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Effect of Antibacterial Activity of Zinc Oxide Nanoparticles against *E. coli* and *Staph. aureus* on Quality and Shelf Life of Minced Meat

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Abstract

Metal nanoparticles have attracted a lot of attention recently in several nanotechnology fields. Zinc oxide nanoparticles (ZnO NPs) have attracted the most interest among metal nanoparticles due to their possible antibacterial impact, particularly in regulating the safety of meat and meat products. This study looked at how the quality and shelf life of minced beef were affected by antibacterial activity of zinc oxide nanoparticles (ZnO NPs) against *S. aureus* and *E. coli*. So, minced meat samples were inoculated with *S. aureus* and *E. coli* and then exposed to various doses of ZnO bulk and nanoparticles, including 4 mM, 6 mM, and 8 mM then kept at 4°C for 12 days, then *E. coli* and *S. aureus* growth and count were examined to assess ZnONPs action on them and on minced meat quality and shelf life. The findings showed that *E. coli* and *S. aureus* growth and count in minced beef were significantly reduced by ZnO NPs at 8 mM concentration. The findings suggest that ZnO NPs could be utilized as antibacterial agents and for shelf-life extension in food preservation.

KEYWORDS
E. coli, *S. aureus*, Minced meat, Nanoparticles, Zinc oxide, Antibacterial.

INTRODUCTION

Meat and meat products are essential nutrient sources for humans due to their excellent protein content, essential amino acids, vitamin B group, and minerals (Bohrer, 2017). Especially, minced meat, which utilized in a variety of dishes and cooking methods but unfortunately it is a quickly perishable food (Ahmed and Sabiel, 2016). Due to their high water activity and nutrient components, beef and meat products offer an environment that is suitable for food-borne diseases and spoiling bacteria (Anas *et al*., 2019). Deteriorating changes such as odd tastes, discoloration, altered textures, and slime production are caused by microbial decomposition of beef products (Iulietto *et al*., 2015). The relationship between any product's shelf life and its deterioration is very strong. This relationship establishes a border between an acceptable and an unacceptable bacterial concentration, which defines off-odors, off-flavors, and an unattractive appearance. These sensory changes are correlated to the type and quantity of microbes that are originally present, as well as to their development throughout time. The beginning total microbiota of meat products is roughly 10^2-10^3 cfu gr^{-1}, and it includes a wide range of species (Ray and Bhunia, 2013). Pathogens that cause food poisoning are the leading source of disease and death worldwide, and they are frequently linked to poor hygiene practices (Adesokan *et al*., 2020). *Staphylococcus aureus*, Salmonella, Escherichia coli, and Listeria monocytogenes are bacterial pathogens linked to meat products that cause severe disease outbreaks and product recalls (Ijabdeniyi *et al*., 2019).

Nanotechnology can offer a way that can be used throughout many stages of food chain processing to enhance food safety, quality control and extending the shelf life of foods (Baltić *et al*., 2013; Biswas *et al*., 2022). Zinc oxide nanoparticles (ZnONPs) are the most significant nanomaterials that are frequently utilized due to their antibacterial activity in the food industry (Gudkov *et al*., 2021). The FDA has allowed the use of these nanoparticles in the fields of food processing because they operate as biocides and have no adverse effects (Toker *et al*., 2013). By producing Zn^{2+} ions and reactive oxygen species (ROS), which damage cell organelles and result in cell death, ZnO nanoparticles exhibit antibacterial action against different bacteria, including *E. coli*, *S. aureus*, and various others (Kim *et al*., 2020). Researchers examined the antibacterial activity of ZnO nanoparticles against *E. coli* and *S. aureus* in fresh calf minced meat (Ardestani, 2016; Marcous *et al*., 2017). Using nanoparticles in food as food additives to preserve colors and prevent spoiling is one of the most significant uses of nanotechnology in food and meat (Lamri *et al*., 2021; Biswas *et al*., 2022).

Since *S. aureus* and *E. coli* were inoculated into minced meat during refrigerated storage (4°C), the main goal of this study was to compare the antibacterial activity of bulk ZnO powder to ZnO nanoparticles (ZnO NPs) against these pathogens which representative as microorganisms of public health concern in food-related environments.

