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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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RESEARCH

Metronidazole-loaded zinc oxide / graphene nanoparticles: synthesis, analysis, drug delivery, and antibacterial efficiency

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Keywords: ZnO; graphene; nanoparticles; instrumental analysis; drug delivery

Summary: In our study, zinc oxide (ZnO) nanoparticles (NPs) were prepared by precipitation (economically and in high quality) at a temperature range of 60°C to 80°C and at pH 8, and were then adorned with graphene (G) plates. To determine its antimicrobial potential, the ZnO/G complex was loaded with metronidazole. The morphology and diameter of the ZnO nanocomposite before and after the loading were validated by scanning electron microscopy. The average size of the ZnO NPs was found to be 20–40 nm, while X-ray diffraction examined how the physical features of these NPs varied from those of its individual components with an average size of 28.1 nm. The assessment of the ZnO/G complex's antibacterial efficacy against Gram-positive and Gram-negative bacteria was the main aim of our work. The agar well diffusion technique was used in order to assess the antibacterial activity of the ZnO/G complex with and without metronidazole. Our study demonstrates that the ZnO/G complex possesses antibacterial activity and might increase the antibiotic action by inhibiting Gram-positive bacteria (more than Gram-negative ones). It is, therefore, concluded that the ZnO/G NPs could be of use in formulating nano-drug conjugates that could act as antimicrobial agents.

الإهداء ....

إلى صاحب الزمان (عجل الله فرجه) وإمامنا الثاني عشر الذي احاطنا بعنايته وتوفيقاته

الى من حصد الأشواك عن دربي ليمهد لي طريق العلم (والدي الحبيب)

إلى من وضعتني على طريق الحياة، وجعلتني رابط الجأش وراعتني حتى صرت كبيرا (أمي الغالية)،

إلى الايادي التي رفعتني من كان لهم بالغ الأثر في كثير من العقبات والصعاب.

إلى جميع أساتذتي الكرام؛ ممن لم يتوانوا في مد يد العون لي

أهدي إليكم بحث تخرجي .....

شكر وتقدير

بسم الله الرحمن الرحيم، والحمد لله رب العالمين الذي وفقنا وأعاننا على إنهاء هذا البحث والخروج به بهذه الصورة المتكاملة، فبالأمس القريب بدأنا مسيرتنا التعليمية، ونحن نتحسس الطريق برهبة وارتباك، إن بحثنا يحمل في طياته طموح شباب يحلمون أن يكون عراقهم شامخاً بعلمه بين الدول

وانطلاقا من مبدأ أنه لا يشكر الله من لا يشكر الناس، فإننا نتوجه بالشكر الجزيل الى الدكتورة (اسماء هاشم الحمداني) التي رافقتنا في مسيرتنا لإنجاز هذا البحث، وكانت لها بصمات واضحة من خلال توجيهاتها وانتقاداتها البناءة وكل من الدكتورة) لينا فاضل الجبوري(التي اشرفت على تعليمنا وتوجيهنا في الجانب العملي والأكاديمي ، و الدكتورة (صبا عبد المنعم القيسي) التي أشرفت على تعليمنا الأمور التقنية والعلمية المتعلقة بالبحث .

كما نشكر عائلاتنا التي صبرت وتحملت معنا ورفدتنا بالكثير من الدعم على جميع الأصعدة، ونشكر الأصدقاء والأحباب وكل من قدم لها الدعم المادي أو المعنوي.

الشكر الموصول ايضاً الى الاساتذة اعضاء اللجنة الذين تفضلوا بقراءة هذا البحث

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CHAPTER ONE

Chapter two : Method and Literature review

Chapter three : Results and discussion

Abstract

In our study, zinc oxide (ZnO) nanoparticles (NPs) were prepared by precipitation (economically and in high quality) at a temperature range of 60°C to 80°C and at pH 8, and were then adorned with graphene (G) plates. To determine its antimicrobial potential, the ZnO/G complex was loaded with metronidazole. The morphology and diameter of the ZnO nanocomposite before and after the loading were validated by scanning electron microscopy. The average size of the ZnO NPs was found to be 20–40 nm, while X-ray diffraction examined how the physical features of these NPs varied from those of its individual components with an average size of 28.1 nm. The assessment of the ZnO/G complex's antibacterial efficacy against Gram-positive and Gram-negative bacteria was the main aim of our work. The agar well diffusion technique was used in order to assess the antibacterial activity of the ZnO/G complex with and without metronidazole. Our study demonstrates that the ZnO/G complex possesses antibacterial activity and might increase the antibiotic action by inhibiting Gram-positive bacteria (more than Gram-negative ones). It is, therefore, concluded that the ZnO/G NPs could be of use in formulating nano-drug conjugates that could act as antimicrobial agents.

Chapter One
Introduction

1.1 Introduction

Nanomedicine is the branch of medicine that utilizes the science of nanotechnology in the preclusion and cure of various diseases using the nanoscale materials, such as biocompatible nanoparticles [1] and nanorobots [2], for various applications including, diagnosis [3], delivery [4], sensory [5], or actuation purposes in a living organism [6].

Drugs with very low solubility possess various biopharmaceutical delivery issues including limited bio accessibility after intake through mouth, less diffusion capacity into the outer membrane, require more quantity for intravenous intake and unwanted after-effects preceding traditional formulated vaccination process. However all these limitations could be overcome by the application of nanotechnology approaches in the drug delivery mechanism.Drug designing at the nanoscale has been studied extensively and is by far, the most advanced technology in the area of nanoparticle applications because of its potential advantages such as the possibility to modify properties like solubility, drug release profiles, diffusivity, bioavailability and immunogenicity. This, can consequently lead to the improvement and development of convenient administration routes, lower toxicity, fewer side effects, improved biodistribution and extended drug life cycle [7]. The engineered drug delivery systems are either targeted to a particular location or are intended for the controlled release of therapeutic agents at a particular site. Their formation involves self-assembly where in well-defined structures or patterns spontaneously are formed from building blocks [8]. Additionally they need to overcome barriers like opsonization/sequestration by the mononuclear phagocyte system [9-10].

Microorganisms are everywhere in the biosphere, and their presence invariably affects the environment where they are growing in. The effects of microorganisms on their environment can be beneficial or harmful or in apparent with regard to human measure or observation. They are in natural symmetry with human body and environment. Therefore control of its harmful effects appears to be most important criteria. To resolve this current problem physicians have recommended many techniques and drugs such as kanamycin, spectinomycin, and penicillin. However, frequent usage of these agents causes the microbes to become resistant against those drugs[11]. Nano materials have been to the research focus of interest in the last decade. Recently strong antimicrobial activity has been revealed by several nanomaterials. The metal and metal oxide nanoparticles such as silver (Ag), silver oxide (Ag_2O), titanium dioxide (TiO_2), gold (Au), calcium oxide (CaO), silica (Si), copper oxide (CuO), and magnesium oxide (MgO) have been reported to possess antimicrobial activity [12].

