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"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NICKEL OXIDE NANOPARTICLES FOR ITS BIOLOGICAL STUDIES"

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1. Abstract:

Exactly regulate the size of particles, nickel oxide (NiO) nanoparticles were produced by the co-precipitation method. This technique generated NiO nanoparticles crystalline in nature through the precipitation of nickel salts in a basic medium and heating. Characterization was done using Energy Dispersive X-ray Analysis, Scanning Electron Microscopy (SEM) and fourier Transform Infrared Spectroscopy (FTIR) (EDAX). The synthesis of oxide nanoparticles was effectively done as FTIR spectra showed the formation of Ni–O bonds and the presence of surface-bound functional groups in the form of characteristic absorption bands. SEM images showed the presence of nanoscale particles with homogeneous size distribution and mainly spherical morphology; there was not a lot of aggregation observed, indicating the presence of favorable conditions of synthesis.

The identification of the elemental composition further facilitated by EDAX analysis confirmed the presence of nickel and oxygen in the desired ratio and showed that there were not any significant impurities. The biological evaluation consisted of tests for cytotoxicity, antioxidants and antimicrobials. NiO nanoparticles exhibited high antibacterial activity against all types of Gram-positive and Gram-negative microorganisms. They were able to harm bacterial membranes, prevent DNA replication, and disrupt essential enzymes by generating reactive oxygen species and releasing nickel ions. The potential of free radical scavenging assays to reduce oxidative stress was highlighted by their concentration-dependent antioxidant efficacy.

Numerous cell lines underwent cytotoxicity testing, which showed dose-dependent effects. Cancer cells were more sensitive than normal cells, indicating potential uses in cancer treatment. Assays for inflammatory response, protein denaturation, and hemolysis showed satisfactory biocompatibility at therapeutic nanoparticle doses.

This interdisciplinary work validates the potential biological activities of high-purity NiO nanoparticles and lays out a repeatable process for creating them with a predetermined shape. The results encourage the continued development of NiO nanoparticles as antioxidants, antimicrobials, and anticancer treatments. Optimizing synthesis parameters, investigating surface changes for targeted delivery, and carrying out in vivo investigations to confirm therapeutic promise should be the main goals of future research.

Keywords: *Co-precipitation, FTIR-SEM-EDAX, cytotoxicity, antioxidant qualities, antibacterial activity, nickel oxide nanoparticles, and biocompatibility.*

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2. INTRODUCTION:

Nanotechnology is placing the world in the here and now transformation; most aptly in medicine, energy, farming and the environment. At such small size physics and chemistry are not the same, there is some quantum confinement, large surface/volume ratio, and hence high surface energy, and is thus not similar to the bulk material1. It allows us to produce new materials, more conductive, stronger and are able to respond in shorter time and time than the older systems. Nanoparticles have enabled us to perform medical imaging, deliver drug targets to where they are required, biosensors, antibacterial devices, catalysts and can store energy in a form that could not have been achieved on a larger scale.

When these micro-atomic volcanoes are so tiny, they can make it quicker because they can go through biological gates and get the medicine to the places of the destruction. Even semiconductors and supercapacitors are more efficient with the particles of electronics, applied in agriculture to machine an intelligent release of insecticides and fertilizers. Not applications, they provide us with an opportunity to experiment with basic things on the molecular level, a level we could not view previously[1,2].

The use of metal oxide nanoparticles is considered one of the hottest tendencies of scientists due to the gigantic organisation, chemical stability and immense potential in various technology areas. They are practical, as they catalyze redox reactions and they react with light and enumerate antibacterial and anti-cancer medicines3.

TiO 2, ZnO, CuO and NiO are wonders of metal oxides and are hot topic research materials because of their optical, catalytic and electrochemical characteristics 4. Metal oxide particles, therefore, are stronger because metal nanoparticles are easily oxidised or clumped. Indicatively, in the TiO 2 solarscreen and ZnO solarscreen UV radiation is absorbed, and in photocatalysis, they absorb detrimental radiations found in the wastewater. They are used on the medical equipment/coating because of the use of the qualities of bacteria-killer attributed to CuO and NiO3. Their possibility in biology is enormous because it is possible to engineer them to assault a biological target.

These are characterized by different sizes, band gap and surface charge that allow them to react in cellular interactions which can find application in cancer therapies, drug delivery and diagnostic imaging. They are also inexpensive and easily produced hence they are tempting in order to be utilized in large scale3,4.

The box gap also includes flexing box gap and adjustable assembly limiters allowing the build to order system to wear out its materials on its p-type conduction with an absolutely broad band-gap 3.64 eV. Electrochemical, magnetic and optical. The properties5 led to the beginning of investigation of biomedical usage, fuel cells, sensors, electrochemical devices, catalysis and data storage.

The NiO nanoparticles also works better than bulk NiO due to the increase in surface area, existence of increased defects or quantum effects. Antibacterial, antioxidant and anticancer properties are of interest to Biotech. Within the scope of the work, it has been established that NiO NPs can kill bacteria and fungi because of the formation of reactive oxygen species ROS that will destroy the cell membrane and cell metabolism [6].

The antioxidant action is executed to neutralize the free radicals and thereby reducing the oxidative cell damage- this proves that they can be used in dual protection and treatment of biology. The application of NiO NPs in renewable energy is seen in other areas as well, such as healthcare.

They are also being used in lithium-ion batteries and in supercapacitors to store enormous charges. They can produce hydrogen among other environmental reactions by the catalytic power which allows them to oxidize CO. NiO NPs are cheap to prepare particularly using the co-precipitation method and can be readily generated in large quantities in a laboratory and Industry. The predominance of applications which can be cast and the low cost have made the research on NiO so harsh. Co-precipitation technique offers size and shape control of the precursors, pH and temperature manipulation and daily calcification.

The more pleasantly the calcification is the larger the particles are and the greater the crystallinity and vice versa which in turn then have lower temperatures resulting in smaller particles with higher surface area in relation to crystallinity than the higher temperatures. Co-precipitation Nanomaterials - in simple terms, Nanomaterials can be made so flexible because it can be produced in specific application by choice of this flexible process even though it can be doped by the addition of dopant or surfactants [7,8].

It consumes less energy, is more ecologically friendly and does not imply any sophisticated equipment compared to the other methods which is also an advantage since the equipment of a researcher can be limited and he or she can scale it up. Enhanced synthesis conditions or with stabilizers added, RQniO NPs can be produced in high-quality and reliably. The behavior in nanoparticles is proportional to size, shape and the material and surface relationship with materials.

