Antibacterial activity of Zinc Oxide Nanoparticle (ZnONP) Biosynthesis by *Lactobacillus plantarium* aganist pathogenic Bacteria

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Abstract

The good capability of zinc-tolerant probiotic of *Lactobacillus plantarum* tolerating high concentrations of $Zinc^{+2}$ and producing Zinc Oxide Nanoparticles high lights the unique characteristics of these bacteria as a natural microbial cell nano-factory for a more efficient and environmentally friendly process of biosynthesis of these nanoparticles. The morphology structure and stability of the synthesized ZnO nanoparticles were studied using structural and morphological Properties technology such as XRD and FE- SEM analysis it also used optical properties such as UV and FTIR spectrum. The results depicted that the synthesized nanoparticles were moderately stable, exagonal phase, roughly spherical with maximum particles in size range within7–19 nm in diameteralso this study include the antibacterial activity of the Zinc Oxide Nanoparticles on some pathogenic bacteria .It is clear in this the study that Zinc Oxide Nanoparticles has stronger inhibitory effect on pathogenic bacteria because The proposed two imaginable mechanisms for the antibacterial action of zinc oxide nanoparticles to bacteria are first, creation of augmented levels of ROS mostly hydroxyl radical and singlet oxygen which destruction the bacterial cell wall. Second nanoparticles deposition on bacterial surface or nanoparticles aggregation either in the periplasm region or in the cytoplasm initiating distraction of cellular function and membranes disorganization .

Keyword: *ZnONPs, Lactobacillus plantarum, Biosynthesis , Characterization and antibacterial activity*

Introduction

Zinc oxide nanoparticles (ZnO NPs) have gained worldwide attention as multifunctional nanomaterials due to their distinctive properties of being versatile semiconductor and piezoelectric properties [1]. Microbial metal nanoparticles synthesis has recently been widely used due to their low cost, biocompatibility and eco-friendliness [2]. ZnO has many important features like chemical and physical stability, high catalysis and efficient antibacterial activity [3]. Symbiotic microorganisms may use NPs as safe source. Microbial nanoparticles synthesis has more benefits than other chemical and physical method. Nanoparticles have many applications in medicine. In addition, the bacterial nanoparticles may also be used for controlling human bacterial pathogens [4]. Among the micro-organisms, lactic acid bacteria (LAB) receive substantial attention because of their safe handling and food-grade status, which are “generally recognized as safe” (GRAS) in the production and preservation of food [5]. A low-cost, unreported and easy method for the biosynthesis of ZnONPs by using reproducible bacteria, *Lactobacillus plantarum* as an eco-friendly reduction and capping agent is described in our current study. *Lactobacillus plantarum* is a non- pathogenic, grampositive, facultative anaerobic bacteria and is the largest of all lactic acid bacteria in its genome. *Lactobacillus plantarum* has a negative electrokinetic potential; which attracts cations easily and this step works as a trigger for the ZnONPs biosynthesis [6]. The mechanisms of cytotoxicity from ZnO-NPs are not yet completely understood, however the generation of hydroxyl radicals (OH•), superoxide anion (O2), and perhydroxyl radicals (HO2) from the floor of ZnO are believed to be fundamental components. When nanoparticles engage with cells, cell protection mechanisms are activated to limit harm. However, if the exceptionally active free radicals manufacturing exceeds the antioxidative protective potential of the cell, it results in oxidative damage of biomolecules which can lead to phone loss of life [7].

Methodology

Bacterial Isolate: *Lactobacillus Plantarum* was selected as a biological model for the synthesis of ZnONPs because it is more efficient for biosynthesis of ZnONPs .We have obtained this isolate from the dairy products which is stored in the advanced Microbiology Lab./University of Babylon. We did culture to the isolate for 24hr. on MRS agar at 38°C. as well as it is iagnosed by Vitek2 system.

Synthesis of zinic oxide nanoparticules by *Lactobacillus plantarum*

Synthesis of zno nanoparticales :- pure culture of *lactobacillus* was inoculate in to the flask contianing de man Ragosa and Sharpe (MRS) broth and incubated at 37c for 24 h at 100rpm . after the incubation period pH of the culture broth was adjusted to using 0.4 M NaOH to delay the process of the transformation.the 0 .1 M ZnSO4 H2O was added to the flask containing culture solution and heated on a water bath up to 80 c for 5- 10 min. A white precipate appears at the bottom of flask indicates the transformation process and the flask was removed from water bath . incubate at 37 c for 12 h so that all the particles gel deposited at the bottom of the flask . the product was filtered and washes with deionised water followed by drying at 40 c in a hot air oven for 4 h [8].