1.2 Graphine oxide Nanoparticals

Graphene oxide (GO) is a unique material that can be viewed as a single monomolecular layer of graphite with various oxygen-containing functionalities such as epoxide, carbonyl, carboxyl, and hydroxyl groups

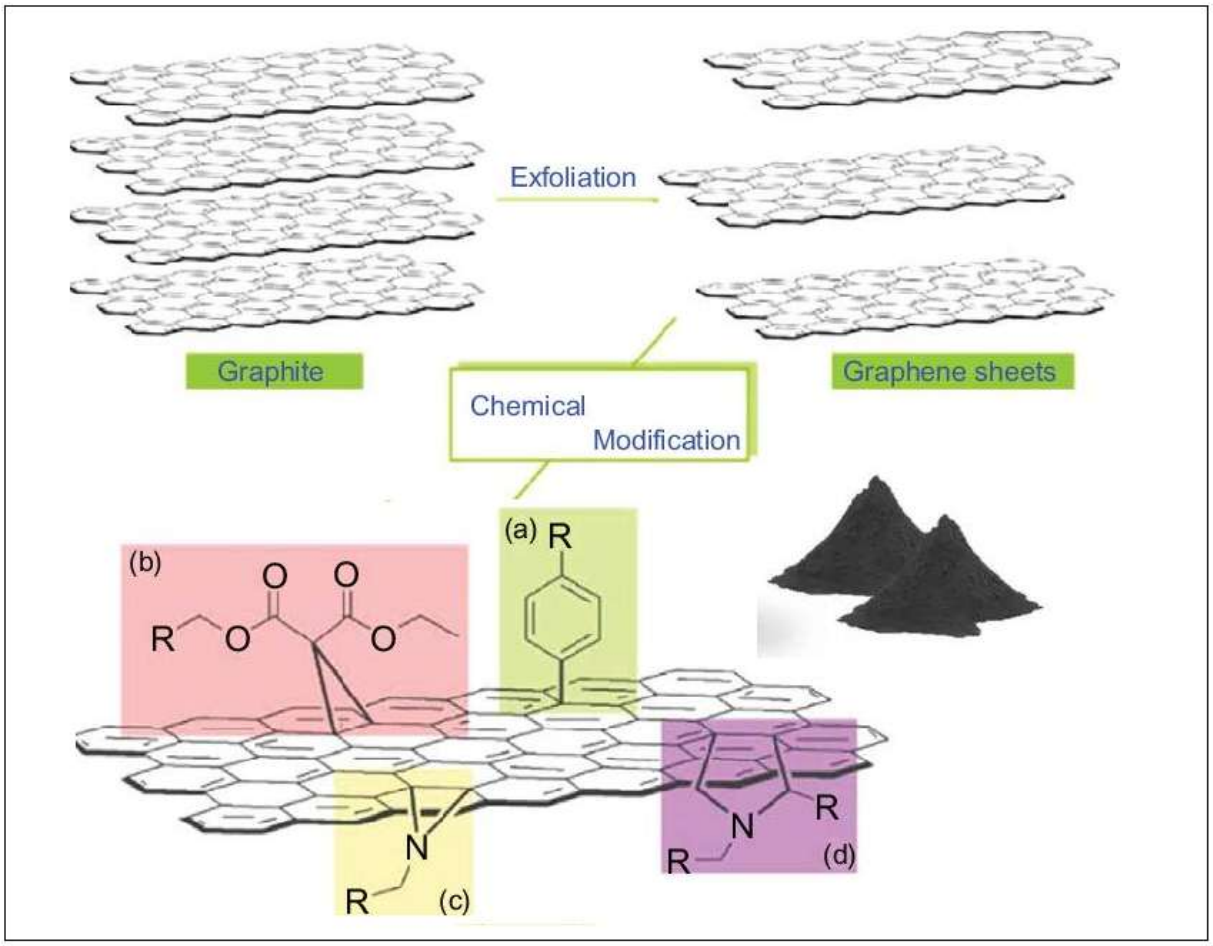

Fig .1 Figure 2.1 General chemical modification routes for exfoliated graphene sheets. from Stergiou et al., 2014

Graphine oxide is its easy dispersability in water and other organic solvents, as well as in different matrixes, because of the presence of oxygen functionalities. GO is highly permeable to cell membranes and shows low toxicity both in vivo and in Ce llular assays. graphene is recorded as having a break strength of 42 N m^{-1}[13].

The massive improvement in mechanical properties can be attributed not only to the strength of the GO filler but the strength of the matrix/filler interface; the OH groups of PVA and the oxygen functionalities of the GO led to a high degree of hydrogen bonding Graphene is an electrically conductive material with high electron mobility (25 m^2 V^{-1} s^{-1}) [14]and electrical conductivity (6500 S m^{-1}) consisting of 2D layers of sp2 carbon one atom thick. Graphene has been shown to greatly improve the electrical conductivity of polymers at low filler contents [15] .Synthesized GO from graphite has a low thermal conductivity of 0.5 W m^{-1} K^{-1} making it not an ideal option for most applications requiring good thermal properties.[16]

1.3 Zinc oxide Nanoparticals

Zinc oxide (ZnO) is a common inorganic compound. It is insoluble in water but soluble in dilute acids and bases. Its melting point is extremely high—1975 ºC, where it also decomposes. ZnO exists in two common crystalline forms: wurtzite and zincblende. The zincblende structure is shown here, but wurtzite is more stable under ambient conditions ZnO occurs in the mineral zincite, but most of the commercial product is made by the high-temperature oxidation of metallic zinc or zinc ores. It is used extensively in diverse industries such as rubber, ceramics, medicine, food, pigments, and coatings. It absorbs ultraviolet light and is probably an ingredient in the sunscreen you used this past summer[17]