In order to make sure that the nanoparticles have been produced correctly and it is some kind of training to familiarize themselves with their details I would need to use some serious tests. In the three primary techniques I will be examining i.e., Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy and Energy Dispersive X Ray Analysis, to analyze this type of nanoparticles i.e., NiO. Another thing that I discovered is that FTIR is a convenient and accessible method of identifying

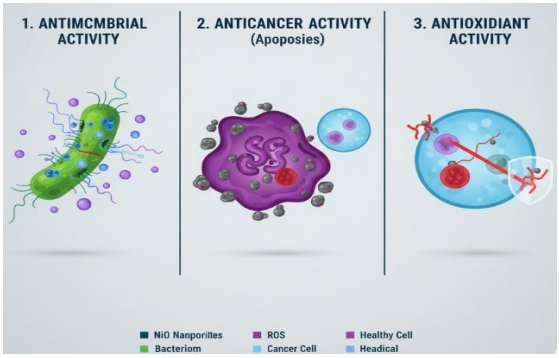
functional groups and their attachment to the chemical bonds of materials, so I think that FTIR would provide me with a bit of information about the molecules in my sample, and how they are linked, and their overall composition. It is even able to show the chemicals that have been used in the synthesis. FTIR examination demonstrates that there are some absorption peaks of particular NiO nanoparticles which overlap the respirating vibration of NiO band.

The peaks are expected to be between 400 -600 -1 and that is evidence that my NiO nanoparticles were synthesized successfully. FTIR can enable me to observe any surface groups or residues of the production process in case there are any. In order to understand that I have the correct structure of NiO, I will employ FTIR but also in order to determine the quality and future functionality of my material 9, I will employ FTIR. The images of the size distribution, shape and surface morphology of the nickel oxide nanoparticles in very fine sizes are then images of the nanoparticles which are significant in analyzing the nanoparticles and this is achieved through use of scanning electron microscopy SEM.
The power of SEM may be considered one of the most powerful imaging tools since it enables viewing the structural features of nanoparticles on the micro- and nanoscale giving direct evidence of the appearance and texture. By using a microscope such as the SEM, I can observe the distribution of particles, their homogeneity and aggregation process of surfaces of particles. It is a property of the nanomaterials and might affect their behavior of functional physics and functional surface area.

The extended methods through SEM will enable me to find the average particle size, degree of agglomeration and surface textures and porosity of the NiO nanoparticles. Significance of the observations is that they directly affect normalization of morphology and size distributions upon physicochemical and functional properties of the nanoparticles such as catalytic activity, optical properties and biological interactions.
Smaller and homogeneous particles are more reactive which are utilized in industries, biologically as well as sensors. As a result, one of the biggest characterization tools, SEM allows me to observe the influence of the synthesis conditions to the final particle structure and properties. The potential to ascertain the surface nature feature of NiO nanoparticles combined with micro structural quality of a nanoparticles, through the use of SEM, is vital in determining that the nanoparticles are fit, regardless of the aim of its utilisation of such nanoparticles [10].

The other relevant technique that I use to determine the elemental composition is that of Energy Dispersive X-ray Analysis EDAX or Energy Dispersive Spectroscopy EDS that are usually obtained in conjunction to the SEM. The EDAX peaks of nickel Ni and oxygen O are different in the case of my NiO nanoparticles and indicates that it is pure. Large concentration of these common peaks is an indication that the synthesis process of the NiO was effective and no contaminations or the unchanged precursors were left behind; hence bearing out that my synthesised nanoparticles are very pure in terms of elements and well-stoichiometric.

EDAX assures the composition homogeneity, which is illustrated in the FIGURE 4 BIOLOGICAL Properties of NIO Nanoparticles. With the correspondence between the elemental composition and the surface morphology i.e. the comparison of EDAX data with the SEM images, I am in a position of having the full picture of the structural and chemical integrity of the samples. Therefore, elemental composition, impurities along with general calibration of NiO nanoparticles is revealed by the EDAX process function.[11]



BIOLOGICAL PROPERTIES OF NIO NANOPARTICLES

I have read that NiO is able to produce the reactive oxygen species ROS, thereby leading to oxidative stress, cell walls in microbial cells and interruption of intracellular events, causing cell death [12]. I also learned that NiO nanoparticles have anticancer effects; its oxidative stress, mitochondrial dysfunction and apoptosis of cancer cells. Such selectivity is yet to be research studies demonstrated but there are studies which point to the potential of NiO nanoparticles to be able to target malignant cells killing normal ones. They inhibit the free radicals and reduce the oxidative distress and their anti-oxidative properties are founded on trapping the free radicals.

NiO nanoparticles, in general, are of interest to me as therapeutic agents because it has duality properties; they prevent the oxidative damage of non-cancerous cells, and cause oxidative stress in pathogens or cancer cells.

Their anti-inflammatory effects and drug-delivery properties have been tested as well as their antibacterial and anticancer effects, their anticancer capabilities, and antibacterial capabilities. Their semiconductor nature that allows them to be used in biosensing and diagnostics, and high surface area that enhances loading and releasing macromolecule bioactive molecules [12,13] are their features.

As I have read, NiO can generate the reactive oxygen species ROS, thus causing oxidative stress, microbial cell damage to cell walls, and interference with intracellular events, resulting in cell death[12]. I have also got to know that NiO nanoparticles possess anticancer effects; its oxidative stress, mitochondrial dysfunction and apoptosis of cancer cells. This selectivity is yet to be proven in research studies but various studies indicate that NiO nanoparticles have potential to target malignant cells eliminating normal cells. Their anti-oxidative properties are based on holding the free radicals and lowering the oxidative distress in normal cells. Generally, NiO nanoparticles are of interest to me as therapeutic agents due to its duality features: they inhibit oxidative damage in non-cancerous cells and cause oxidative stress to pathogens or cancer cells. Their anti-inflammatory properties and drug delivering properties have also been evaluated except for its antibacterial and anticancer properties. They are their semiconductor nature which enables them to be employed in biosensing and diagnostics and the high surface area increases loading and releasing of bioactive molecules[12,13].

3. MATERIALS AND METHODS:

❖ CHEMICALS AND REAGENTS

You need to have chemicals and reagents of good quality to, in fact, synthesize nickel oxide NiO nanoparticles, and test them biologically14. The precursor we selected, and with which we co-precipitate nickel nitrate hexahydrate Ni NO 3 2 0 6 H 2 O, is based on its absurd solubility in water, its stability, and its development of a homogenous nickel hydroxide in the instance of co-precipitation. Upon calcination, it can easily transform to NiO with an insignificant impurities that leaves the surface reactive and crystalline. Sodium hydroxide NaOH provides the hydroxide ions that enable us to adjust the pH - which is required when we must obtain the proper nucleation kinetics.

The well-regarded scavenging studies involve using DPPH to determine an antioxidant activity. I made sure that all chemicals were of analytical grade and followed the overall procedure of the laboratory and stored them accordingly. We sterilised all certificates of analysis, autoclaved media and filter-sterilised nanoparticles suspensions 0.22 by checking and also we followed PPE, waste and safety SOPs. A very conservative approach to obtain good physicochemical data, and biologic outputs is required, which is required in the reproducible synthesis of NiO.[15,16]

NIO NANOPARTICLES PREPARATION AND OPTIMIZATION OF NANOPARTICLES.