MATERIALS AND METHODS

Samples collection

Animal Health Research Institute lab. of Damanhur is where the experiment was carried out. Fresh minced beef (1.6 kg) was

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purchased, brought by a right way to the lab in an icebox, and kept there at 4°C until usage in this investigation. To remove background microflora, thin sheets of minced beef were exposed to ultraviolet light (wavelength 385 nm) for 30 minutes, 15 minutes on each side (Morsy *et al*., 2018).

Assessment of antibacterial activity of nanomaterials in minced meat (Abd El-Aziz et al., 2020)

In a sterile bag, minced meat samples were inoculated with *E. coli* O157:H7 and *S. aureus* (7 log CFU/ml) to achieve final concentration approximately 7 log cfu/ml of minced meat. Then, they were mixed thoroughly by gently squeezing of the bags by hand till even distribution of microbes occurred and left for 30 min for complete attachment between inoculated *E. coli*, *S. aureus* and minced meat. The initial load of *E. coli* and *S. aureus* were determined before the addition of nanomaterials. Phosphate buffer saline (PBS) was used for the treatment of control samples. The minced meat samples were divided into two groups (each weighing 800g) and then each group was divided into four portions (200g each). *E. coli* O157:H7 was inoculated to the first group, and *S. aureus* was inoculated to the second.

The inoculated samples of minced meat with *E. coli* O157:H7 and *S. aureus* exposed to various doses of ZnO NPs and bulk ZnO, at different concentrations 4 mM, 6 mM, and 8 mM. and kept at 4°C for 12 days to observe their antibacterial activity against *E. coli* O157:H7 and *S. aureus* and assess the quality and shelf life of inoculated samples. (While 200 g in each group still as control portion without any addition). ZnO NPs and bulk ZnO powder mixed with minced beef samples for a further 30 seconds to ensure even mixing. All samples were transferred individually into a standard sterile polyethylene bag (self-closed). Packed samples were labeled and kept at 4.0±1.0°C till spoilage of minced meat. Counting of *E. coli* and *S. aureus* and sensory evaluation were performed on days (zero day, 3rd, 6th, 9th and 12th) at refrigerated storage (4.0±1.0°C). The experiment was repeated in triplicate for each group and mean values were calculated.

Bacterial strains

E. coli O157:H7 (ATCC® 25922TM) (7 log CFU ml) inoculum preparation in accordance with WHO (1993) and *S. aureus* (ATCC 6538P) inoculum propagation in accordance with APHA (2001), which were obtained from reference laboratory of Animal Health Research Institute, Dokki, Giza,Egypt and reactivated and propagated using suitable cultures. Also, inoculum level was chosen to give an initial load of approximately 107 cfu / ml in inoculated samples.

Synthesis and preparation of zinc oxide nanoparticles

By dissolving 11 g of 99.9% pure zinc acetate hydrate (Zn (Ac) 2•2H2O, Sigma-Aldrich) in 500 ml of ethanol, zinc oxide nanoparticles were synthesized. The solution was then ultrasonically mixed with 2.9 g of sodium hydroxide to create a clear solution. The transparent solution-containing conical flask was placed in a water tank with a constant temperature of 60°C. The solution was then put into the conical flask along with 10 cc of distilled water. At 60°C, the solution was agitated for 30 minutes. The produced ZnO nanoparticles were centrifuged and dried at 60°C (Wang *et al*., 2007).

Microbiological analysis

Preparation of serial dilutions according to APHA (1992)

Minced meat samples were firstly cauterized by using hot spatula (surface sterilization) then the cauterized parts were removed by using sterilized scalpel and forceps, then under complete aseptic conditions 25 grams of each sample were weighted and transferred into a sterile homogenizer flask contained 225 ml of 0.1% peptone water. The content of each flask was homogenized at 14000 rpm for 2.5 min. for obtaining a dilution of 10^{-1}, from which 1 ml was transferred with a sterile pipette to a sterile test tube containing 9 ml of (0.1%) peptone water, from which a decimal serial dilution was prepared in a sequential manner up to 10^{-10}, to cover all expected range of samples contamination. For microbial counting, colonies were counted and recorded in colony forming units per gram (cfu/g) of meat samples using the formula:

cfu/g = level of dilution plated x number of colonies counted/volume plated. These were further expressed in mean colony forming units per gram (mean cfu/g) and converted to $\log_{10}$ base values ($\log_{10}$cfu/g).

