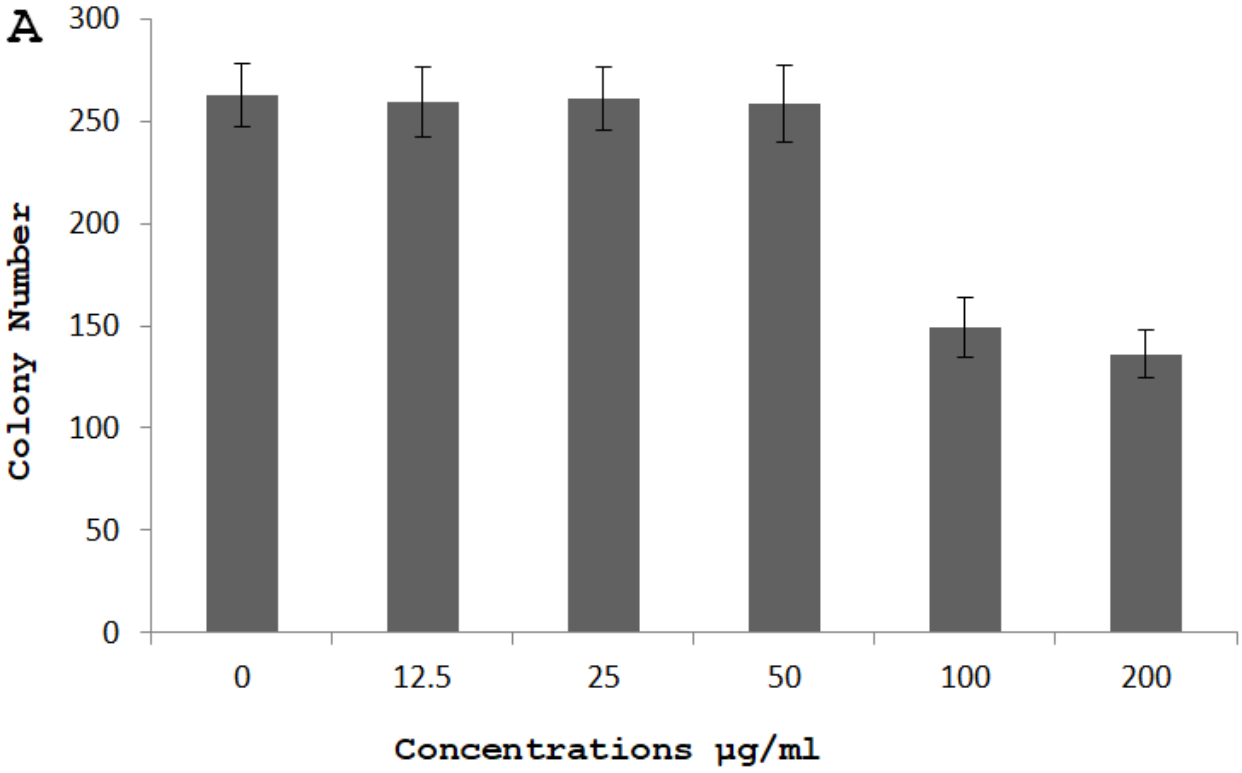


Figure 1. Effect of different concentrations of GO Nanosheet on *S. typhi* growth. Growth comparison was conducted by colony counts (A) and OD measurement (B).