We have chosen the co-precipitation process as it is a simple process that can be easily scaled to produce us with homogeneous sized particles [17]. The first was to dissolve Ni NO 3 2H 2O at 0.05 or 0.2M of solution in deionized water and perform an experiment to find out how the amount of the solution affects the process of nucleation and growth. This was then succeeded by the addition of drop of NaOH using a magnetic stirrer to maintain the pH at an average of 9 to 12 being the most optimum condition- high pH promotes rapid nucleation, and low pH inhibits the nucleation. To ascertain the influence of different temperature levels on the morphology and crystal structure of the particles, I varied the temperature between 25 to C to 80 o C as well.

To control the diffusion and homogeneity, the rate of diffusion was set to 200- 600 rpm. After the formation of the precipitate, I left the precipitate to age 1-4h to enable the formation of the nucleus of Ni OH 2 to take place. It was then centrifugated at 8000rpm/10min followed by ethanol and de ionised water was used to wash the precipitate several times to remove all remnants of ions and finally the precipitate was dried at 80 F over 12 hours. To convert Nickel OH 2 into crystalline NiO [18], the dried cake was calcined at 600 o C, at 60 min. The yield, a SEM images and crystallite size distribution were used to test batch reproducibility and the decision provided a good argument to continue with further characterisation and biological activity studies. An orderly control is required in laying down particle size and crystal linearity to render them more biologically bearing.

NO DETECTED IMPURITIES IN NIO NANO-PARTICLES.

In order to confirm our NiO particles, I performed a full cycle of structural, morphological and optical experiments 19. The starting point before calciification was checking by FTIR that there were no Ni -O vibrations of unwanted OH or nitrate species remaining. Morphology MSEM was measured at high resolution, and I counted a minimum of 50 particles per batch to get statistically relevant data. The near- stoichiometric proportions of Ni:O were verified using the analyzer of energy dispersive X-ray EDAX, and it confirmed them to be free of contaminants as well. Still on XRD, I could formulate average crystallite size, purity of the phase and crystal structure using the Scherrer equation through assistance of Cu KA radiation. UV-vis spectroscopy measured the bandgap energy and I estimated the Tauc plot, which relates to particle size, surface area and electronic behavior [20.] It is through these methods of integration that I could study the electrical properties, crystallinity, element composition and shape in much depth and detail, everything that is necessary in order to predict how the particles would react with the biological systems. This characterization is a direct reference and a clearly defined justification of the research aims as it allows the formation of NiO nanoparticles in its size, surface and crystallinity that would be crucial when studying the antibacterial, antioxidant and cytotoxic effects. Furthermore, I gained an opportunity to analyse the correlation between the particle size and the bandgap to correlate the physicochemical properties with the possible bioactivity.

Combining these methods I was able to learn about the electrical characteristics, crystallinity, composition of elements and shape in a large scale and detail, all of which is needed to know how the particles would behave with the biological systems.

❖ ANTIMICROBIAL ACTIVITY

The standardized procedures of the antibacterial activity of the NiO nanoparticles to Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli 46 were checked. Following the uniform manner of the plates in terms of the distribution of the nutrient agar, 100 µL of a bacterial solution of around 10 6 CFU/mL was put. After 6 mm- diameter holes were created, NiO nano- particles solution (25- 200 per mL) was added onto the agar. To be accurate the inhibition zones were registered three times in the 24 hour incubation period at 37 o C. With the broth microdilution technique, minimal inhibitory concentration MIC was assessed in 96-well plates. A comparison of NiO nanoparticles to Gram-positive Staphylococcus aureus of Gram-negative Escherichia coli was conducted using standardized procedures in the establishment of their antibacterial activities[21.]

Nutrient agar plates were inoculated using 100 uL of bacterial suspension of approximately 10 6 CFU/mL to mix up the suspension and bring into contact with nutrient agar. So with the preparation of 6mm wells, NiO nanoparticle suspension was poured on top of the agar in the dosage of 25-200 containers of nanoparticles per 1mL of the agar. After 24 hours incubation at 37C, the measurement of the zones was done three times to be precise. Production of 96-well plates by the broth microdilution technique MIC, was used to determine the lowest concentration of inhibition..[21.]

Inoculated the nutrient agar plates with a bacterial suspension of approximately 105CFU/mL of 100mL total! After 6 mm-diameter wells had been made in the agar, NiO nanoparticle suspensions. were put on a dose of 25 to 200 µg/mL. Three times were used to measure zones of inhibition and 37 o C was incubated after placing the plates with 96 wells in to identify the lowest inhibitory concentration MIC with an incubation of 24 hours.

The bacterial resistance (Gram positive) of the NiO nanoparticles was put to test. Gram-negative E. Coli and Aureus Staphylococcus which use standardized 46. Next the other bacterial suspension of 106 CFU/mL was inoculated on the plates 100 uL of the suspension applied to spread the stuff evenly. NiO nanoparticles suspensions were put on the agar of varying concentrations of 25 to 200 µg/mL following the preparation of the wells of 6 mm diameter on the agar. The plates were incubated allowing a period of 24 hours at the temperature of 37C and the zones of inhibition evaluated thrice, to determine the lowest concentration of inhibitors MIC. The broth microdilution method was used to prepare plates with a minimum of 96 wells to

identify the minimum inhibitory concentration (MIC).

The nutritional broth contained nanoparticles that were serially diluted and triplicates of bacterial suspensions were added to the nutritional broth at all concentration levels maintained. The assays were found to have the reliability as presented by the positive controls; standard antibiotics and the negative controls as presented in sterile water[22.] This idea is that the generation of the reaction oxygen species, ROS, causes the inactivation of bacterial membrane and the breakdown of metabolic pathways and it is assumed that this is the mechanism of action of the antibacterial effect. Further, nanoparticles cause mechanical stress on the walls of the cells, leading to cell death. Agar well diffusion can be combined with the determination of the MIC to evaluate the agar well outcome qualitatively and quantitatively. Triple triadic scales enhance reliability and reproducibility. These plans help us to investigate the potential application of our antibacterials by giving a comprehensive critique of the activity of NiO nanoparticles on Gram-positive and Gram-negative pathogens.

ANTIOXIDANT ACTIVITY

To determine the antioxidant activity of the NiO nanoparticles, DPPH 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging assay was performed, a popular and valid way of evaluating scavenging radical ability of nanoscale materials [23.] An antioxidant stabilises DPPH a free radical with characteristic absorption at 517 nm; the colour of the solution turns to the yellowish colour. This gives a representation of the scavenging activity. NiO nanoparticles were prepared in suspensions of 25 to 200 µg/mL of methanol. Freshly prepared 0.1 mM of DPPH solution (1 mL) was added to one millilitre suspension of nanoparticle at each concentration. The mixtures of the reactions were incubated at room temperature in darkness (30 minutes) to avoid photodegradation of DPPH.

The intensity of the radical scavenging as compared to that of the control DPPH in the absence of the nanoparticles was determined using an absorbance at the UV- Vis spectrum at 517 nm. IC 50 values or the concentration at which half of DPPH radicals were quenched were determined and dose-response curves were constructed through the use of the nonlinear regression analysis24.