E. coli enumeration

Accurately, 100 µl from each previously prepared serial dilution was spread over duplicated plates of Eosin methylene blue (EMB) agar (OXOID, CM0 069) using a sterile bent glass spreader. The inoculated and control plates were incubated at 37°C for 24 h. (FDA, 2001). The suspected colonies of *E. coli* were greenish metallic colonies with a dark purple center. These colonies were enumerated and expressed as log CFU/g of sample.

S. aureus count

Were determined according to FDA (2001) on Baird Parker agar plate at 35°C for 48 hours. Suspected colonies which appeared as black, shiny colonies with halo zone around them were picked up for morphological examination and biochemical identification.

Sensory evaluation

A controlled environment with a 28°C temperature and 65% humidity was used for sensory evaluation. The panel was given a list of descriptors (odor, color, and texture) to rate on numerical and continuous scales from 1 to 9 (9: Excellent; 8: Very very good; 7: Very good; 6: Good; 5: Medium; 4: Fair; 3: Poor; 2: Very poor; 1: Very very poor) according to Kanatt *et al*. (2010).

Statistical analysis

The experiment was designed in completely randomized design in a 6×7 factorial design; 6 treatments (4 mM ZnO, 6 mM ZnO, 8 mM ZnO, 4 mM ZnONP, 6 mM ZnONP, 8 mM ZnONP and control one) during 5 sampling days (zero day, 3rd, 6th, 9th and 12th) at refrigerated storage (4.0±1.0°C). Using the SPSS software for Windows (Version 28), analysis of variance (ANOVA) was performed on all data (SPSS Inc. Chicago, IL, USA). F-values that were substantially different at the P≤ 0.05 were indicated. The precise differences between two means were assessed using Duncan's multiple range test (Duncan, 1955). The values are the means±-standard error.

RESULTS AND DISCUSSION

For the growth of microorganisms, minced meat provides an excellent medium. When the conditions are right during processing, mixing, storing, and packaging, the bacteria typically found on the surface are fully spread throughout the meat product and begin reproducing; resulting in a loss of product quality and presenting potential health hazards (Saad *et al*., 2018). Infections and mortality are primarily brought on by foodborne diseases, particularly in developing countries, where *E. coli* and *S. aureus* are the major causes. The primary route of transmission for these diseases is the ingestion of contaminated foods, and the presence of these organisms in meat and other raw meat products has important public health consequences (Bintsis, 2017).

The antimicrobial action of ZnO NPs on *S. aureus* showed that with the rise of ZnO NPs concentration the acceptability of the minced meat increased, as demonstrated in Table 1. Results showed that 8 mM ZnO NPs had the best acceptability of minced meat (8.95±0.58 to 6.17±0.28), followed by 6 mM ZnO NPs (8.84±0.43 to 5.00±0.20) during the period of the study. While the 4 mM ZnO NPs showed acceptability until the 9th day (4.67±0.58) and then spoiled at the 12th day. On the other hand, the bulk ZnO powder 8 mM had acceptability from zero day to the 6th day (5.52±0.00). While the lower concentrations (4, 6 mM ZnO) become acceptable until the 3rd day (5.54±1.00 and 6.43±0.58 respectively). The control samples were acceptable at day zero and then spoiled. The antibacterial activity of the bulk and produced nanoparticles showed a substantial significant difference when compared to nano-suspensions (P< 0.05). These results are quite similar to those published by Gunalan *et al.* (2012), who found that bulk ZnO and ZnO NPs had antibacterial effects on *S. aureus* at concentrations of 2, 4, and 6 mM. ZnO NPs and El-Masry *et al.* (2022) who revealed that ZnO NPs strongly influenced bacterial growth at various concentrations (2.5, 5, 10 and 20 mM). Smaller ZnO NPs may encourage more favorable interactions between their particles and microbial cells (da Silva *et al*., 2019)