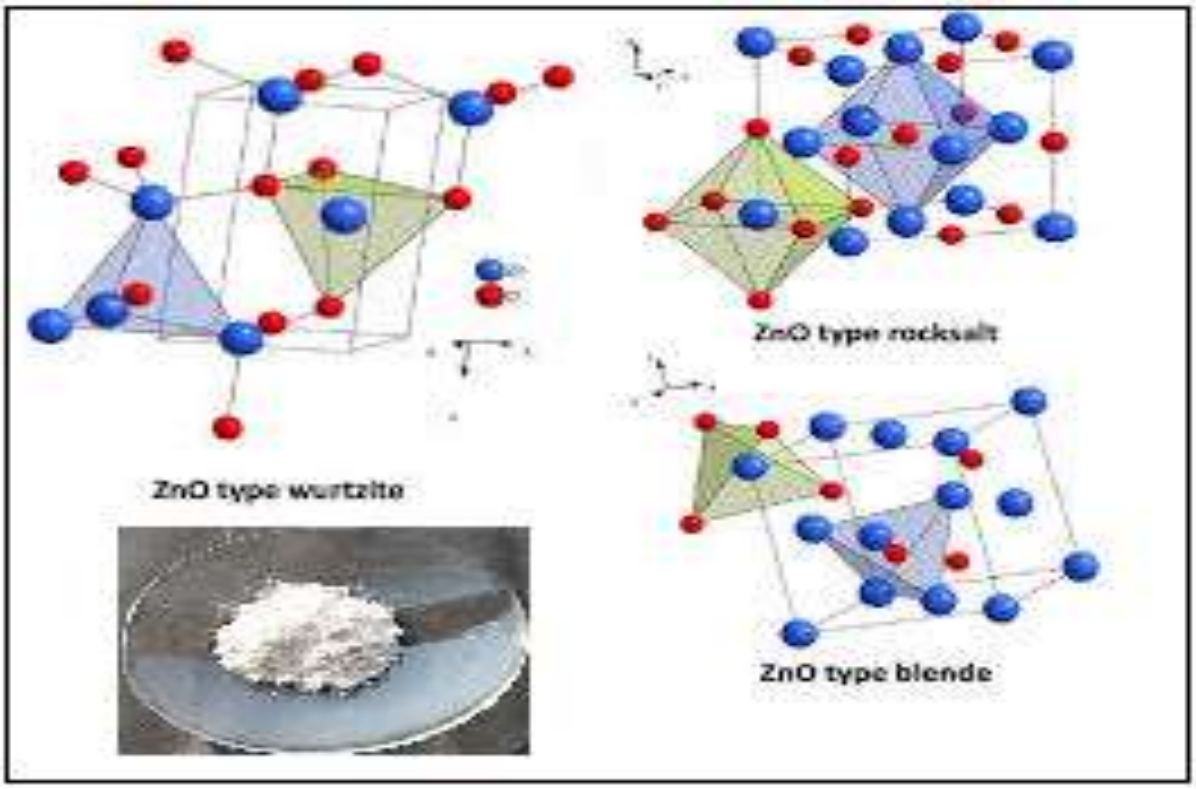

Fig .2 ZnO crystal structures: rocksalt, zinc blende and wurtzite

ZnO is a key technological material. The lack of a centre of symmetry in wurtzite, combined with large electromechanical coupling, results in strong piezoelectric and pyroelectric properties and the consequent use of ZnO in mechanical actuators and piezoelectric sensors. In addition, ZnO is a wide band-gap (3.37 eV) compound semiconductor that is suitable for short wavelength optoelectronic applications. The high exciton binding energy (60 meV) in ZnO crystal can ensure efficient excitonic emission at room temperature and room temperature ultraviolet (UV) luminescence has been reported in disordered nanoparticles and thin films. ZnO is transparent to visible light and can be made highly conductive by doping. ZnO is a versatile functional material that has a diverse group of growth morphologies, such as nanocombs, nanorings, nanohelixes/nanosprings,nanobelts, nanowires and nanocages. The objective of this article is to review the unique nanostructures that have been grown for ZnO and their corresponding growth mechanisms. The potential applications and novel nanodevices demonstrated for ZnO and SnO_2 nanostructures will be reviewed.[18]

1.4 Nanoparticals used in drug delivery antibactiral

In the recent past, the targeted drug delivery has gained more attention for various advantages. Amongst the plethora of Avenues explored for targeted drug delivery. Nanoparticles are particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site- specific action of the drug at the therapeutically optimal rate and dose regimen. Drugs with very low solubility possess various biopharmaceutical delivery issues including limited bio accessibility after intake through mouth, less diffusion capacity into the outer membrane, require more quantity for intravenous intake and unwanted after-effects preceding traditional formulated vaccination process. However all these limitations could be overcome by the application of nanotechnology approaches in the drug delivery mechanism[19].Controlled drug delivery systems (DDS) have several advantages compared to the traditional forms of drugs. A drug is transported to the place of action, hence, its influence on vital tissues and undesirable side effects can be minimized. Accumulation of therapeutic compounds in the target site increases and, consequently, the required doses of drugs are lower. This modern form of therapy is espe- cially important when there is a discrepancy between the dose or the concentration of a drug and its therapeutic results or toxic ef- fects. Cell-specific targeting can be accomplished by attaching drugs to specially designed carriers[20]

The way of conjugating the drug to the nanocarrier and the strategy of its targeting is highly important for a targeted therapy. A drug may be adsorbed or cova- lently attached to the nanocarriers surface or else it can be encapsulated into it. Covalent linking has the advantage over other ways of attaching as it enables to control the number of drug molecules connected to the nanocarrier, i.e., a precise control of the amount of therapeutic compound delivered. Cell-specific target- ing with nanocarriers may be accomplished by using active or passive mechanisms. The first strategy relies on the attraction of a drug – the nanocarriers conju- gate to the affected site by using recognition ligands, attached to the surface of conjugates antibodies, low molecular ligands, e.g., folic acids, peptides, etc. The active strategy can be also achieved through a ma- nipulation of physical stimuli (e.g., temperature, pH, magnetism). Passive targeting is a result of enhanced vascular permeability and retention (EPR) which is characteristic of leaky tissues of tumors .[21]

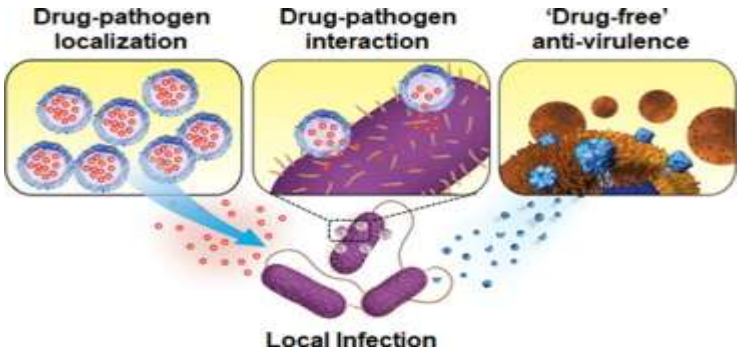

Fig . 3. Nanocarriers used for drug delivery in anti- bacteria .

Nanocarriers used for medical applications have to be biocompatible (able to integrate with a biological system without eliciting immune response or any negative effects) and nontoxic (harmless to a given

biological system). Undesirable effects of nanoparti- cles strongly depend on their hydrodynamic size, shape, amount, surface chemistry, the route of admini- stration, reaction of the immune system (especially a route of the uptake by macrophages and granulo- cytes) and residence time in the bloodstream. Due to a number of factors which may affect the toxicity of nanoparticles, their estimation is rather difficult and, thus, toxicological studies of each new DDS formula- tion are needed. However, with respect to their size, one can make some generalizations – smaller particles have a greater surface area, thus, they are more reactive and, in consequence, more toxic. It is generally accepted that nanoparticles with a hydrodynamic diameter of 10–100 nm have optimal pharmacokinetic properties for in vivo applications. Smaller nanoparticles are subjects to tissue extravasations and renal clearance whereas larger are quickly opsonized and removed from the bloodstream via the macrophages of the reticuloendothelial system [22].

1.5 Metronidazole Drug

Metronidazole is a member of the class of imidazoles substituted at C-1, -2 and -5 with 2-hydroxyethyl, nitro and methyl groups respectively. Metronidazole is a commonly used antibiotic, belonging to the nitroimidazole of antibiotics. It is frequently used to treat gastrointestinal infections as well as trichomoniasis and giardiasis, and amebiasis which are parasitic infections. Metronidazole has been used as an antibiotic for several decades, with added antiparasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections. It is available in capsule form, tablet form, and topical form, and suppository preparations for the treatment of various infections.[23]

1.6 ZnO/GO Composites

In recent years, many works have been reported about the combination of ZnO nanorods (NRs) with graphene oxide for developing various photonic and optoelectronic applications [24]. Improved ZnO NR/GO layers through a chemical bath deposition (CBD) method, for volatile organic compound detection [25]. Developed polysulfone (PSF)-nanohybrid (membranes using a ZnO–GO composite in order to obtain enhanced performance with an improved permeability rate [26]. Improved the catalytic activity of GO/ZnO nanorod films by UV irradiation [27] . Presented a cost-effective method for the preparation of a GO–ZnO nanocomposite for a UV detection application.Studied the performance of ZnO/GO hybrids as an anode for lithium ion batteries. Reported the synthesis of a Mg-doped ZnO:GO nanocomposite and its properties for acetic acid-sensing applications{28-29].