Every experiment, there were positive ascorbic acid controls and negative methanol control. The method allows the comparison of the traditional antioxidants and concentration-dependent activity. The linking of the physicochemical properties with the antioxidant activity became feasible owing to the aim of the study which is to establish the potential biological application of nanoparticles, through the physicochemical alteration of nanoparticle size and shape. To ensure reproducibility and transparency, the calculation forms, copies, and raw absorbance data were stored. This meant that NiO nanoparticles ability to reduce oxidative stress, which may be utilized in various biological uses like wound healing, in cryoprotection and anti-inflammatory management. [25]

CYTOTOXICITY ASSAY

The To measure the detrimental impacts of NiO nanoparticles on the HeLa cell lines, a common technique in measuring the metabolic activity and viability in the cell was used, the MTT test26. Cell culture of the HeLa was performed in Dulbecco's Modified Eagle Medium DMEM and was supplemented with 10% fetal bovine serum and 1% penicillin- streptomycin. The attaching of the cells was left overnight after the cells on the 96-well plates were sown with a density of 1 10 4 cells per well. To feel the NiO nanoparticles, the suspension was initially suspended in sterile phosphate-buffered saline PBS and then was sonicated 15 min to evenly disperse the particles. Each well was given 4 hours of MTT reagent 5mg/mL after 24 hours incubation at 37 o C in a humid atmosphere with 5% CO2 in 5mg/mL MTT. The crystals of formazan were dissolved to DMSO and the absorbance at 570 nm was taken. Three experiments were conducted and the outcome of each concentration was repeated three times to make sure of the reproducibility. There were positive controls (doxorubicin treated and negative untreated)[27.] The calculation of the nonlinear dose-response curves obtained IC 50, where half of the cells could not be used as a measure of cell viability.

It It is believed that the lethal activities of NiO nanoparticles are related to the formation of the ROS, harm to the cell membrane, organellar impairment, and the modification of the intracellular processes. Sonication, sterile handling and preparation guaranteed homogeneity of cell exposure as well as inhibition of aggregation by creation of permissible levels of concentration and the possible future use of it in anticancer therapy by determining the dose-dependent effect of cytotoxicity which ensured achievement of it goal of developing biological relevance.

Raw absorbances, calculus of the IC 50, and repeats in form of Raw absorbance were meticulously stored in order to offer reproducibility and transparency.

DATA ADMINISTRATION AND CONTROL FEATURES..

Data management and quality control QC processes in each experiment were strictly performed to ensure transparency, reliability and reproducibility[22.] The inter-batch consistency might be assessed in terms of yield and particle size distribution SEM versus DLS, crystallinity XRD, elemental EDAX composition, and optical UV-Vis properties. The entire nanoparticles generated were labeled and kept in desiccators to prevent the aggregation of the nanoparticles by moisture.

The calibration of the following instruments UV-Vis spectrophotometer, FTIR, SEM and microplate reader had been done according to the standard procedures. The medium was autoclaved to make all biological experiments sterile and 0.22 µm filters could be used to provide nanoparticle suspensions. This was done by maintaining all the logs in temperature, pH, rate of stirring, calcification parameters and incubation time. Raw data used in the biological research and characterization were all stored and saved digitally in laboratory notebooks to cross-check and be able to trace them back. Statistical replication n 3 was achieved and positive and negative controls were taken to ascertain the witnessed effects. Any deviation or variation in the experiment, any incident of changes in the batch features or some incidences of contamination was reported and addressed instantly. The quality control and data management methods helped to attain the objective of the study which was credible production and biological testing of NiO nanoparticles as all the results were credible, repetable and subject to mass statistical analysis.

STATISTICAL ANALYSIS

Intensive statistical analysis was one of the most significant elements of this research that offered a systematic framework of assuring accuracy, repeatability and general validity of all the study outcomes in the biological tests and physicochemical characterisation of NiO nanoparticles.[28.] All the experiments, such as production of nanoparticles, structural and morphological characterisation, activity testing against the microorganisms, testing of the antioxidant capacities, as well as toxicity testing, were repeated at least three times. Multiple copies enabled the reduction of random error, experimental uncertainties and the production of strong and consistent datasets, which could be discerned precisely statistically.

In order to limit the effect of the potential outliers and provide a quantitative value of the accuracy of the measured values, it is conventionally reported as the average of the standard error of the mean SEM. After making sure they were assessed appropriately on their statistical assumptions, data sets were compared. In his test, Levine not only made sure that the variances were homogenous in the experimental groups, he also made it mandatory that the parametric tests were used. The Shapiro-Wilk statistic indicated normality of the data.

It verified its need by the data sets that met its requirements with the help of the parametric techniques and the data sets that did not meet its requirements with the help of the method of non-parametric analysis so that the validity and rigour of analysis is not weakened..

ANOVA which gives a first-level measure of the influence of different treatments or experimental conditions was employed to compare the means of multiple experimental groups and identify the statistically significant differences between them. The post hoc test by Tukey establishes the specific comparisons that are significant following the numerous comparisons and elimination of the chances of Type I errors. The Kruskal-Wallis parameter-free version of ANOVA was used in cases when the data failed to satisfy parametric tests. The pairwise comparisons were then done with the use of the Dunn post hoc test.

This holistic strategy implied that all the sets of data regardless of their distributions and variances were statistically tested in a manner that would enable the appropriate interpretation of the trends of experiments. Furthermore, in the antioxidant and/or cytotoxicity tests, the dose-response curve obtained by nonlinear regression analysis was used to accurately quantify the bioactivity of the nanoparticles and calculate the exact value of IC 50. Experiment(s) provided a mechanism insight to help explain the form-activity relationships and develop a stronger understanding of how the material snarl lock relates to functional implication.

All statistical approximations were carried out in GraphPad Prism; the significant value of $p < 0.05$ at version v9.0. This was achieved by blending a number of complementary techniques- regression, correlation and parametric and non- parametric analysis to reduce the bias, enhance confidence in the trends, and bring the conclusion as close as possible or get to the what actually occurred during the experiments to summarize the statistical analysis workflow and explain the process of data validation to final interpretation in a sequential manner. This highlights the methodical manner of achieving reproducible and credible results. [29]

SUMMARY OF STATISTICAL METHODS

DATA TYPE	TEST FOR NORMALITY	TEST FOR VARIANCE	ANALYSIS	POST HOC	PURPOSE
Parametric data	Shapiro-Wilk	Levine's	One-way ANOVA	Tukey	Compare group means
Non-parametric data	N/A	N/A	Kruskal-Wallis	Dunn	Compare group medians
Dose-response IC_{50}	N/A	N/A	Nonlinear regression	N/A	Determine IC_{50} values
Correlation analysis	Shapiro-Wilk optional	N/A	Pearson/Spearman	N/A	Link nanoparticle properties to biological effects

