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Antimicrobial Activity of Locally Synthesized Carbon Nanosphere on Some Pathogenic Species of Bacteria and Parasites

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Abstract:

Antibacterial activity of CNSs against *Staphylococcus aureus* and *Escherichia coli* was estimated. Higher inhibition zone of 18 mm and 20 mm were observed against *S. aureus* and *E.coli*, respectively, at a concentration of 2 mg/ml of carbon nanosphere after 24 hrs of incubation at 37 ºC. *In vitro* cytotoxicity experiment was performed on two parasite strains of *Leishmania donovani* and *Leishmania tropica* by using MTT assay. *L. donovani* revealed more sensitiv to the CNSs than *L. tropica*. An intermediate level of cytotoxicity of 51.31 % was observed when 2.4 mg/ml of CNSs was incubated with *L. donovani*, while weak cytotoxicity of 37.20 % was shown when the same concentration of CNSs was used against *L. tropica* within 24 hr at 37 ºC.

Key words: Antimicrobial Activity**,** Bacteria, Carbon Nanospheres, Cytotoxicity, Parasites.

Introduction:

Nanomaterials have been synthesized with their excellent applications. Carbon nanomaterials have unique chemical and physical properties that make them promising as nano-medicines and therapeutic agents (1,2). Spherical carbon nanomaterials have properties that can be exploited in the diagnosis and treatment of cancer and infectious agents. The increasing resistance of the microorganisms (i.e. Bacteria and parasites) towards antimicrobial agents has led to serious health problems and increase the investigations to find new agents which can effectively prevent severe morbidity and mortality (3,4). The study of the antibacterial properties of carbon nanomaterials against Gram negative or positive bacteria (i.e. *E. coli* and *S. aureus*) provides fundamental information on the possible toxicity and environmental impact of these materials (5). Moreover, visceral leishmaniasis (VL) and cutaneous leishmanaisis (CL), caused by *Leishmania donovani* and *Leishmania tropica*, are the most important neglected parasite diseases. The treatment of leishmaniasis by pentavalent antimonials has a broad limitations concerning their therapeutic safety and high toxicity. A wide research for novel drugs used to treat leishmaniasis is absolutely important and demand (6,7,8,9).

On the other hand, cancer disease is the main reason of deaths in the world, it caused more than 8.2 million deaths worldwide (10). Organic or inorganic nanoparticles (NPs) can improve the pharmacological properties against cancer. NPs should be up to 100 nm to cross the particular vascular structures and reach the tumor tissue. Moreover, small NPs can be accumulated inside the tumors by enhancing permeability and retention effect (EPR). The size and surface area of NPs affected their activity against pathogens or improving their activity for interaction with target cells (11,12).

The aim of the present work, is to study the effect of the previously synthesized and characterized carbon nanosphere (CNSs) on the antimicrobial activity. The antibacterial activity of CNSs against Gram positive and Gram negative bacteria was determined. Moreover, *In vitro* cytotoxicity of prepared nanoparticles was estimated against two parasite strains, *Leishmania*

donovani and *Leishmania tropica*, using MTT assay.

Materials and Methods

Synthesis of Carbon Nanospheres (CNSs)

The carbon nanospheres used in the present work was previously synthesized without catalyst via chemical vapor deposition technique (CVD) (13). Briefly, a single tube furnace was used for the pyrolysis of acetylene gas as a hydrocarbon source under nitrogen gas environment. Nitrogen and acetylene gases with flow rates of 93 sccm and 7sccm respectively were introduced through the quartz tube from one side while the outlet gas was exhausted through water bubble on the other side of the tube. The hydrocarbon gas was introduced carefully into the preheated furnace at a temperature of 650ºC at the center. The pyrolysis was employed at 650ºC for 60 minutes.

Antibacterial Activity of CNSs

A fifty microliter of each examined sample of CNSs of 0.25, 0.5,1 and 2 mg/mL concentrations was spotted, separately, on Muller Hinton agar well, previously seeded with the *E. coli* or *S. aureus,* purchased from Biotechnology division / Applied Sciences / University of Technology. 0.5 MacFarland tube contains 10^8 cells/ml. Then, the plates were incubated for 24 hrs at 37 ºC. The antimicrobial activity was determined by measuring the growth inhibition zones diameters in mm (14).