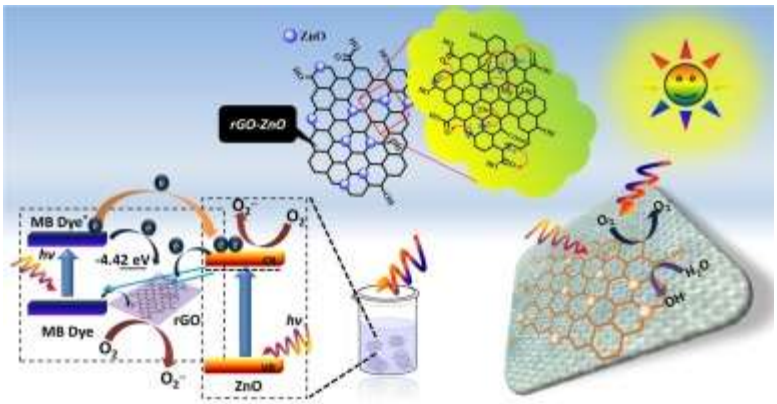

Fig. 4 Zinc oxide NPs / Graphine oxide NPs Composites

1.7 Drug delivery

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. For the treatment of human diseases, nasal and pulmonary routes of drug delivery are gaining increasing importance. These routes provide promising alternatives to parenteral drug delivery particularly for peptide and protein therapeutics. For this purpose, several drug delivery systems have been formulated and are being investigated for nasal and pulmonary delivery. These include liposomes, proliposomes, microspheres, gels, prodrugs, cyclodextrins,among others. Nanoparticles composed of biodegradable polymers show assurance in fulfilling the stringent requirements placed on these delivery systems, such as ability to be transferred into an aerosol, stability against forces generated during aerosolization, biocompatibility, targeting of specific sites or cell populations in the lung, release of the drug in a predetermined manner, and degradation within an acceptable period of time[4,21].

Drug delivery systems can in principle provide enhanced efficacy and/or reduced toxicity for anticancer agents. Long circulating macromolecular carriers such as liposomes can exploit the enhanced permeability and retention' effect for preferential extravasation from tumor vessels[7].

Liposomal anthracyclines have achieved highly efficient drug encapsulation, resultingin significant anticancer activity with reduced cardiotoxicity and include versions with greatly prolonged circulation such as liposomal daunorubicin and pegylated liposomal doxorubicin.Pegylated liposomal doxorubucin has shown substantial efficacy in breast cancer treatment both as monotherapy and in combination with other chemotherapeutics. Additional liposome constructs are being developed for the delivery of other drugs. The next generation of delivery systems will include true molecular targeting; immunoliposomes and other ligand-directed constructs represent an integration of biological components capable of tumor recognition with delivery technologise.[30]

1.8 Anti bacteral activity

1.8.1 Anti bacteral activity of zinc oxide

Highly reactive oxygen species (ROS) are released from the surface of zinc oxide nanoparticles (ZnO NPs) which cause damage to the microorganisms. ROS may break down the cell wall and cell membrane of the bacteria leading to the leakage of cell contents causing their death. Antibacterial effect of ZnO NPs synthesized from plants' extracts has been seen against several grampositive (Bacillus coagulans, Staphylococcus aureus, Bacillus subtilis) and gramnegative bacteria (Shigella dysenteriae, Sphingomonas paucimobilis, Salmonella typhimurium, Escherichia coli, Bacillus pumilus, Salmonella typhi). Zinc ions (Zn^{2+}) freed from ZnO NPs may attack DNA and proteins of the cell, thus hindering the growth of the bacteria. The negative charge on the surface of some bacteria (E. coli, S. typhi) interacts with positively charged particles in ZnO NPs inhibiting the growth of these bacteria.

The rate of antibiotic activity of ZnO NPs depends on size, the concentration of ZnO NPs, and the type of surfactant used in the synthesis of these ZnO NPs. Due to their antibiotic property, ZnO NPs can be used as antibacterial coating of inner surfaces of refrigerators and dishwashers, plastic food containers, in the lining of food cans used for packaging and preservation of food to prevent their spoilage.[31]

1.8.2 Antibacterial activity of GO

GO showed strong antibacterial activity against the Gram-positive and Gram-negative MDR pathogens tested, by forming zone of inhibition in an agar well diffusion. The antibacterial activity of GO was compared with that of the commercial antibiotics GEN, AZM, IMP, COT, CFM, CIP, AMX and CTR . All the pathogens used in the study are sensitive to GO but they are totally resistant to CFM, COT, AMX and IPM. GEN, AZM, CTR and CIP produced a zone of inhibition in the range of 7–29 mm, whereas, GO generated a clear zone of inhibition in the range of 27–41 mm, indicating that GO is an attractive antibacterial agent. Upon direct contact with the bacterial outer surface, this may puncture the cell wall and the membrane. This may lead to lipid injuries , where large amounts of phospholipids may be extracted from the microbes by further van der Waals and hydrophobic bond formation with the charged groups of GO [32]. As a result, the pathogens may suffer from perturbation of membrane integrity which can disable many essential functions, such as respiration, materials transport, osmotic balance and energy transduction [33-36]If the surface of the bacterial cell is surrounded by sufficient GO sheets, the cell becomes biologically inactive and finally dies, leading to the formation of a clear zone of inhibition in the culture plate. Secondly, cell entrapment might be another potential mechanism for the antibacterial activity of GO. The bacterial cells may be trapped by GO sheets upon their contact. The trapped bacteria might be detached from the external microenvironment and their access to nutrients might be restricted causing growth inhibition. Herein, the size of GO may have a significant influence on cell entrapment, with sheets of large lateral size showing better inhibition [37]

1.8.3 Antibacterial Activity of Zinc Oxide/Graphene Oxide Composites

materials with good antibacterial activity and less toxicity to other species attract numerous research interest. Taking advantage of zinc oxide (ZnO) and graphene oxide (GO),Thesynergistic effects of GO and ZnO NPs led to the superior antibacterial activity of the composites. GO helped the dispersion of ZnO NPs, slowed the dissolution of ZnO, acted as the storage site for the dissolved zinc ions, and enabled the intimate contact of E. coli with ZnO NPs and zinc ions as well. The close contact enhanced the local zinc concentration pitting on the bacterial membrane and the permeability of the bacterial membrane and thus induced bacterial death. In addition, the ZnO/GO composites were found to be much less toxic to the cells, compared to the equivalent concentration of ZnO NPs in the composites. The ZnO/GO composites are promising disinfection materials to be used in surface coatings on various substrates to effectively inhibit bacterial growth, propagation, and survival in medical devices.[38]

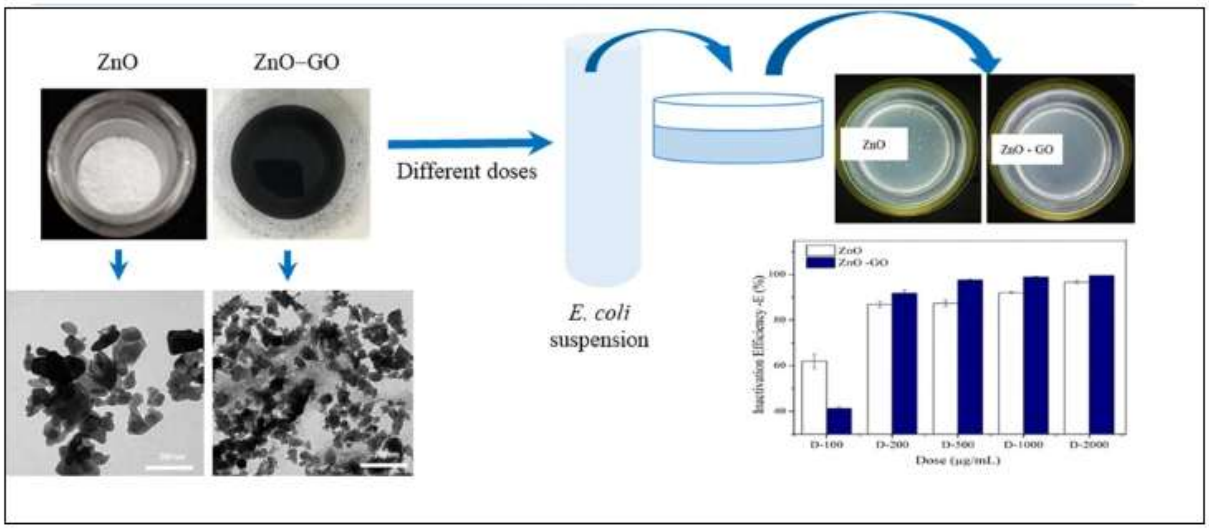

Fig 5. Antibacterial Activity of ZiO /GO Composites

Chapter two

Method

2.1 Synthesis of ZnO nanoparticles

The direct thermal precipitation technique has been used in preparing zinc oxide NPs. Using deionized water, the preparation of KOH and zinc nitrate (0.4M and 0.2M, respectively) took place at room temperature, marginally adding the aqueous solution to the zinc nitrate while constantly stirring, followed by controlling the temperature at 60 oC for 120 min, forming a white precipitation. The resulting mixture is centrifuged at 500 rmp for 20 minutes, followed by a triple wash in deionized water and absolute alcohol. Kept at 300, 500oC for 2 hours, the formation of zinc oxide is facilitated using a custom prepared tubular muffle furnace with no calcination.[39]