Structural and Morphological Measurements

The X-Ray Diffraction Measurements

Strong non-destructive technique for material characterization is XRD, via XRD can determined the crystal structure and particle size. These characterization is generally carried out (achieved) with a typical wavelength of X-ray that is comparable to the interatomic distance in a crystal. The constructive interference occurs when the path difference is an integer multiple of the wavelength. This is the Bragg condition for diffraction. The intensity of the reflected beam has sharp peaks in the corresponding directions. They are called Bragg peaks (9). The Bragg peak can be found by varying the angle 2θ of the detector.

2□□□□□=□λ 2-1

Where n is integer

The particle size (D) is measured by Scherer's formula:

□=0.9□Δ(2□).cos (□) 2-2

where Δ (2θ) is the full width at half maximum (in radians) of the peak, θ is the Bragg angle. The samples structure was analyzed with a Shimadzu 6000 X - ray diffractometer (made in JAPAN) using Cu Kα radiation (λ=0.15406 nm) in reflection geometry. A proportional counter with an operating voltage of 40 KV and a current of 30 mA was used. XRD with scanning speed of 8 degrees/min over 2θ range 5° - 80° (Shimadzu XRD-6000-Japan). The XRD patterns display no peaks indicating that the samples structure was in the amorphous phase .

The Field Emission-Scanning Electron microscopes (FE- SEM) Measurements

Scanning Electron Microscope utilizes a highly energetic beam of electrons for taking the images of sample by scanning it (10).).The topography of the prepared powder was studied by Field Emission Scanning Electron Microscopes (SEM) type- S-1640 HITACHI company (Japan) with different magnification powers and detectors . It was also used for particle size measurements. Prior to the test, the samples should be dried and cleaned. The FE-SEM will take 3 to 4 minutes to pump down to 10-5 mbar. The extra high tension (EHT) (beam voltage) for gas panel is set to the desired value, normally starts with 5 KV. The sample's surface covered with a gold coating to facilitate the discharging of the surface charge.

Optical Measurements

UV -Vis Diffuse Reflectance Measurements

The optical properties of semiconductor were studied using a Varian Carry 5000 model UV- VIS-NIR spectrophotometer with an integrating sphere, using PTFE as the reference disk. Diffuse reflectance spectra were taken in the range of 200-800 nm supplied by Shimadzo Corporation (JAPAN) and in air at room temperature. The diffuse reflectance of the sample was related to the Kubelka- Munk function F(R). Diffuse reflectance data were transformed using Kubelka-Munk function by the relation:

F(R)=(1−R)22R …… 2-3

Here R is the diffuse reflectance of the sample.

The Kubelka-Munk theory allows us to calculate the energy gap of the thin or powder According to graph of F(R) versus energy values.

Fourier Transform Infrared (FTIR) Measurements

Mid-IR spectra, from 4000 to 400 cm-1, were obtained for some pure samples and all doped samples using FTIR - Spectrometer, supplied by Shimadzu, on KBr pellets of the samples.

Antibacterial activity of ZnONP

Disc Diffusion Method.

1. Concentrations were taken of each bacterial isolates and compared to McFarland solution to get the right concentration for each of them.

2. The appropriate concentration 0.1 ml of each bacterial isolates were added to dishes containing Muller Hinton agar is spread on the surface of the dish-by spreader and left the dishes for an hour.

3. Wells were made by using cork borer 6 mm diameter as it was equal distance between the well and the other.

4- The Nanoparticles were dissolved with distal water to get various concentrations ranged from (2.5-0.625) µg/ ml.