Antiparasites Activity of CNSs

Cell cytotoxicity was determined by colorimetric assay using 3-[4, 5 – dimethylthiazoyl]-2, 5- diphenyltetrazolium bromide (MTT dye). Two parasites strains of *L. tropica* and *L. donovani*, purchased from Biotechnology Research Center / Al Nahrain university, were used in this work. In brief, cell suspension (100 µl) was added onto culture plate wells and the volume was completed to 200 µl by adding sample and DMSO to get final concentration of 0.15, 0.3, 0.6. 1.2 and 2.4 mg/ml of CNSs, then incubated for 24 hrs, at 37 ºC with 5% CO_2, and dead cells were removed centrifugally. MTT dye was added (50 µL/well) and incubated for 4 hrs. Then 50µL of DMSO were added to each well. The experiment is carried in triplicate, and the absorbance of the color obtained from living cells were read at wavelength of 620 nm by ELISA reader. Thereafter, the absorbance mean of each group was calculated, and cells viability or cytotoxicity percentages were obtained as follows:

Cell viability (%): the absorbance mean of treated sample/ Mean Absorbance of non-treated sample multiplied by 100) , while cell cytotoxicity (%): equals to 100 - cell viability. Non-treated cultures, only cells in the medium, represent the experimental control (15,16).

Results and Discussion:

Antibacterial Activity

Antibacterial activity of prepared CNSs against Gram positive and negative bacteria was gauged in Muller Hinton agar medium. Higher antibacterial activity revealed when 2 mg/ml of CNSs was examined against both bacteria. The inhibition zone of 18 mm and 20 mm were observed against *S. aureus* and *E. coli,* respectively, after 24 hrs of incubation at 37 ºC (Tab.1). The growth inhibition zone is clearly demonstrated the potential activity of CNSs against both tested bacteria as shown in Fig.1a, 1b.

Table 1. Determination of bacterial growth inhibition zone caused by different CNSs concentrations.

Concentration (mg/ml)	Zone of inhibition (mm)	
	E. coli (Mean ± SD)	*S. aureus* (Mean ± SD)
0.25	14.70 ± 0.02	13.40 ± 0.03
0.50	18.00 ± 0.07	16.00 ± 0.05
1	18.80 ± 0.05	17.50 ± 0.06
2	20.00 ± 0.04	18.00 ± 0.03
Control	0	0

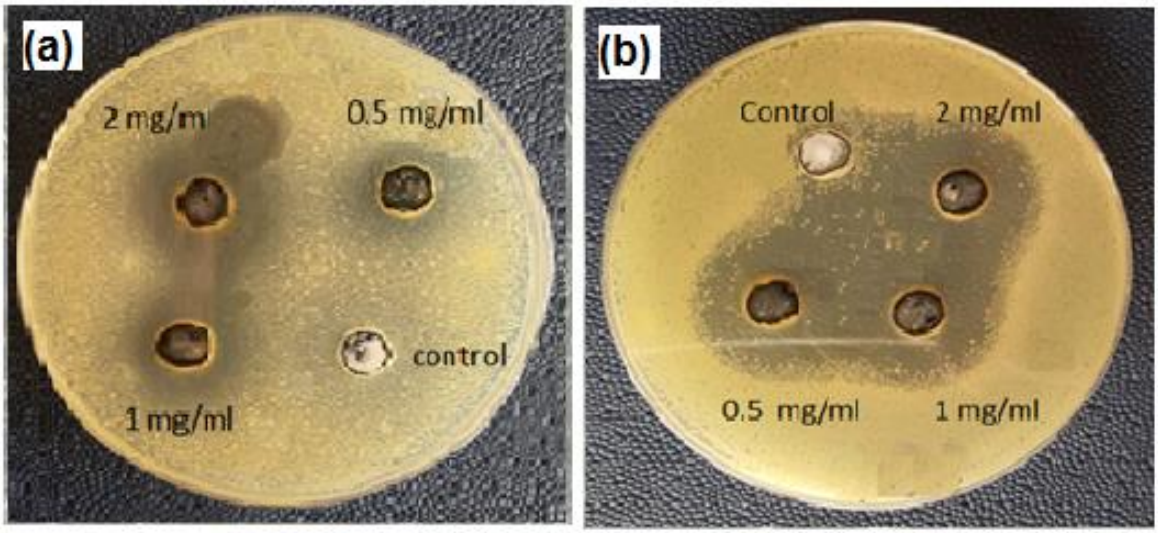

Figure 1. Antibacterial activity of CNSs against (a) *S. aureus* and (b) *E. coli*, after 24 hrs of incubation at 37 ºC.

It seems that the increment in CNSs concentration lead to increase in the inhibition growth of examined bacteria. The number of carbon-based nanomaterials have been found to possess powerful bactericidal properties toward different pathogens. The toxicity of CNSs is influenced by chemical and physical properties (17). Fullerenes structure can absorb light and subsequently generate reactive oxygen species (ROS) (18). The increasing in ROS production leads to an extensive damage in cellular components, such as lipids and proteins (19). On the other hand, the oxidative stress can be triggered by means of graphene without ROS generation, and interference with a specific bacterial process through oxidization and disruption of vital biological structures (20). Moreover, CNSs differed from other carbon type like carbon nanotubes and graphene by causing minimal damage to the cells because of their low cytotoxicity and no sharp edges. For this reason, CNSs was selected as a nanomaterial to study their activity against bacteria, parasites and tumor cells.