2.2 Synthesis ZnO/GO Composites

Prepare the ZnO/GO composite, approximately 10 mg GO was dispersed into 150 mL of distilled water and sonicated for 10 min, Zinc Oxide powder was added to the GO suspension with continuous stirring. After that, it was sonicated for 60 min. , the suspension containing GO and ZnO particles. After that, the composite was processed under stirring for 12 h. The composites were prepared using ratios of ZnO and GO. This mass ratios was (25:1) [40].

2.3 loading metronidazole on ZnO/GO NPs

The loaded ZnO nanoparticles with metronidazole . To prepare the ZnO/ GO/ metronidazole , approximately 7.5 mg of metronidazole were dissolved in 10 mL of a mixture of metronidazole and 20 mg ZnO and sonicated for 4 h. The solutions were stirred for 24 h at room temperature [39].

The Antibacterial activity

To investigate the antibacterial efficacy of gram-positive bacteria ***Staphylococcus aureus*** and gram-negative bacteria ***E.coli***, “the agar well diffusion” technique was selected, and cultured in a media of Mueller- Hinton agar. After 24 hours, one hundred microliters of old mature cultured media were swabbed using the L-shaped rod on the medium. The wells were made with a sterile cork tool (6 mm). “Zone of inhibition” (ZOI) was calculated in millimeters. In each Petri dish, three wells were prepared and fifty µl of each was added individually [39].

Chapter three
Results and Discussion

3.2 X-ray diffraction pattern (XRD) :

Structural Analysis. In order to gain the crystal structure information of ZnO and ZnO-GO as well as the influences from different preparation conditions, the XRD measurements of the catalysts were conducted, as shown in Figure 10. The characteristic peaks at 2θ = 31:7° , 34.4° , 36.2° , 47.5° , 56.7° , 63.0° , 66.4° , 68.1° , and 69.3° were observed from the XRD patterns of ZnO and ZnO-GO nanocomposites, corresponding to the planes (100), (002), (101), (102), (110), (103), (200), (112), and (201), respectively (Figure 10), indicating the existence of ZnO . No existence of other phases or impurity was found, indicating the high purity of catalysts[41-42]. It is notable that the characteristic diffraction peak of GO at 2θ = 24:6° was not found in the XRD pattern of ZnO-GO, which was probably because ZnO crystals were covered by limited amounts of GO that changed its structure[43]. According to related literatures[44], the presence of grain boundaries in ZnO and ZnO-GO nanocomposition can be proved owing to the existence of amorphous superficial and intergranular layers betwee

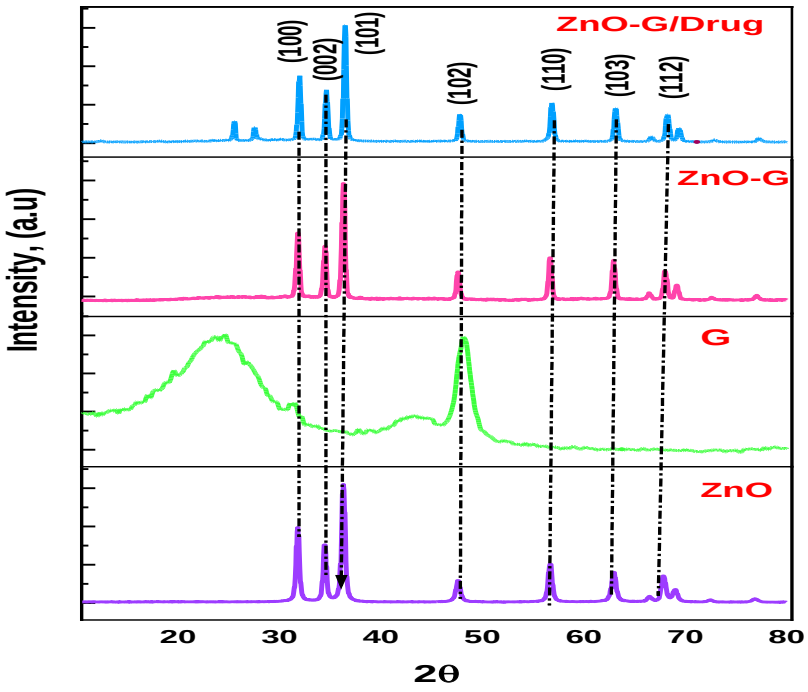

Fig 6: X-ray diffraction (XRD) spectra of ZnO ,GO, ZnO-GO, and loaded ZnO-GO/ metronidazole

3.1 Morphological Analysis SEM

The SEM was used to study the morphologies of the as-prepared GO, ZnO, and ZnO/GO nanomaterials, Figure 8(A) displays the SEM images at a higher magnification, and demonstrates the formation of particles with a size of rang (35-70)nm by Image J software. It also provided a clearer idea about the particle separation, as the particles are seen to be separated smoothly, without being highly affected by agglomeration. The graphene sheets are not perfectly flat but intrinsically microscopic roughening and out-of-plane deformations (wrinkles). Besides, some dispersed GO sheets could connect randomly to each other that brought about a porous structure with numerous cavities or holes, which provides additional possibilities to form ZnO-GO nanohybrid between precursor and GO. Figure 8(B) clearly shows that ZnO nanoparticles appear as granule-like nanostructures and the powder aggregation at different levels. It can be seen from Figure 8(C) that the surface of GO is covered rigorously by ZnO crystals, demonstrating a good combination between GO sheet and ZnO nanoparticles. The ZnO-GO catalyst shows a cross-linked flaky structure and excellent dispersibility without agglomeration, and the structure presents an average thickness of about rang (20-40) nm. It can be observed obviously that the ZnO-GO nanocomposites collapsed into a smaller average sizes. It can be seen that the calcined samples have better dispersion.