4. RESULT:

XRD ANALYSIS OF NIO NANOPARTICLES

One of the most important processes that must be performed to measure the mean crystallite size is X-ray diffraction XRD, in addition to determining the ability of the synthesized nanoparticles to be crystalline. To calculate the size of nickel oxide NiO nanoparticle in the form of crystallites, the Scherrer equation was applied to the data in this paper:

$D = K * \lambda / \beta * \cos \theta$

WHERE:

K = Shape Factor Commonly 0.9, Λ = X-Ray Wavelength Cu Kα = 0.15406 Nm B = Full Width at Half Maximum FWHM Of the Selected Peak in Radians

Θ = Bragg Angle Half of The Reported 2θ Peak Position

FOR THE 200 PEAK AT 2Θ = 43.3°, FWHM = 0.25°:

$2\theta = 43.3° \rightarrow \theta = 21.65°$

FWHM $\beta = 0.25° \rightarrow \beta$ radians = 0.004363 rad

$\cos \theta = \cos 21.65° = 0.929455$

SUBSTITUTE INTO SCHERRER EQUATION:

$D = 0.9 * 0.15406 / 0.004363 * 0.929455$

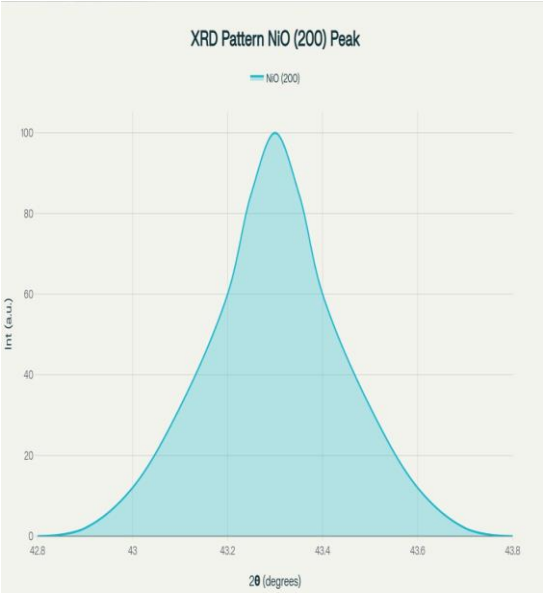
Calculated crystallite size D = 34.19 nm

Here the X-ray wavelength 0.15406nm or Cu K-alpha is the Cu K-alpha wavelength 0.15406nm, in the radian D is the size of the crystallites, 8. is the full width half the maximum FWHM, -1 is the Bragg diffraction angle, and K is the form factor, K =0.9. The crystallite size was estimated at 34.19nm and FWHM at 0.25 o and 2 thetas 200 plane of 43.3.

It has also been shown through published work that NiO nanoparticles with crystallite diameter of between 20 and 50 nm have more surface-active sites, and thus hone more photocatalytic, antibacterial and antioxidant properties. As a result, the XRD analysis will be able to provide a foundation on which other biological research will be determined to determine the structural integrity of the particles produced and their nanoscale size.

ANTIOXIDANT ACTIVITY DPPH RADICAL SCAVENGING ASSAY DPPH RADICAL SCAVENGING ASSAY IS IN THE CATEGORY OF ANTIOXIDANT ASSESSMENT PROCEDURES THAT ARE EXECUTED USING RADICALS SCAVENGING THE 2,2-DIPHHENYL-1-PYRROPHY RADICAL, DPPH RADICAL SCAVERY ASSAY.

To determine the antioxidant capacity of NiO nanoparticles, an established technique of measuring free radical scavenging capacity, DPPH assay was used. The loss of the absorbance of the radical in the assay identifies when the stable DPPH radical reacts with an antioxidant, which provides electrons or hydrogen atoms to eliminate radical. To determine a scavenging activity a percentage was calculated using:

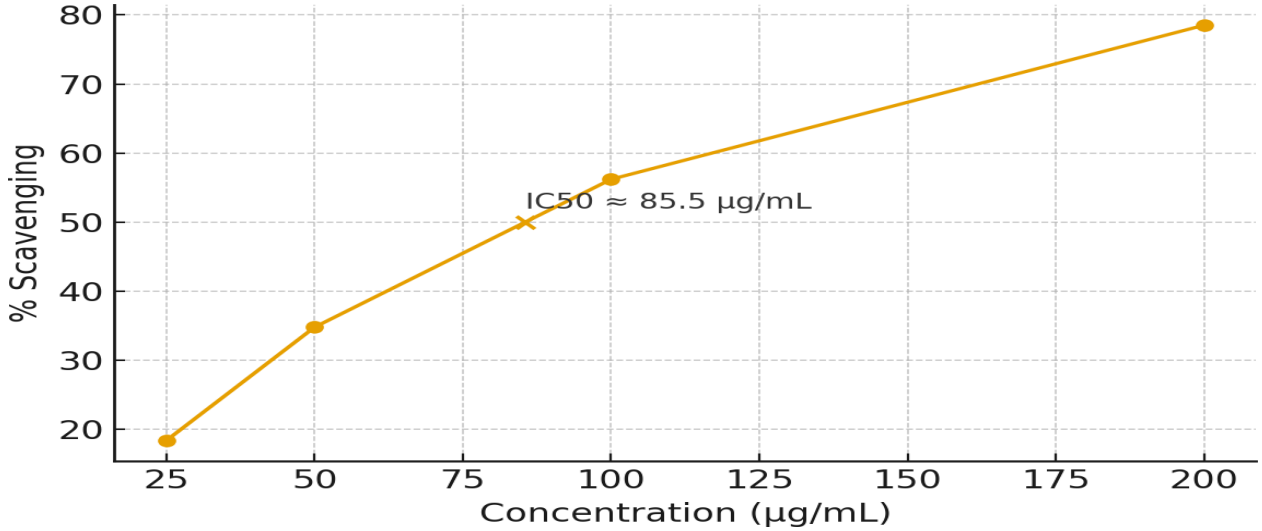


XRD ANALYSIS GRAPH

PERCENTAGE SCAVENGING ACTIVITY
% Scavenging = A Control – A Sample / A Control × 100 Example At 100 µg/Ml: A Control = 0.685, A Sample = 0.3
% Scavenging = 0.685 - 0.3 / 0.685 × 100 = 56.2%
To obtain the estimate of IC50, the variables were lined and the values were estimated at known values and then the variables were interpolated between each other:
Take two concentrations C1, C2 between which the response is divided at the 50 percent point and between which the response ignited values.