5- Nanoparticles inhibition zones were measured by a ruler, (11)

Results and Discussion

Biosynthesis of ZnO NPs by *Lactobacillus plantarium*

Lactobacillus plantarum was confirmed The formation of nanoparticles by observing change in color of solution during synthesis of ZnO nanoparticles showin Figure 1 explain the reduction of Zinc Oxide into zinc nanoparticles during exposure to the bacterial extract is followed by the color change from brown color to yellow during 24h also show in figure B increase of reduction througt 48h from incubation peroid by the color change from brown color to pale yellow (12)

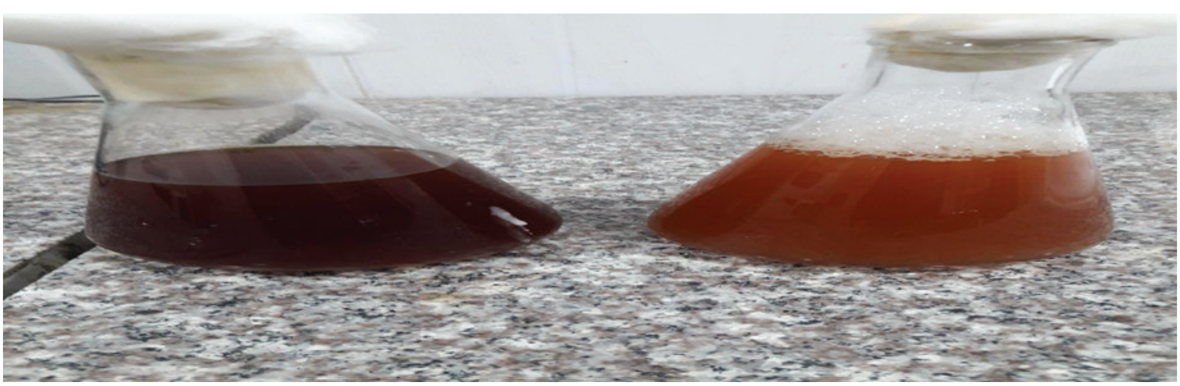

Figure-1 -Color change indicates the formation of ZnO nanoparticles compare with control after 24h .

The white color deposition at the bottom of flasks figure 2. This result was agree with (13) by using *Lactobacillus sporogens* and also agree with the result obtained by (14) regarding to the outcomes achieved. (15) was showed that synthesis of Zinc oxide nanoparticles (ZnO NPs) using culture supernatant of endophytic bacterial isolate *Sphingobacterium thalpophilum* in the process of reduction aqueous zinc oxide being extra-cellular which lead to the development of an easy bioprocess for synthesis of ZnO NPs It is clear that , *Lactobacillus plantarum* acts a crucial part in the synthesis of ZnO nanoparticles.

Figure 2: Biosynthesis of ZnO NPs from *Lactobacillus plantarum*

Structural and Morphological Properties of Zn- ONPs biosynthesized by *Lactobacillus plantarum*

X-ray diffraction analysis

Figure 3 shows X-ray diffraction (XRD) patterns of the biogenic ZnONPs synthesized using *Lactobacillus plantarum* . XRD analysis reveals that the nanoparticles synthesized were pure and crystalline innature. The peaks at 2θ = 31.7694, 34.4211, 36.2521, 47.5376 , 56.6016 , 62.8624 , 66.3782 , 67.9610 , 69.0982, 72.5631 , and 76.9671 wereas signed to the (100), (002), (101), (102), (110), (103), (200), (112),(201), (004), and (202) respection lines of spherial ZnO Particles respectively. The average particle size of ZnO was estimated by applying the Scherrer equation(17), as shown in equation (3-1) for the peak (101) reflection at 2θ by using the full width at half maximum (FWHM) (17, 18)

□= K□ (□)………………………3−1

Bcos□.

The calculated average particle diameter is 7 nm. Line broadening of the diffraction peaks is anindication that the synthesized materials are in the nanometer range.The synthesis of the nanoparticles obtained is too small; this may bedue to the biological synthesis method adopted to prepare the nanoparticles .