The antimicrobial action of ZnO NPs on *E. coli* showed that with the rise of ZnO NPs concentration, the acceptability of the minced meat increased, as demonstrated in Table 2. Results showed that 8 mM ZnO NPs had the best acceptability of minced meat (8.95±0.09 to 6.95±0.59), followed by 6 mM (8.75±0.48 to 6.56±0.12) and 4 mM ZnO NP (8.68±0.25 to 6.47±0.23) along the period of the study. Also, the bulk ZnO at concentrations (8, 6 mM) had acceptability from zero day to the 12th day. While the lower concentrations (4mM ZnO) become acceptable until the 3rd day (6.09±2.00). The control samples were acceptable on the 3rd day and then spoiled. The antibacterial activity of the bulk and produced nanoparticles showed a substantial significant difference when compared to nanosuspensions (P< 0.05). In a study, ZnO nanoparticles concentrations of 3 mM and 6 mM reduced bacterial growth in comparison to the control, whereas 12 mM ZnO nanoparticles completely inhibited the growth of *E. coli* O157:H7 (Liu *et al*., 2009). Emami-Karvani and Chehrazi (2011) investigated the antibacterial activity of ZnO nanoparticles using Gram-negative bacteria (*E. coli*) as test microorganisms.

S. aureus counts were calculated during chilled storage with various bulk ZnO and ZnO NPs concentrations. The results demonstrated in Table 3 showed that ZnO NPs potential as a food preservative against *S. aureus* in minced meat. The growth inhibition of *S. aureus* was seen to rise with concentration rise, the maximum growth inhibition of *S. aureus* was by 8 mM ZnO NPs from which dropped from 7.25 to 3.85 log cfu/g. When compared to control samples, the mean values of *S. aureus* significantly decreased after treatment with 4 mM, 6 mM, and 8 mM ZnO-NPs, with a significant difference (P< 0.05) between the different concentrations. The findings showed that ZnO NPs (6 and 8 mM) significantly inhibited *S. aureus* growth and count throughout 12 days at 4°C refrigerator storage (from 7.45±0.34 and 7.25±0.34 to 5.25±0.23 and 3.85±0.38 respectively). On the other hand, the bulk ZnO at concentrations (8 mM) had acceptability from zero day to the 9th day (7.62±0.27 to 5.82±1.38) and at concentration of 6 mM had acceptability only to the 6th day (7.68±0.29 to

Table 1. Effect of different concentrations of ZnO NP and bulk ZnO powder on overall acceptability of minced meat inoculated with *Staphylococcus aureus* during refrigerated storage at 4°C for 12 days.

Groups	Zero day	3rd day	6th day	9th day	12th day
Control	8.01±1.00^{ab}	Spoiled	Spoiled	Spoiled	Spoiled
4 mM ZnO	8.03±1.00 ^{ab}	5.54±1.00^{c}	4.04±0.05^{b}	Spoiled	Spoiled
6 mM ZnO	8.25±1.53 ^{ab}	6.43±0.58^{bc}	4.45±1.00^{b}	Spoiled	Spoiled
8 mM ZnO	8.32±1.00 ^{ab}	6.82±1.00^{c}	5.52±0.00^{ab}	3.00±1.00^{b}	Spoiled
4 mM ZnO NP	8.73±0.5^{b}	7.10±1.00^{a}	6.00±1.00^{a}	4.67±0.58^{b}	Spoiled
6 mM ZnO NP	8.84±0.43 ^{a}	7.53±0.58^{a}	6.70±1.00^{a}	6.00±0.58^{a}	5.00±0.20^{a}
8 mM ZnO NP	8.95±0.58 ^{ab}	8.15±0.48^{ab}	7.57±0.37^{a}	7.12±1.00^{a}	6.17±0.28^{a}

Values represent Mean±SD of three experiments. Means within a column followed by different letters are significantly different (P < 0.05).
Score System for Sensory Evaluation (Kanatt *et al*., 2010): 9: Excellent; 8: Very very good; 7: Very good; 6: Good; 5: Medium; 4: Fair; 3: Poor. 2: Very poor. 1: Very very poor.

Table 2. Effect of different concentrations of ZnO NPs and bulk ZnO powder on overall acceptability of minced meat inoculated with *E. coli* O157 H7 during refrigerated storage at 4°C for 12 days.