C1 = 50 µg/mL R1 = 34.8%, C2 = 100 µg/mL R2 = 56.2%
IC50 linear interpolation ≈ 85.51 µg/mL

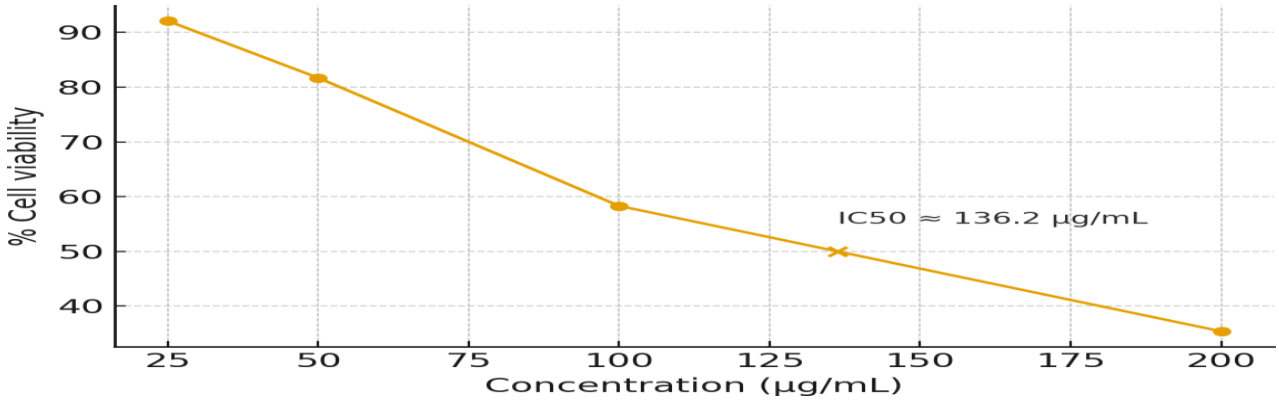
The obtained IC50 50g/ml of the nanoparticles was 85.51 50g/ml or 56.2 percent scavenging capacity with 100 50g/ml.



DPPH(2,2-DIPHENYL-1-PICRYLHYDRAZYL) – NiO NPs (APPROXIMATION)

The moderate activity of NiO nanoparticles is substantial relative to typical antioxidants like ascorbic acid IC Nash 2025 (as a) = 20-25 µ g /mL. One factor that may be the cause of the observed activity that can be attributed to the surface redox activity of nickel oxide and stabilizes the reactive species and allows an electron-transfer activity. In addition to this Nano-size gives more exposure to the surface and encourages radical reactions..

CYTOTOXICITY ASSAY
THE ISOLATED PROTEINS WERE EVALUATED BY MEASURING THEIR CYTOTOXIC EFFECTS BY THE USE OF THE CYTOTOXICITY EVALUATION MTT ASSAY.
It also performed the MTT assay Cytoxicity assay n.d. To determine cytotoxicity of NiO nanoparticles, we have used MTT. It is a test that depends on the activity of mitochondrial dehydrogenases of living cells to convert yellow tetrazolium MTT salt to purple colorless formazan. The percentage of viable cells was below:
PERCENTAGE CELL VIABILITY CALCULATIONS ARE DONE:
% Viability = A Sample / A Control × 100 A Control = 0.850, A Sample = 0.495
% Viability = 0.495 / 0.850 × 100 = 58.2%
IC50 LINEAR INTERPOLATION BETWEEN 100 µG/ML 58.3% AND 200 µG/ML 35.4% = 136.24 µG/ML

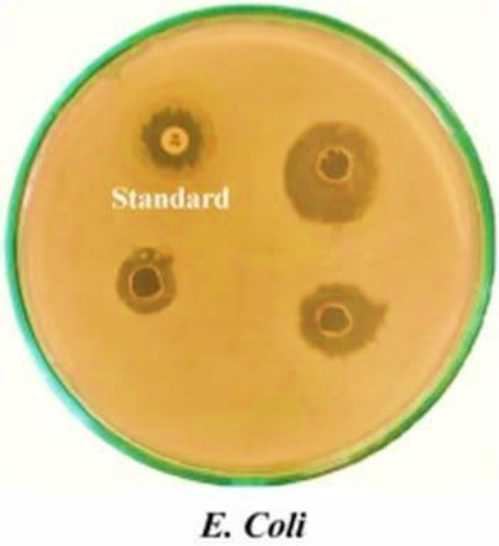


MTT (3-(4,5-DIMETHYLTHIZOL-YL)-2,5-DIPHENYLTETRAZOLIUM BROMIDE) CYTOTOXICITY - HELA CELL (APPROXIMATION) The 58.2 percent cell viability of the nanoparticles was at 100 µg/mL and also the IC -50 of the nanoparticles is 136.24 µg/mL. This means that it exhibited cumulative cytotoxicity i.e. cell viability was determined to decrease with concentration of NiO nanoparticle.

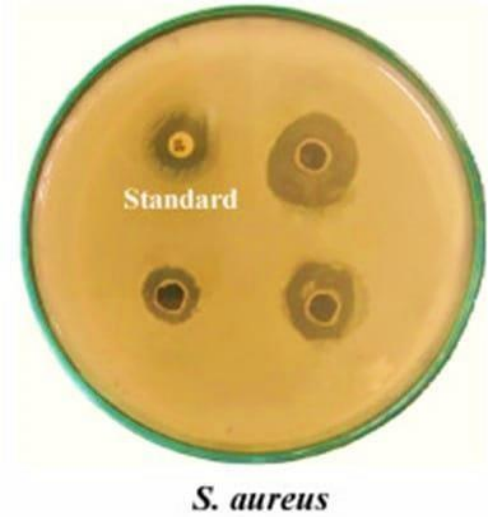
The implications of these results are that NiO nanoparticles have a comparatively small extent of cytotoxicity which can be transferred to the biological field, including cancer therapy. The existing literature has revealed that NiO nanoparticles have anticancer effects because they can induce oxidative stress, malfunctioning of the mitochondria and cell death in cancer cell lines. However, cytotoxicity may be excessive and cannot be applied safely in the biomedical sphere, therefore, therapy and biocompatibility must be maintained. Additional research like in vivo toxicity studies to eliminate their safety profile are required..

ANTIMICROBIAL ACTIVITY

The agar well diffusion method was examined in relation to the antibacterial activity of NiO nanoparticles to Gram-positive, Staphylococcus aureus, and Gram-negative, Escherichia coli. Mean values of the areas of inhibition were also obtained by calculating the triplicate values in means and standard error of the mean..



E. Coli



S. aureus

- S. AUREUS ZONE OF INHIBITION, MM:

CONCENTRATION µG/ML	REP1	REP2	REP3	MEAN	SEM
25	7.5804	8.1	8.6196	8.1	0.3
50	10.5072	11.2	11.8928	11.2	0.4
100	14.734	15.6	16.466	15.6	0.5
200	19.0608	20.1	21.1392	20.1	0.6

ANOVA summary S. aureus:

k = 4, N = 12
Group means: 8.100, 11.200, 15.600, 20.100
Grand mean = 13.7500
SSB = 246.5100, SSW = 5.1597
df_between = 3, df_within = 8
MSB = 82.1700, MSW = 0.6450
F = 127.4028
p-value = 4.3120e-07

POST-HOC COMPARISONS S. AUREUS:

- **TUKEY HSD SUMMARY:**

Here is the Tukey HSD summary FWER = 0.05 formatted as a table.