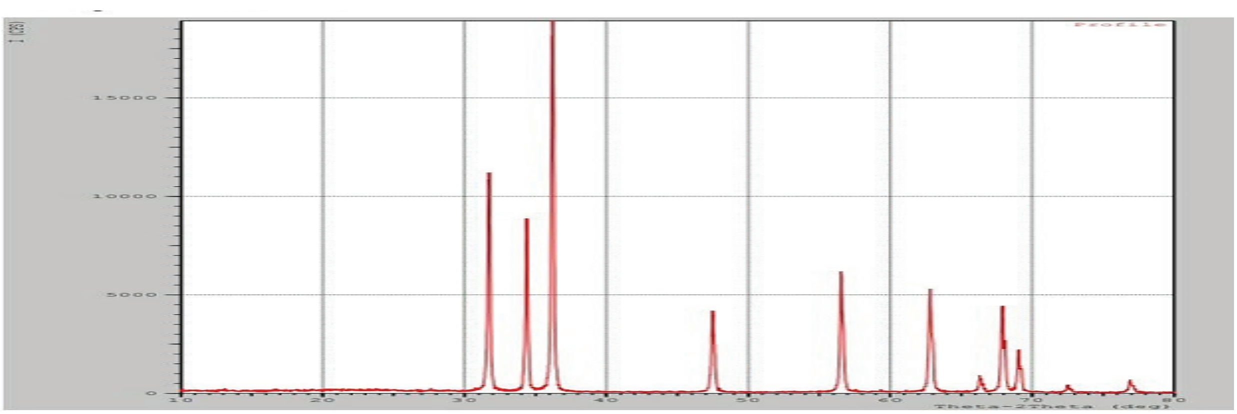

Figure 3: XRD analysis of ZnO nanoparticles synthesis by *Lactobacillus plantarum*

Field Emission - Scanning Electron Microscopy (FE-SEM) Analysis

The FE-SEM photographs of the ZnO NP biosynthesis by *Lactobacillus plantarum* were prepared by biological method and Calcinated at 500 Co for one hour was shown in figure 4

. Scanning electron microscope was used to decide size, location and shape of the Zinc oxide nanoparticles. These images demonstrated that zinc oxide nanoparticles are hexonal in shape and their sizes are about (56.14nm, 62.98nm , 64.97nm) indicating the diameters of NPs were accurate and appropriate as ZnONPs. [10] .

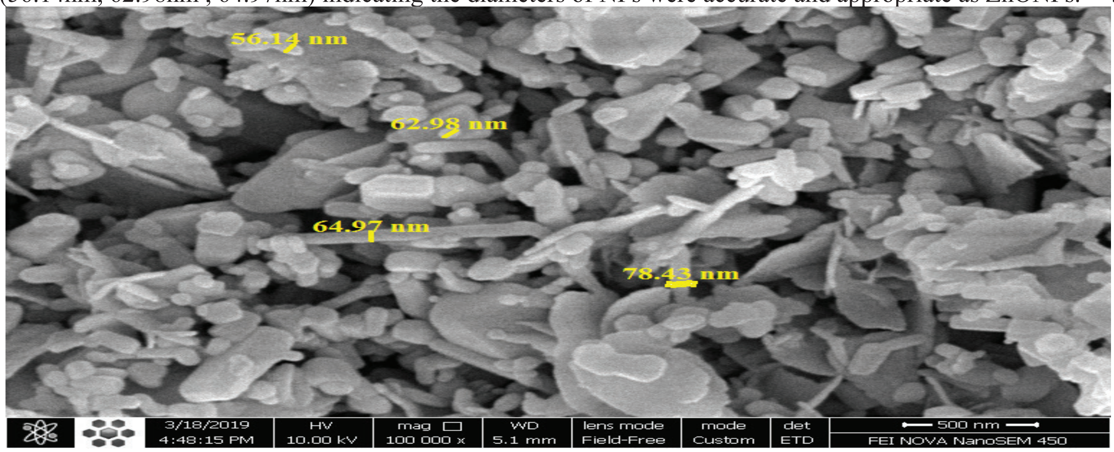

Figure 4: FE-SEM images of biological synthesis ZnO nanoparticles by *Lactobacillus plantarum*

Fourier Transform Infrared (FTIR)

FTIR spectra is used to access the details of functional groups involved in the biosynthesis of ZnONPs . The obtained FTIR spectra of ZnO NPs exhibited prominent peaks at 3460 , 1718 , 1627 , 1047 and 584 cm . The broad vibrational band observed at 3460 cm is attributed to the symmetric stretching mode of water molecules . the band observed at 1718 , 1627 and 1047cm is assigned to the bending vibrational mode of water molecules. Figure 5 reveals the peak observed at 584-441 cm corre-sponds to the stretching vibrations of ZnO NPs .