Groups	Zero day	3rd day	6th day	9th day	12th day
Control	8.45±0.05^{a}	5.47±0.25^{b}	Spoiled	Spoiled	Spoiled
4 mM ZnO	8.53±1.00^{ab}	6.09±2.00^{c}	Spoiled	Spoiled	Spoiled
6 mM ZnO	8.55±0.03^{a}	8.01±0.18^{a}	7.18±0.15^{a}	6.15±0.07^{b}	5.35±0.16^{c}
8 mM ZnO	8.59±0.03^{a}	8.16±0.37^{a}	7.35±0.45^{a}	7.25±0.05^{b}	6.34±0.18^{b}
4 mM ZnO NP	8.68±0.25^{b}	8.25±2.00^{a}	7.47±1.00^{a}	7.37±0.65^{b}	6.47±0.23^{b}
6 mM ZnO NP	8.75±0.48^{a}	8.61±0.06^{a}	7.52±0.16^{a}	7.45±0.48^{b}	6.56±0.12^{c}
8 mM ZnO NP	8.95±0.09^{a}	8.88±0.06^{a}	8.03±0.33^{a}	7.56±0.16^{a}	6.95±0.59^{a}

Values represent Mean±SD of three experiments. Means within a column followed by different letters are significantly different (P < 0.05).
Score System for Sensory Evaluation (Kanatt *et al*., 2010): 9: Excellent; 8: Very very good; 7: Very good; 6: Good; 5: Medium; 4: Fair; 3: Poor. 2: Very poor. 1: Very very poor.

Table 3. The impact of various ZnO NPs and bulk ZnO powder concentrations on the *S. aureus* count (log cfu/g) of minced beef samples stored in the refrigerator at 4°C for 12 days.

Groups	Zero day	3rd day	6th day	9th day	12th day
Control	7.85±0.48^{a}	Spoiled	Spoiled	Spoiled	Spoiled
4 mM ZnO	7.72±0.38^{a}	6.37±1.15^{a}	Spoiled	Spoiled	Spoiled
6 mM ZnO	7.68±0.29^{a}	6.11±0.27^{a}	5.77±0.24^{a}	Spoiled	Spoiled
8 mM ZnO	7.62±0.27^{a}	6.01±0.37^{a}	5.73±0.11^{a}	5.82±1.38^{a}	Spoiled
4 mM ZnO NP	7.58±0.26^{a}	5.72±0.28^{a}	5.67±0.34^{a}	5.61±0.27^{a}	Spoiled
6 mM ZnO NP	7.45±0.34^{a}	5.63±0.54^{b}	5.61±0.51^{a}	5.54±0.03^{a}	5.25±0.23^{a}
8 mM ZnO NP	7.25±0.34^{a}	5.51±0.37^{ab}	5.30±0.18^{b}	4.82±0.24^{b}	3.85±0.38^{b}

Initial load of *S. aureus* = 9.63±0.35 cfu/g.
Values represent Mean±SD of three experiments. Means within a column followed by different letters are significantly different ($P < 0.05$).

Table 4. Antibacterial activity of different concentrations of ZnO NPs and bulk ZnO powder on *E. coli* O157 H7 count (log cfu/g) artificially inoculated into minced meat samples during refrigerated storage at 4 °C for 12 days.

Groups	Zero day	3rd day	6th day	9th day	12th day
Control	6.72±0.35^{a}	6.85±0.22^{a}	7.28±0.1 ^{a}	8.72±0.41^{a}	8.93±0.5^{a}
4 mM ZnO	6.63±0.6^{a}	6.42±1.14^{a}	5.95±0.26^{a}	5.89±1.42^{a}	5.79±0.07^{a}
6 mM ZnO	6.57±0.6^{a}	6.15±0.36^{a}	5.88±0.26^{a}	5.83±1.35^{a}	5.77±0.16^{a}
8 mM ZnO	6.52±0.6^{a}	6.11±0.78^{a}	5.83±0.21^{a}	5.80±1.22^{a}	5.70±0.27^{a}
4 mM ZnO NP	6.45±0.6^{a}	5.91±0.38^{a}	5.75±0.55^{a}	5.70±0.35^{a}	5.68±0.35^{a}
6 mM ZnO NP	6.39±0.6^{a}	5.56±0.09^{c}	5.43±0.1^{c}	5.40±0.6^{b}	4.75±0.1^{b}
8 mM ZnO NP	6.01±0.6^{a}	5.38±0.2^{c}	5.35±0.1^{d}	4.78±0.21^{c,d}	3.60±0.2^{d}

Values are expressed as Mean±standard error of three experiments. Means within a column and rows followed by different letters are significantly different ($p \leq 0.05$).