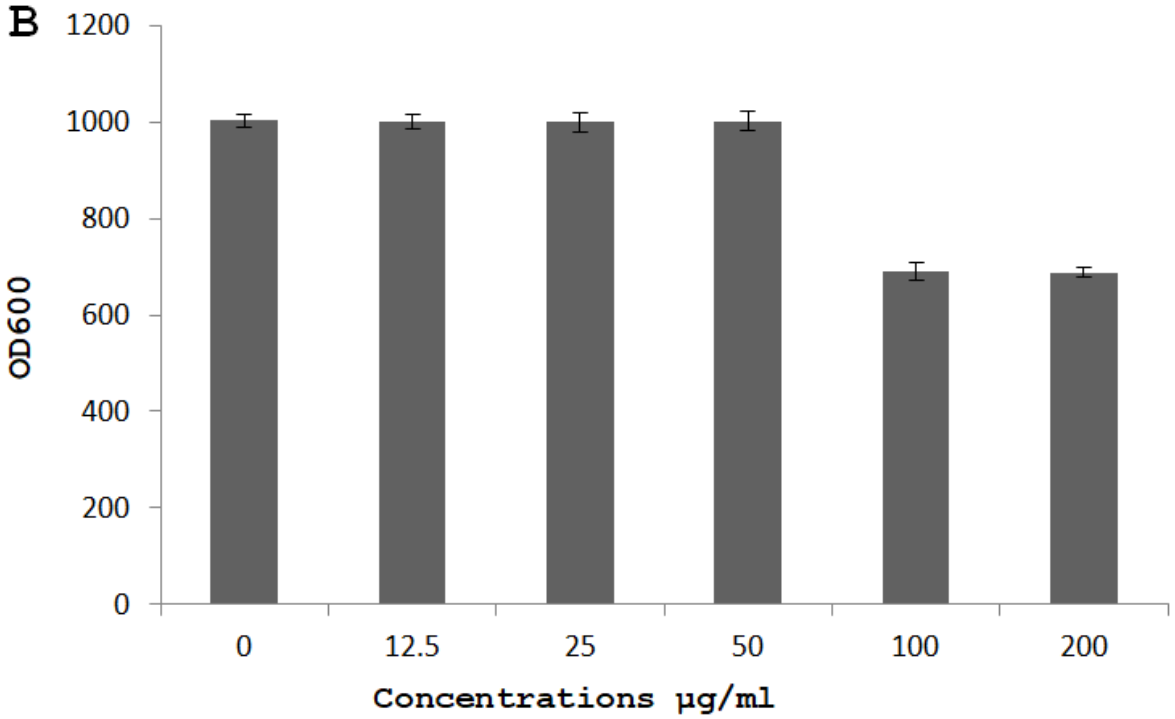


Figure 2 Effect of different concentrations of GO Nanosheet on *S. paratyphi* growth. Growth comparison was conducted by colony counts (A) and OD measurement (B).

Effect of GO Nanosheet on *Salmonella* muscle attachment

GO Nanosheet significantly affected the attachment of *salmonella* strains on chicken muscles. 25 µg/ml concentrations of GO nanosheet were highly effective in preventing of *S. typhi* attachment to chicken muscles (Figure 3). Also, GO Nanosheet significantly reduced *S. paratyphi* attachment (Figure 4). Our results demonstrated that GO Nanosheet significantly reduced *salmonella* attachments to chicken muscles.

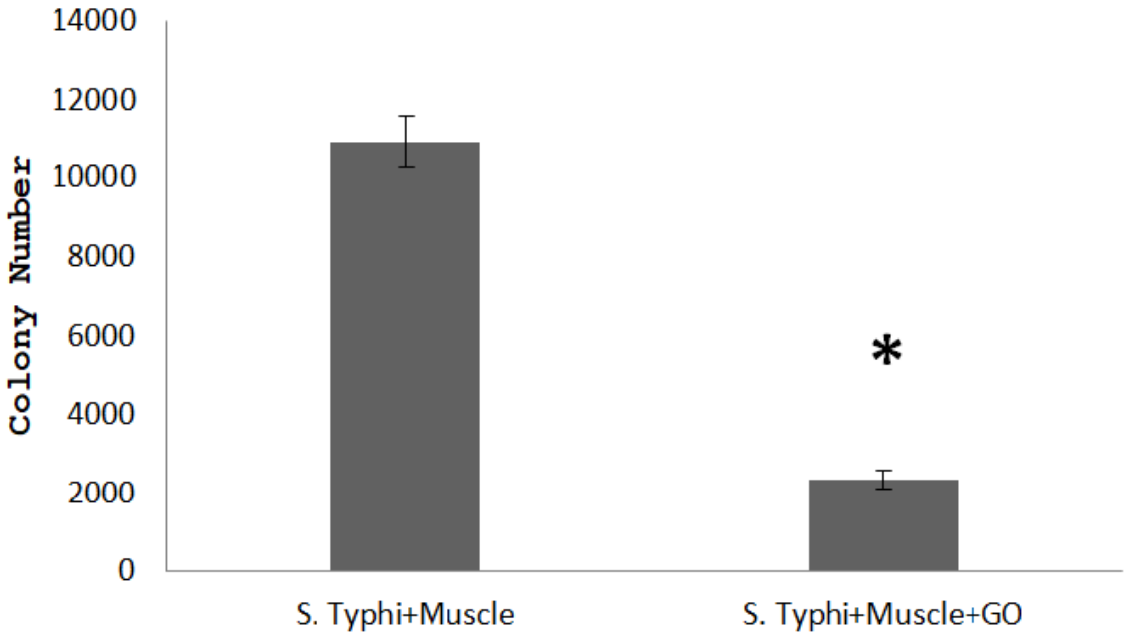


Figure 3. Effect of GO Nanosheet on *S. Typhi* attachment to chicken muscle. (*) significant (P values < 0.001)