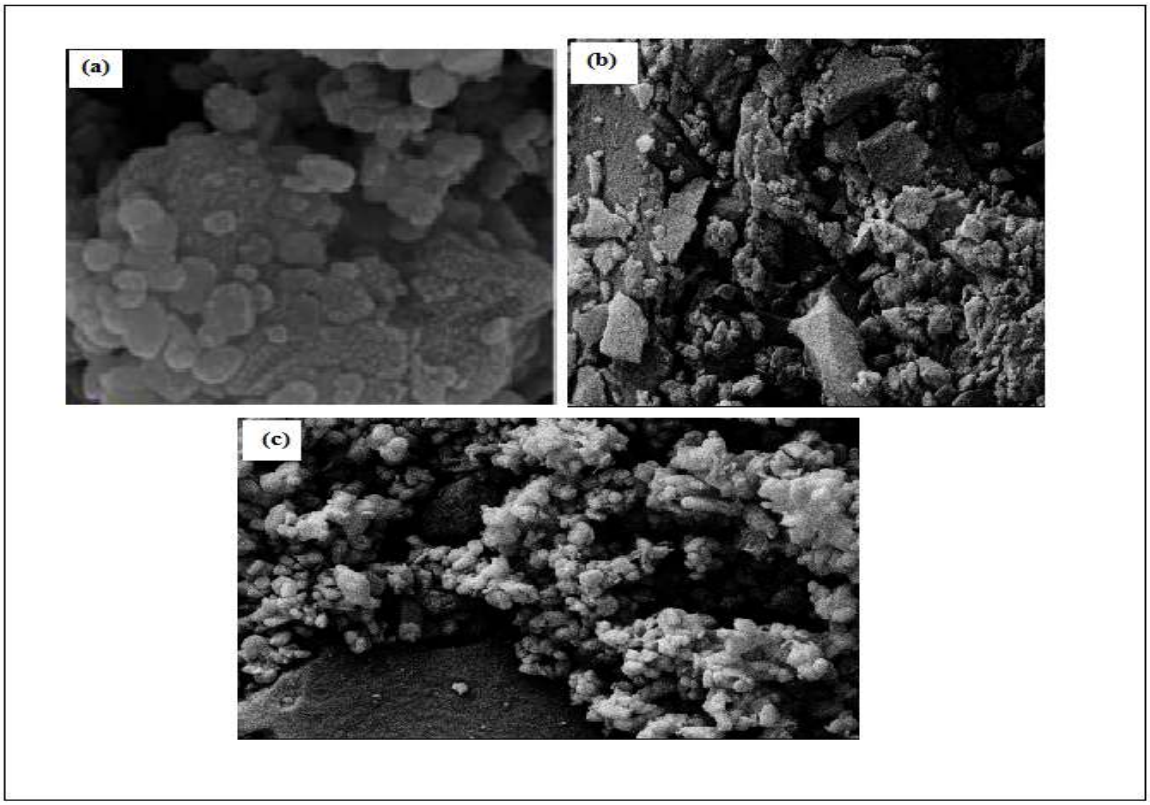

Fig 7: SEM images of (A) GO, (B) ZnO, (C) ZnO-GO nanocomposite

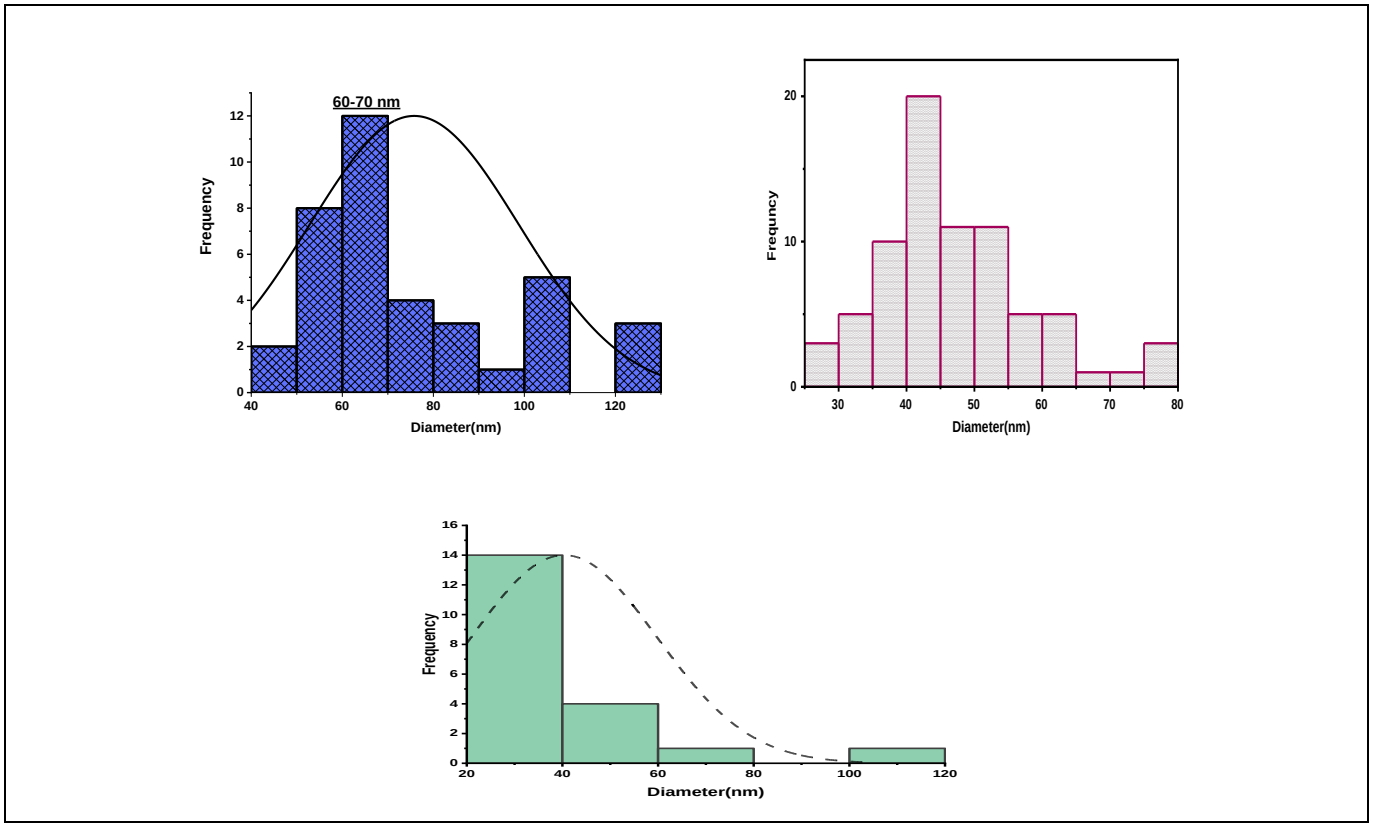

Fig 8: Histogram show the distribution of (a) GO, (b) ZnO, (c) ZnO-GO nanocomposite

Fig. 9 exhibits the SEM imaging of the metronidazole- ZnO-GO. The surface shape of metronidazole has altered after being loaded with ZnO-GO. As we can see in Fig. 9B, the NPs manifested as granules with a light color which are evenly spread over the surface of metronidazole. In Fig. 9B, we can see the SEM micrograph of the ZnO-GO after reaction with metronidazole. The morphology of these ZnO-GO was altered completely, and the greatest available holes and pores on the surface of ZnO-GO were occupied by metronidazole particles. When comparing Fig. 9A with Fig. 9B, we can see the significant change in the morphology of metronidazole-ZnO-GO .