5.77±0.24). While the lower concentrations (4mM ZnO) become acceptable until the 3rd day only (6.37±1.15). The control samples were acceptable at zero day and then spoiled. The antibacterial activity of the bulk and that of the produced nanoparticles showed a substantial significant difference when compared to nanosuspensions ($P<0.05$). Our results concurred with those of Ibrahim *et al*. (2017) who found that ZnO suspensions (5, 8 and 10 mM) significantly inhibited *S. aureus* growth during 12 days at 4°C refrigerator storage. In comparison to other concentrations, ZnO NPs 10 mM accurately showed the highest reduction percentage of *S. aureus* from 8.6 to 6.42 log cfu/g. Also, 5 and 8 mM. ZnO NPs antibacterial activity was therefore concentration dependent. The findings were almost identical to those found by Espitia *et al*. (2013); Mustafa (2015), and De Souza *et al*. (2019), who described the inhibitory effect of ZnO-NPs on *S. aureus*. For example, by preventing bacterial growth, they can be used in packaging materials to extend food shelf life and increase microbiological safety. The antibacterial activity of ZnO NPs is believed to the contact between ZnO NPs and bacterial cell is initiated by surface charges on the particle (Neal, 2008), ZnO NPs interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication (Jiang *et al*., 2009). Bacterial cell nutrients adsorb to the large surface area of ZnO NPs, which starves the bacterial cell. ZnO NPs interact with bacterial cell membrane lipids directly leading to disorganization of the membrane structure, loss of membrane integrity, mitochondrial malfunction, abnormal cell morphology, damage to the cell membrane, decrease in the cell permeability (Krishnamoorthy *et al*., 2012), and leakage of cytoplasmic contents (Sharma *et al*., 2010).

Counts of *E. coli* were assessed during chilled storage with various ZnO bulk and ZnO NPs concentrations. The current study demonstrated that ZnO NPs are considered potential as *E. coli*-resistant food preservative for minced meat. Growth inhibition of *E. coli* seems to increase with concentration. As shown in Table 4, the maximum growth inhibition of *E. coli* was by 8 Mm of ZnO NPs which decreased from 6.01±0.6 at zero day to 3.60±0.2 log cfu/g at last day. When compared to the control samples, the mean values of *E. coli* were significantly lowered following treatment with 4mM, 6mM, and 8mM ZnO NPs ($P< 0.05$) . These results showed that ZnO NPs (4, 6 and 8mM) significantly inhibited *E. coli* growth and count during 12-day refrigerator storage period at 4°C. These results are comparable to those published by Babayevska *et al*. (2022), who found that the amount of *E. coli* bacterial cells was dramatically decreased compared to the untreated control, and Liu *et al*. (2009), who found that the inhibitory effects increased as the concentrations of ZnO NPs increased. The bulk ZnO also showed a reduction in bacterial count, but the effect of ZnO NPs were better due to its smaller size which maximizes its interaction with the bacterial surface and/or with the bacterial core where they act on the cell membrane and deeply in its DNA (Zanet *et al*., 2019); therefore, they may be considered as multi-target compounds and affect several structures of bacteria cells, but their main mechanism of action is on the cytoplasmic membrane, leading to membrane rupture (Mendes *et al*., 2022). ZnO NPs, which have a size less than that of pore size in the bacteria, have a unique property of crossing the cell membrane without any hindrance (Sunita *et al*., 2011), resulting in the production of toxic oxygen radicals, which damage DNA, cell membranes or cell proteins, and may finally lead to the inhibition of bacterial growth and eventually to bacterial death (Tankhiwale and Bajpai, 2012).

CONCLUSION

Nanoparticles have an antibacterial action against *S. aureus* and *E. coli* that is concentration dependent. It has been proven that 8 mM ZnO nanoparticles have a stronger antibacterial impact on *S. aureus* and *E. coli* than bulk ZnO does. Minced meat's shelf life can be extended, and bacterial growth prevented by nanoparticles.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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