The well resolved intense and broad transmission band below ~590 cm−1 was attributed to the stretching vibration of zinc-oxygen bond (18,19). A slight peak shift observed at metal doping in the present study (between 584 and 441 cm−1) may be attributed to the change in particle size as a function of metal doping.

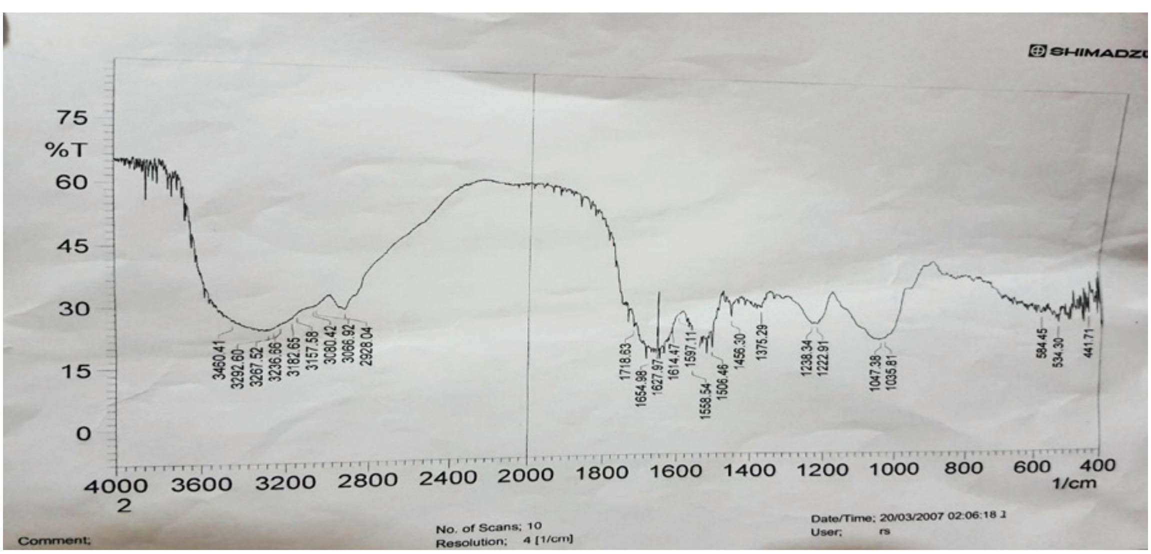

Figure 5 : FTIR spectra of biosynthesis of Zno nanoparticles by *Lactobacillus plantarum*

UV -Vis Diffuse Reflectance

The samples, separation of biomass eliminated the interference in reading UV/Vis absorbance (420 nm) of colloidal ZnO nanoparticles. It was assumed that sample by centrifugation could be useful. However the absorbance (420 nm) of colloidal suspension of nanoparticles decreased greatly to near zero, and the color (brown to yellow) disappeared. It might be due to the aggregation of nanoparticles or their entrapment in the biomass. Therefore, centrifuging the samples showed negative effects on nanoparticles and should be avoided. their absorbance was read at 420 nm, and corrected for the absorbance before the start of the reaction.