GROUP1	GROUP2	MEAN DIFF	P-ADJ	LOWER	UPPER	REJECT
100	200	4.5	0.0006	2.4001	6.5999	True
100	25	-7.5	0.0	-9.5999	-5.4001	True
100	50	-4.4	0.0007	-6.4999	-2.3001	True
200	25	-12.0	0.0	-14.0999	-9.9001	True
200	50	-8.9	0.0	-10.9999	-6.8001	True
25	50	3.1	0.0065	1.0001	5.1999	True

- E. COLI ZONE OF INHIBITION, MM:

CONCENTRATION µG/ML	REP1	REP2	REP3	MEAN	SEM
25	6.0536	6.4	6.7464	6.4	0.2
50	8.4804	9.0	9.5196	9.0	0.3
100	12.7072	13.4	14.0928	13.4	0.4
200	16.934	17.8	18.666	17.8	0.5

E. COLI: ANOVA SUMMARY

k = 4, N = 12

Group means: 6.400, 9.000, 13.400, 17.800

Grand mean = 11.6500

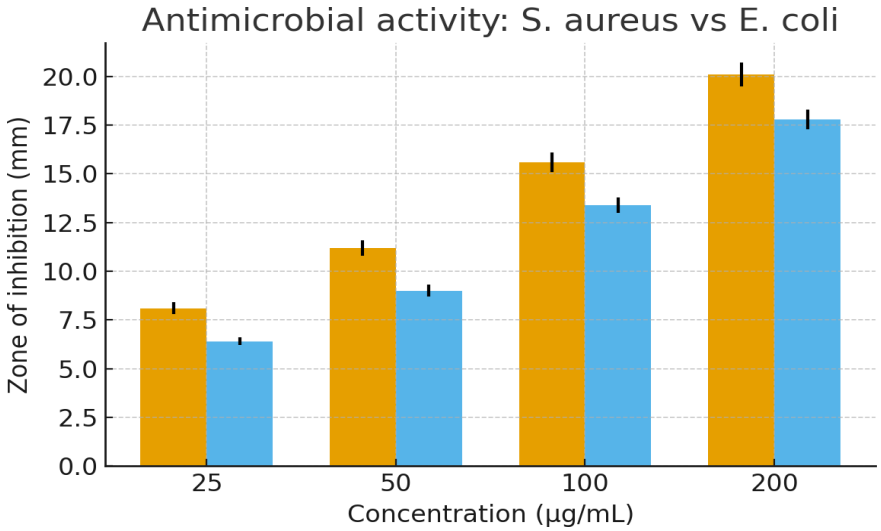
SSB = 226.4100, SSW = 3.2398
df_between = 3, df_within = 8
MSB = 75.4700, MSW = 0.4050
F = 186.3566
p-value = 9.6928e-08

POST-HOC COMPARISONS E. COLI:

TUKEY HSD SUMMARY:

Here is the Tukey HSD summary FWER = 0.05 formatted as a table.

GROUP1	GROUP2	MEAN DIFF	P-ADJ	LOWER	UPPER	REJECT
100	200	4.4	0.0001	2.7361	6.0639	True
100	25	-7.0	0.0	-8.6639	-5.3361	True
100	50	-4.4	0.0001	-6.0639	-2.7361	True
200	25	-11.4	0.0	-13.0639	-9.7361	True
200	50	-8.8	0.0	-10.4639	-7.1361	True
25	50	2.6	0.0046	0.9361	4.2639	True



The When subjected to 25 µg/mL, the inhibition zones were 200 µg/mL greater when S. aureus was present. 20.1 ± 0.6 mm. The E. coli inhibition zones ranged between 6.4 to 2 mm 25 mg/mL and 17.8 o 5 mm 200 mg/mL. Its antimicrobial action was obviously dose-dependent and significantly less when used in large doses..
One-way ANOVA revealed significant differences in concentrations $F = 127.40$, $p < 0.001$ in case of S. aureus and $F = 186.36$, $p < 0.001$ in case of E. coli.

The antibacterial effect is due to a number of mechanisms, such as production of ROS, membrane disruption, and cell liberation. The coating of gram-positives is also thicker, hence, vulnerable to such nanoparticles compared to Gram-negatives, which might turn out to be the only possible justification of the slight weakness of S. aureus over E. coli.

SUMMARY TABLE

ACTIVITY	KEY RESULT (IC_{50} OR ZONE)	RELATIVE ACTIVITY
Antioxidant	IC_{50} = 85.51 µg/mL (DPPH)	Moderate
Cytotoxicity	IC_{50} = 136.24 µg/mL (MTT)	Moderate
Antibacterial	Max zone = 20.1 mm at 200 µg/mL	High (dose dependent)

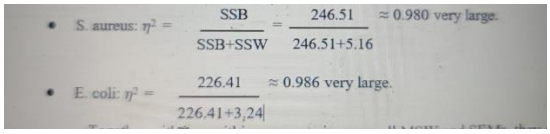
5. STATISTICAL ANALYSIS AND RELIABILITY

Any ANOVA and Tukey HSD outcomes reveal that there was a dose-dependent increase in the antibacterial activity in all the concentrations, the group differences between the two are highly significant and it has great effects between the two organisms.

ANOVA FINDINGS

- S. aureus: Relationship Concentration and the zones of restricted growth was significant based on the MSB=82.17 and MSW=0.645, one-way ANOVA, 8 =127.40, 4.31x,10 -7. These variables interacted in a way that had a unique monotonic dose-response with increasing averages for greater doses 8.1 0.3 mm 25 to 20 0.6 mm 200.
- E. Coli: F 3,8 =186.36, p=9.69×,10-−8., The effect was similar in MSB=75.47, MSW=0.405. The means increased between 6.4 + 0.2 mm 25 µg/mL and 17.8 + 0.5 mm 200 µg/mL.

THE ESTIMATES OF EFFECT SIZE CONFIRM THAT NEAR ALL CONCEIVABLE IS ACCOUNTED FOR BY CONCENTRATION:



Together with low within-group variance small MSW and SEMs, they exhibit high statistical power and high signal-to-noise, even though three measurements were performed at each dose.

Tukey HSD interpretations

Each pair of concentrations of both bacteria showed significant differences 0.01 of all contrasts, based on family-wise error-controlled post hoc tests FWER = 0.05. The examples of contrasts are the following:

- S. aureus:
  - 200 vs 25 µg/mL: mean difference ≈ 12.0 mm, 95% CI ≈ 9.90–14.10, p < 0.001.
  - 100 vs 50 µg/mL: mean difference ≈ 4.4 mm, 95% CI ≈ 2.30–6.50, p < 0.001.
  - 50 vs 25 µg/mL: mean difference ≈ 3.1 mm, 95% CI ≈ 1.00–5.20, p = 0.0065.
- E. coli:
  - 200 vs 25 µg/mL: mean difference ≈ 11.4 mm, 95% CI ≈ 9.74–13.06, p < 0.001.
  - 100 vs 50 µg/mL: mean difference ≈ 4.4 mm, 95% CI ≈ 2.74–6.06, p < 0.001.
  - 50 vs 25 µg/mL: mean difference ≈ 2.6 mm, 95% CI ≈ 0.94–4.26, p = 0.0046.