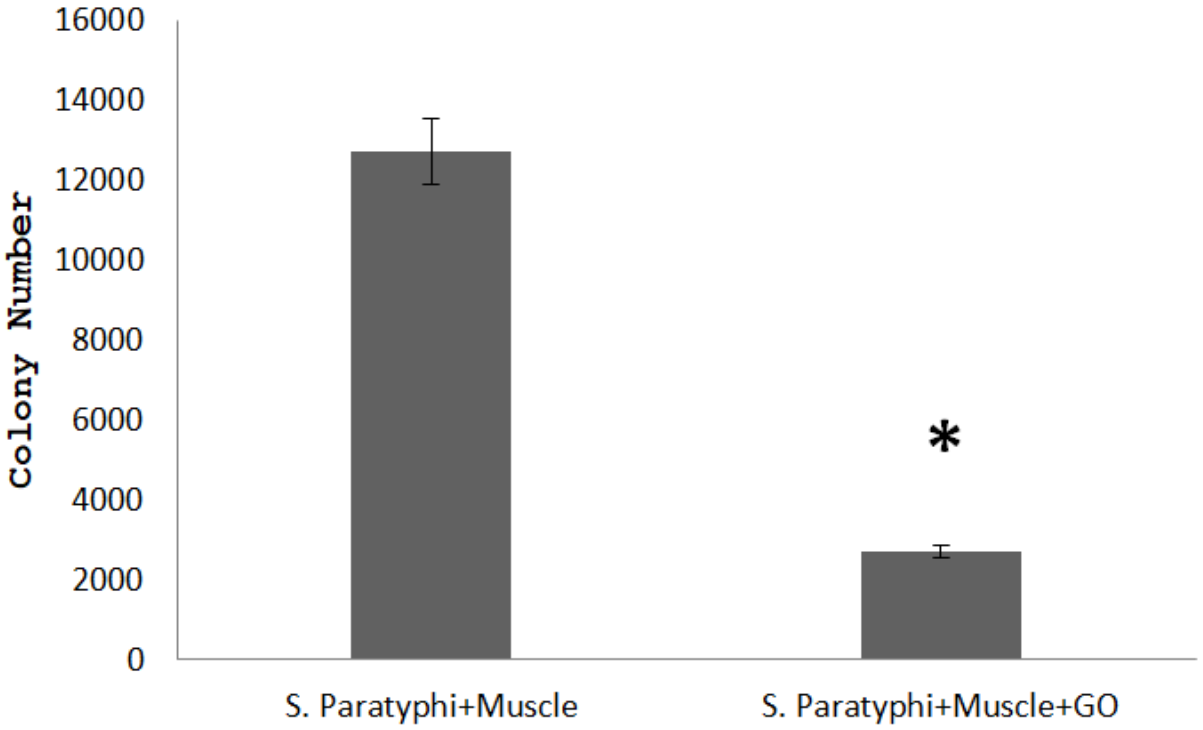


Figure 4. Effect of GO Nanosheet on *S. Paratyphi* attachment to chicken muscle. (*) significant (P values < 0.001)

Discussion

Salmonella species are common organisms for food-borne illness. One approach to prevent the problem of *salmonella* associated food contamination is the prevention of bacterial cell adhesion. Therefore, the aim of current research was to evaluate the effect of GO Nanosheet on *salmonella* attachment to chicken muscles. We determined the optimal concentration of GO Nanosheet that is not effecting the *salmonella* growth and used it in our attachment experiments. It was observed that 0, 12, 25, and 50 µg/ml concentrations of GO Nanosheet had no significant inhibition of *salmonella* growth. In contrast, GO Nanosheet concentration at 100 and 200 µg/ml reduced the bacterial growth. However, GO Nanosheet concentration at 25 µg/ml had no effect on *salmonella* strains growth and it was significantly reduced the attachment of *Salmonella* strains to chicken muscle. These results indicated that the GO nanoparticle may have an effective binding capacity to our bacterial strains. Therefore, GO Nanosheet had a potential role in prevention of bacterial contamination in food.

The possible explanation of our results is that GO Nanosheet interact with the bacterial cell wall and disrupts the integrity of the bacterial membrane, which subsequently mediates the release of functional enzymes such as β-D-galactosidase from the bacterial cell and ultimately kills the cell. In another study, GO Nanosheet show high efficiency in trapping bacteria between clusters graphene sheets which resulted in prevention of bacterial

adhesion [18, 19].

It was also shown that graphene nanoparticle has effective antimicrobial properties [20, 21]. Previous research on GO shows its ability of destructive extraction of lipids from bacterial lipid bilayer [22]. High antibacterial activity of GO Nanosheet may also due to its ability to act as an electron transport pathway in which electrons from bacterial membrane are believed to travel through the GO and are accepted by the conducting substrate under a negative membrane potential. This will lead to disruption of the electron transport within the bacterial cell, hindering ATP production and consequently leading to bacterial death [23-25]. Upon contact with GO Nanosheet and due to its small size, bacterial cells may trap and the trapped bacteria may be separated from the external microenvironment. This will limit bacterial access to the nutrients resulting in bacterial growth inhibition. To avoid the risk of bacterial food contamination, the bacterial adhesion should be controlled, keeping the microbial population low.

Conclusion

We determined the antibacterial activity of GO Nanosheets on *S. typhi and S. paratyphi*. Our results showed that GO Nanosheets have the predominant antibacterial behavior against our bacterial strains and significantly reduced bacterial adherence to chicken muscle. However, this work suggests that GO Nanosheets had very significant effects on the viability of *Salmonella* attachment to chicken muscle, which could provide an alternative strategy for reducing *Salmonella* contamination in frozen food products.

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