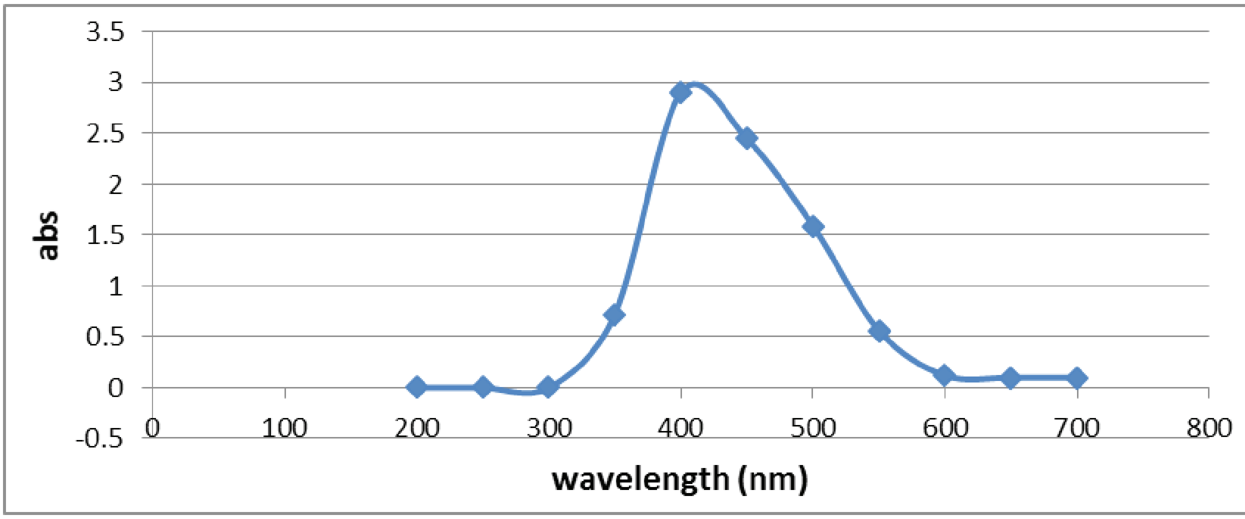

Figure 6 : UV- Vis spectrum of ZnO NPs synthesized by *Lactobacillus plantarum*.

Antibacterial activity of Zn-ONPs against Pathogenic Bacteria.

The antibacterial activity of biosynthesis ZnONPs examined against four pathogenic bacteria as shown in table 1.

Table (1): Pathogenic bacterial isolates

Bacteria Isolated	Gram stain	Type of sample	Source Obtained
Escherichia coli	Negative	Urine	Kufa university – college of science
Staphylococcus aureus	positive	Blood	Babylon University – College of Medicine
Klebsiella Pneumoniae	Negative	Swap (sputum)	Al-Hilla teaching hospital
Pseudomonas aeruginosa	negative	Swap (sputum)	Hillah public health Lab

The antibacterial activity of ZnO nanoparticles was tested by the disc diffusion agar methods table 2 show These results indicate that Zinc Oxide nanopaticles had antibacterial effects on four pathogenic bacteria include *(Escherichia .coli , Staphylococcus aureus , Klebsiella pneumoniae , pseudomomas aeruginosa*) , the antibacterial concentrations were inconsistent. Results of(20) . indicated that 0.625 mg/ml zinc oxide nanoparticles have low inhibit the growth of all bacteria and 2.5mg/ml higher concentrations completely inhibited the growth all these bacteria . concentrations may be that the different microbes and Zinc Oxide or its nanoparticles were used. Also these result showe Pseudomonas aeruginosa have inhibition efficency in 2.5 mg more than other bacteria while klebsiella pneumonia have low inhibition zone at same concentration Interestingly .

Table (2): Shows the diameter of inhibition zone of ZnONPs for some pathogenic bacteria.

	ZnONPs Con. (mg/ml)	ZnONPs(mm) E.coli	ZnONPs(mm) Staph .aureus	ZnONPs(mm) Klebsiella Pneumonia	ZnONPs(mm) Pseudomonas aeruginos
1	2.5	20	19	18	22
2	1.25	18	16	15	18
3	0.625	14	12	12	16

On the other hand, the ZnO NPs were exhibited antibacterial activity against MRSA (21) so, the result obtained agree with these findings. The proposed two imaginable mechanisms for the antibacterial action of zinc oxide nanoparticles to bacteria are first, creation of augmented levels of ROS mostly hydroxyl radical and singlet oxygen which destruction the bacterial cell wall. Second nanoparticles deposition on bacterial surface or nanoparticles aggregation either in the periplasm region or in the cytoplasm initiating distraction of cellular function and membranes disorganization. Also the result showed the same result found by (22) which showed that ZnO nanoparticles had antibacterial activity against bacteria .

Conclusion

Symbiotic microorganisms can use as safe source of nanoparticles. The microbial synthesis of nanoparticles is advantageous more other chemical and physical methods. Nanoparticles applications in medicine and sensors are envisaged .ZnONPS biosynthetic by *Lactobacillus Plantarum* using these it as antibiotics give us wonderful results, low complications, very low cost and low resistance in comparison with ordinary antibiotics. The results represent a great potential benefit for a wide numbers of medical applications in the battle against antibiotic- resistant bacterial pathogens .

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Mustaqbal University College and all experiments were carried out in accordance with approved guidelines.

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