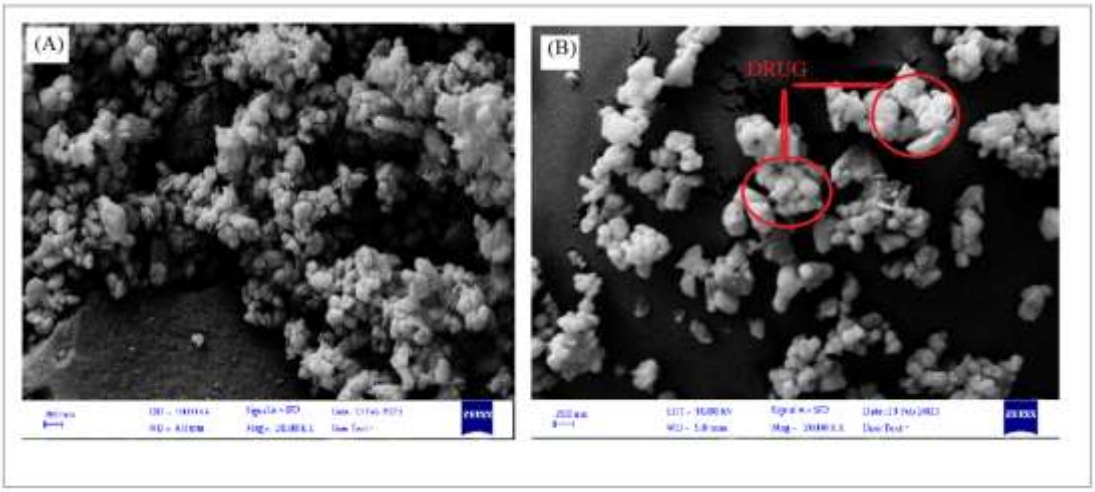

Fig 9: SEM images of(A) ZnO-GO nanocomposite, and (B) loaded ZnO-GO/ metronidazole

3.3 Antibacterial activity of ZnO/GO NPs

Antibacterial activity of ZnO/graphene NPs was tested against two bacterial strains at one concentration with metronidazole as a standard. The highest antibacterial activity of ZnO/graphene NPs with drug was noted against ***S. aureus*** at 5 mm. While the other bacteria ***E.coli*** was not affected.(figure 5).

Zinc oxide (ZnO) has an innate advantage of broad antibacterial activities against bacteria fungus and virus. Food and Drug Administration of the United States had recognized zinc oxide as a safe material. Release of zinc ions from ZnO was suggested as one of the primary antibacterial mechanisms, moreover the penetration of a bacterial membrane upon contact with ZnO particles also contributed to the antibacterial ability of ZnO NPs[11].

The inhibition zone was observed to assess the potent bactericidal activity of nanocomposite against the two tested microorganisms. It seems that the graphene sheet facilitates the deposition of ZnO nanoparticle on the bacterial cell wall, leading to the internalization of zinc oxide nanoparticles that induce ROS production. The ROS generated by internalized ZnO-NPs starts damaging the DNA and other cellular machinery components of the bacteria.

Several mechanisms have been proposed for bactericidal activities of ZnO NPs, including the direct contact with cell membrane, release of metallic ions, generation of ROS, and internalization of ZnO NPs. For direct contact killing mechanism, ZnO NPs tend to disrupt the cell membrane function, and interfere electron transport chain upon attachment on the cell wall, leading to the the ROS production .

In particular, ZnO NPs with very small sizes (ca $\leq$ 10 nm) can easily penetrate into cytoplasm, inducing the DNA damage and apoptosis. Generally, Gram-positive and Gram-negative bacteria have different sensitivity towards ZnO NPs due to the difference in their cell wall structures. Gram-positive bacteria have thick layers of peptidoglycan (20–80 nm), which are anchored to underlying cytoplasmic membrane via lipoteichoic acid (LTA). In addition, peptidoglycan is relatively porous, and does not form a permeability barrier for small substrates. Together with teichoic acid and LTA containing phosphate groups, the cell wall of Gram-positive bacteria is a highly charged anionic polymer, thus favoring electrostatic attraction of positively charged nanoparticles. In this respect, there is a greater affinity of ZnO to Gram-positive bacteria than Gram-negatives. On the contrary, peptidoglycan of Gram-negatives is thinner (<10 nm), and surrounded by an outer membrane containing lipopolysaccharides (LPS). LPS is a complex macromolecule, being impermeable to hydrophobic antibiotics. So outer membrane blocks the entry of numerous toxic compounds and prevents hydrophobic antibiotics from entering the organisms. Therefore, Gram-negative bacteria with a thinner peptidoglycan layer and an outer membrane are more resistant to ZnO NPs than Gram-positive [45].

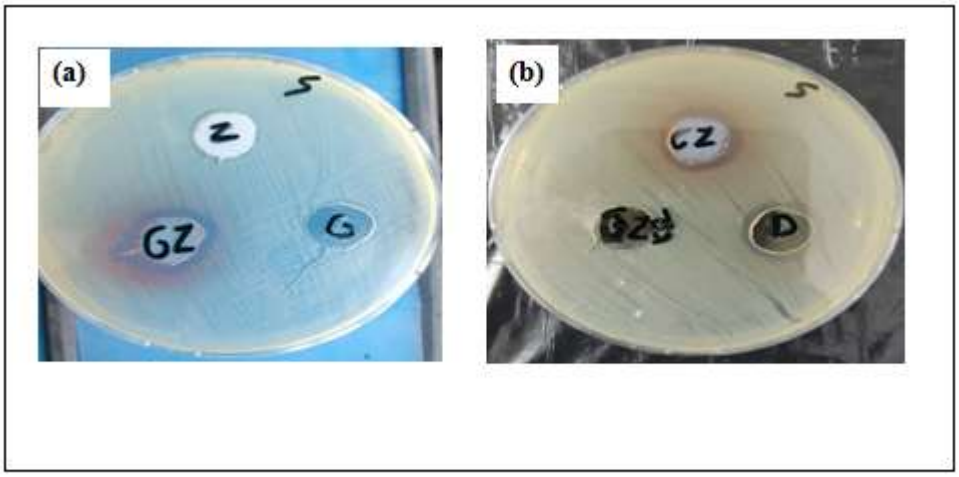

Fig 10: Antibacterial activity of (a) ZnO, GO, ZnO/GO and (b) ZnO/GO, drug, ZnO/GO- drug on Bacterial isolates by Agar Diffusion Method

Conclusion

ZnO-graphene nanostructu with positive surface charge are able to adhere and attach on negatively charged membrane via electrostatic interaction when they come in contact with bacteria. This effect disrupts bacterial cell membrane function, interferes electron transport chain, and deactivates bacterial enzyme, leading to final cell death. Apart from contact killing effect, other mechanisms such as the ROS production and released zinc ions have also been reported to be responsible for bactericidal activity of ZnO nanomaterials. The antimicrobial activity of ZnO nanostructures is size-, shape-, and concentration- dependent.

Reference

References:

1- McNamara, Karrina, and Syed AM Tofail. "Nanoparticles in biomedical applications." Advances in Physics: X 2.1 (2017): 54-88.

2- Saadeh, Yamaan, and Dinesh Vyas. "Nanorobotic applications in medicine: current proposals and designs." American journal of robotic surgery 1.1 (2014): 4-11..

3- Oliveira Jr, Osvaldo N., et al. "Nanomaterials for diagnosis: challenges and applications in smart devices based on molecular recognition." ACS applied materials & interfaces 6.17 (2014): 14745-14766.

4- De Jong, Wim H., and Paul JA Borm. "Drug delivery and nanoparticles: applications and hazards." International journal of nanomedicine 3.2 (2008): 133-149.

5-Holzinger M, Le Goff A, Cosnier S. Nanomaterials for biosensing applications: a review. Front Chem. 2014;2:63. doi: 10.3389/fchem.2014.00063

6- Golovin, Yuri I., et al. "Towards nanomedicines of the future: Remote magneto-mechanical actuation of nanomedicines by alternating magnetic fields." Journal of Controlled Release 219 (2015): 43-60.

7- Lu, Haijiao, et al. "Recent progress on nanostructures for drug delivery applications." Journal of Nanomaterials 2016 (2016).

8- Zhang, Yu, et al. "Recent advances in poly (α-L-glutamic acid)-based nanomaterials for drug delivery." Biomolecules 12.5 (2022): 636.

9- Finbloom, Joel A., et al. "Engineering the drug carrier biointerface to overcome biological barriers to drug delivery." Advanced Drug Delivery Reviews 167 (2020): 89-108.