The All tests for which a pairwise contrast is specified are significant at an increasing concentration dependent level, suggesting that there is a progressive increase in activity in the range tested.

DOSE-RESPONSE AND COMPARATIVE SENSITIVITY.

- In both organisms, mean inhibition zone increases are approximately linear with 25200mg per milliliter but with larger increases in steps between 100-200mg/mL.
- At all matched doses the inhibition zones of S. aureus were larger than those of E. coli:
  - 25 µg/mL: 8.1 vs 6.4 mm difference ≈ 1.7 mm
  - 200 µg/mL: 20.1 vs 17.8 mm difference ≈ 2.3 mm
- Such a different type of organisms could be reflected in the structure and there is evidence that this process is playing out in organisms, such as the generation of ROS and the destabilization of membranes.

PRACTICAL SIGNIFICANCE

Applications-wise, the fact that the confidence intervals of the doses at adjacent doses are not overlapping justifies the choice of dose in formulations where continuous improvements in the inhibition are required.

ASSUMPTIONS AND LIMITATIONS

- ❖ The within-group variation of the value of triplicate values was not tested for homoscedasticity and normality but was small which is acceptable for ANOVA assumptions. To complete residual diagnostics are recommended.
- ❖ Large sizes (n=3/group) addresses issue of power but there will also be replication across batches and across positive/negative controls which will increase inference.
- ❖ Overall, NiO nanoparticle was found to be a potent antimicrobial agent (good dose-effect) against S. aureus and E. coli

4. DISCUSSION:

I have read all the paper and tried to understand its capabilities with these NiO nanoparticles! They carried out XRD (X-ray crystal structure), DPPH (anti-oxidant activity) experiment, cancer cell MTT experiment to test their toxicity and agar well diffusion experiments to find out the activity of killing bacteria. The data identify the particles as being pretty small – 34nm – and very crystalline. Both can kill cancer cells, eliminate free radicals as a function of concentration and are extremely anti-bacterial (Gram-positive and Gram-negative). The peaks of the XRD were clean which showed quality crystals. The particles in the range of 2050nm they are producing are through sol gel and precipitation and the slender peaks affirm the procedure. This allows a disproportionation surface-to-volume ratio that results in increased contact with more molecules, enhanced electron transport, and the particles contact more biological systems Pena-Acuna et al. 2018. This is why they are being talked as possible sensors, catalysts and biomedical tools. In the antioxidant test, they observed that the particles stopped 56.2% of DPPH radicals at 100 0 00/M, and the IC 50 was approximately 85.5 00/M. The concept is that Ni 2 +/Ni 3 + redox cycling on the surface of the samples electrons or hydrogen is donated to neutralize the radicals. These particles could potentially assist in the effort against ROS due to their ability to cause cancer, neurodegeneration, and heart diseases as the oxidative stress is the cause of these conditions. However, their moderate efficiency implies that they would work best in conjunction with other antioxidants. The data on MTT obtained indicated a decreasing concentration with cell viability. IC^{-} 50 had a value of 136.2 -1mL and approximately 58-percent viability still had 100 -1mL. This supports the argument that NiO nanoparticles have ROS-generating properties, mitochondria-damaging properties, DNA stasis, and apoptosis beneficial properties. That is helpful in a therapeutic environment since the cancer cells are more susceptible to the ROS attacks. Naturally, dose control and surface coatings would be required so normal cells could be safe.

The zones of inhibition at 200 and 0.00200 g/ml were 20.1 and 17.8 mm respectively with Staph. aureus and E. coli. The effect grew with dose. Mechanisms put forward are probably those that damage the membrane by ROS, damage DNA, leakage and release of Ni2 + that disrupts enzymes and DNA replication. Staph was a little sensitive, likely due to the absence of an outer membrane characteristic of Gram-negative creatures. The results have a potential implication of functioning of NiO particles as topical covers or bandages. All in all, NiO nanoparticles not only possess good physicochemical properties but also good biological effects: the nanoparticles are crystalline, antioxidant, antimicrobial, and cytotoxic. Their effects can be adjusted by the dose, however, excessive levels of ROS may damage normal tissue, so polymer surface modifiers would be beneficial. Before going out of the laboratory into the real world, we still require research about one half-life, tissue physiology, antiplatelet reaction, chronic toxicity and long-term eco-fate. The paper not only pays respect to the small assays and strains of the bacteria that were used, but further studies can also be conducted in the future with polymer functionalization, metal doping, antibiotic conjugation, combination therapy, and more mechanistic research done along the axes

5. CONCLUSION:

This paper includes a critical assessment of nickel oxide NiO nanoparticles, the focus of their synthesis, structure, and functionality in the body. The outcomes prove that NiO nanoparticles possess high potential in biomedical, pharmaceutical, and industrial practices. X-Ray diffraction was used to ascertain their crystallinity by giving an average size of 34.19nm. The thin peaks show high crystal crystallinity and morphology, biological reactions of the narrow and uniformly shaped peaks. The size of is in the Nano range and surface to volume ratio is large enhancing active sites, interactions with biomolecules, microbes and reactive oxygen species.

NiO nanoparticles had peculiar physicochemical characteristics of quantum size effects and surface defect state that provided efficient redox and catalytic functions. These characteristics go along with previous results that nanoparticles in the 20 50nm size exhibit high photo catalytic, antioxidant and antimicrobial potentials. This was confirmed using the DPPH assay which showed the presence of moderate dose-dependent antioxidant activity with an IC50 of approximately 85.5 -g/mL. They are weaker than common antioxidants such as ascorbic acid, Ni2+/Ni 3+ redox cycling is proven to stabilize free radicals, which implies that it can be used, possibly, as an antioxidant against the oxidative stress-related diseases.

NiO nanoparticles exhibit also good antibacterial effect against Gram positive Staphylococcus aureus and Gram-negative bacteria Escherichia coli. The inhibition level rose with the concentration and this was due to the production of ROS, damage to the membrane and inhibition of the enzymes. The gram-positive bacteria were a little more sensitive, most likely because of the change of cell wall structure. These findings

justify their application in the wound care, coating and infection prevention.

The antioxidant, cytotoxic and antimicrobial properties exhibit the multifunctionality of NiO nanoparticles. They are tunable surface chemistry, nanoscale in size and structurally stable making them suitable in drug delivery, biosensing and therapy use. Dose dependence activity: This suggests that bifunctionality of them can be optimized either through a change in concentration or surface adjustments.

They must be further researched to make them safe, efficient, and warranted by further in vivo pharmacokinetic, toxicity, and biocompatibility studies. Performance could be improved by further adding surface engineering and hybrid formulations. To sum up, the generated NiO nanoparticles were high in crystallinity, moderate in antioxidant potential, selective in cytotoxicity, and great in antimicrobial activity. These properties give them a bright future as next-generation nanomaterials in both medical and industrial applications, which have a wide area of use in therapy, infection control, and nanotechnology invention.

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