10- Taffe, Michael A., and Nicholas W. Gilpin. "Racial inequity in grant funding from the US National Institutes of Health." Elife 10 (2021): e65697.

11- Zhang, Xin, et al. "Preparation of polycrystalline ZnO nanoparticles loaded onto graphene oxide and their antibacterial properties." Materials Today Communications 28 (2021): 102531.

12- Hou, Wenjia, et al. "Application of fullerenes as photosensitizers for antimicrobial photodynamic inactivation: a review." Frontiers in Microbiology 13 (2022): 957698.

13- Matuyama, E. Pyrolysis of graphitic acid. J. Phys. Chem. 58(1954) 215–219.

14- Hiew, B.Y.Z.; Lee, L.Y.; Lee, X.J.; Gopakumar, S.T.; Gan, S.; Lim, S.S.; Pan, G.T.; Yang, T.C.K.; Chiu, W.S.; Khiew, P.S. Review on synthesis of 3D graphene-based configurations and theiradsorption performance for hazardous water pollutants. Process. Saf Environ. Prot. 116(2018)262–286.

15-. Marcano, D.C.; Kosynkin, D.V.; Berlin, J.M.; Sinitskii, A.; Sun, Z.; Slesarev, A.; Alemany, L.B.; Lu, W.; Tour, J.M. Improve synthesis of graphene oxide. ACS Nano 2010, 4, 4806–4814.

16- Zhang, C.; Lv, W.; Xie, X.; Tang, D.; Liu, C.; Yang, Q.-H. Towards low temperature thermal exfoliation of graphite oxide for graphene production. Carbon 62(2013) 11–24.

17- Baig, Abdul Mannan. "Updates on what ACS reported: emerging evidences of COVID-19 with nervous system involvement." ACS chemical neuroscience 11.9 (2020): 1204-1205.

18- Tinacba, Erin Joy C., et al. "ZnO/Zn and ZnO film deposited via microwave atmospheric plasma jet as photo-catalyst for rhodamine 6G dye degradation." Thin Solid Films 624 (2017): 197-200.

19- .Kannadasan, M., et al. "A review: nano particle drug delivery system." Journal of Pharmaceutical Sciences and Medicine (IJPSM) 5.12 (2020).

20- Wilczewska, Agnieszka Z., et al. "Nanoparticles as drug delivery systems." Pharmacological reports 64.5 (2012): 1020-1037.

21-Nevozhay D, Kañska U, Budzyñska R, Boratyñski J: Current status of research on conjugates and related drug delivery systems in the treatment of cancer and other dis- eases (Polish). Postepy Hig Med Dosw, 61(2007)350–360.

22- Cole AJ, Yang VC, David AE: Cancer theranostics: the rise of targeted magnetic nanoparticles. Trends Biotech- nol, 29(2011)323–332

23- Sayers, Eric W., et al. "Database resources of the national center for biotechnology information." Nucleic acids research 49.D1 (2021): D10.

24-Vessalli, B.A.; Zito, C.A.; Perfecto, T.M.; Volanti, D.P.; Mazon, T. ZnO nanorods/graphene oxide sheets prepared by chemical bath deposition for volatile organic compounds detection. J. Alloy. Compd. (2017)996-1003, 696.

25- .Chung, Y.T.; Mahmoudi, E.; Mohammad, A.W.; Benamor, A.; Johnson, D.; Hilal, N. Development of polysulfone-nanohybrid membranes using ZnO-GO composite for enhanced antifouling and antibacterial control. Desalination (2017)402, 123–132.

26- .Rokhsat, E.; Akhavan, O. Improving the photocatalytic activity of graphene oxide/ZnO nanorod films by UV irradiation. Appl. Surf. Sci. 371(2016) 590–595.

27.- Alamdari, S.; Ghamsari, M.S.; Afarideh, H.; Mohammadi, A.; Geranmayeh, S.; Tafreshi, M.J.; Ehsani, M.H.Preparation and characterization of GO-ZnO nanocomposite for UV detection application. Opt. Mater. (2019)250–243 ،92

28- Qi, Y.; Zhang, C.; Liu, S.; Zong, Y.; Men, Y. Room-temperature synthesis of ZnO@GO nanocomposites as anode for lithium-ion batteries. J. Mater. Res. 2018, 33, 1506–1514.

29-Khorramshahi, V.; Karamdel, J.; Yousefi, R. High acetic acid sensing performance of Mg-doped ZnO/rGO nanocomposites. Ceram. Int. 45(2019) 7034–7043.

30- Setia, Aseem, et al. "Current advances in nanotheranostics for molecular imaging and therapy of cardiovascular disorders." Molecular Pharmaceutics 20.10 (2023): 4922-4941.

31- Thanuja, P., G. L. Meena, and Nikita Khoisnam. "Distribution and tenancy relationship of operational land holding in different Agro-climatic Regions of Rajasthan."

32- Tu, Yusong, et al. "Destructive extraction of phospholipids from Escherichia coli membranes by graphene nanosheets." Nature nanotechnology 8.8 (2013): 594-601.

33- Akhavan, O., and E. Ghaderi. "Escherichia coli bacteria reduce graphene oxide to bactericidal graphene in a self-limiting manner." Carbon 50.5 (2012): 1853-1860.

34- Pham, Vy TH, et al. "Graphene induces formation of pores that kill spherical and rod-shaped bacteria." ACS nano 9.8 (2015): 8458-8467.

35- Chong, Yu, et al. "Light-enhanced antibacterial activity of graphene oxide, mainly via accelerated electron transfer." Environmental Science & Technology 51.17 (2017): 10154-10161.

36- Li, Ruibin, et al. "Identification and optimization of carbon radicals on hydrated graphene oxide for ubiquitous antibacterial coatings." ACS nano 10.12 (2016): 10966-10980.

37- Liu, Shaobin, et al. "Lateral dimension-dependent antibacterial activity of graphene oxide sheets." Langmuir 28.33 (2012): 12364-12372.

38- Wang, Yan-Wen, et al. "Superior antibacterial activity of zinc oxide/graphene oxide composites originating from high zinc concentration localized around bacteria." ACS applied materials & interfaces 6.4 (2014): 2791-2798.

39- Habeeb, Saba Abdulmunem, et al. " Green synthesis of metronidazole or clindamycin-loaded hexagonal zinc oxide nanoparticles from Ziziphus extracts and its antibacterial activity." *Pharmacia* 69.3 (2022): 855-864.

40-Lin, Yi, et al. "Green synthesis of ZnO-GO composites for the photocatalytic degradation of methylene blue." *Journal of Nanomaterials* 2020 (2020): 1-11.

41- El Hamidi, A., et al. "Refractive index controlled by film morphology and free carrier density in undoped ZnO through sol-pH variation." Optik 158 (2018): 1139-1146.

42- Hernández-Carrillo, M. A., et al. "Eu-modified ZnO nanoparticles for applications in photocatalysis." Catalysis Today 349 (2020): 191-197.

43- Wu, Yunyan, et al. "Light-induced ZnO/Ag/rGO bactericidal photocatalyst with synergistic effect of sustained release of silver ions and enhanced reactive oxygen species." Chinese Journal of Catalysis 40.5 (2019): 691-702.

44- Straumal, Boris, et al. "Influence of texture on the ferromagnetic properties of nanograined ZnO films." physica status solidi (b) 248.7 (2011): 1581-1586.

45- Li, Yuchao, Chengzhu Liao, and Sie Chin Tjong. "Recent advances in zinc oxide nanostructures with antimicrobial activities." International Journal of Molecular Sciences 21.22 (2020): 8836.