Antiparasites Activity

In order to evaluate the antiparasites activity of CNSs, *in vitro* cytotoxicity experiment was performed on two of parasite strains by using MTT assay. The cytotoxicity level was estimated by using an intensity scale for tested cell line as follows: weak cytotoxic activity (<50%), intermediate activity (51%–75%) and high activity (> 75%) (21).

Antiparasites activity of CNSs was determined against promastigotes of two species of *Leishmania* (Fig. 2 and 3). *L. donovani* revealed more sensitive to the CNSs than *L. tropica*. Intermediate level of cytotoxicity of 51.31 % was observed when 2.4 mg/ml of CNSs was incubated with *L. donovani*, while weak cytotoxicity of 37.20 % was shown when the same concentration of CNSs was used against *L. tropica* within 24 hrs at 37 ºC (Table 2).

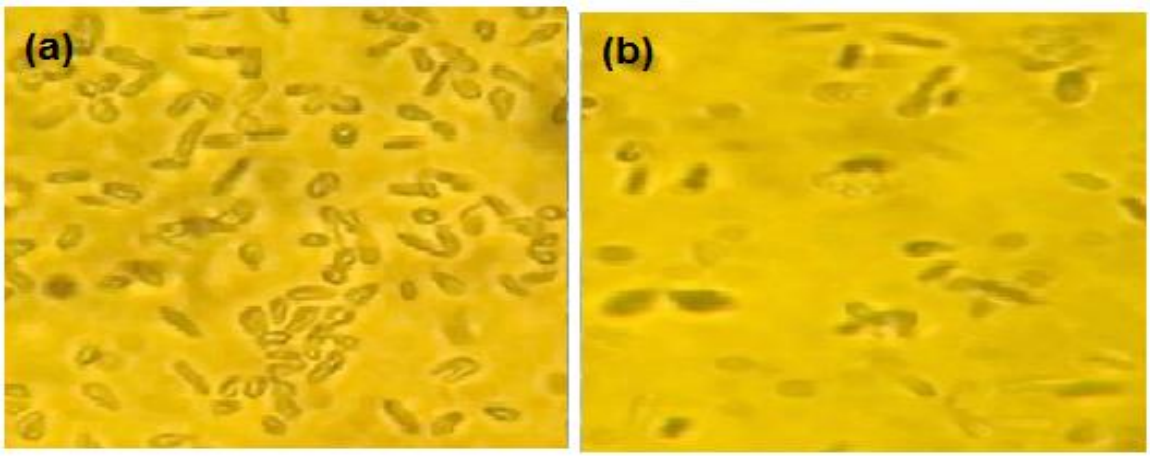

Figure 2. Promastigotes of *L. tropica* observed under inverted microscope at power 400 (a): control and (b) *L. tropica* treated with 2.4 mg/ml of CNSs.

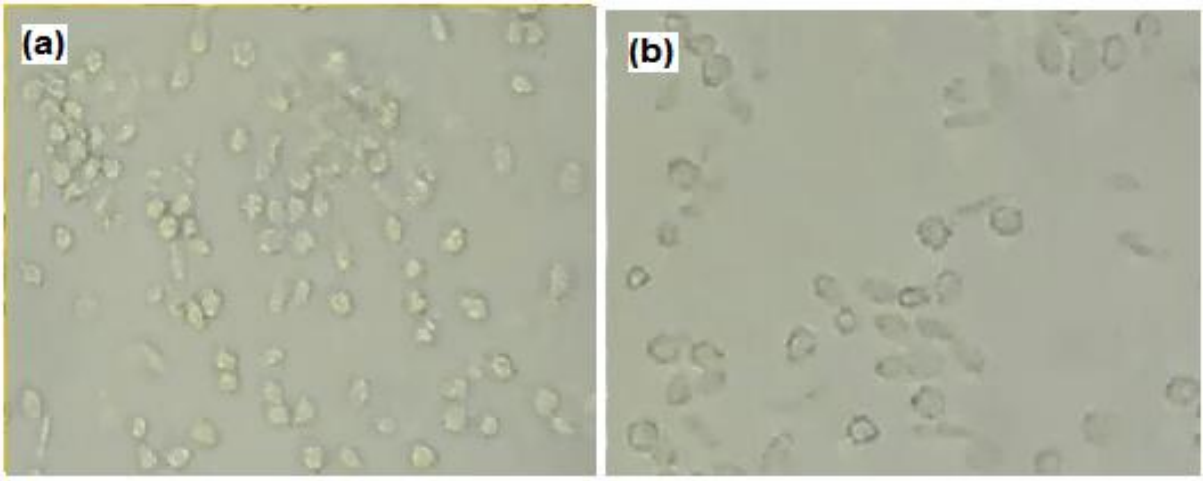

Figure 3. Promastigotes of *L. donovani* observed under inverted microscope at power 400; (a) control and (b) *L. donovani* treated with 2.4 mg/ml of CNSs.

Table 2. Antiparasites activity of CNSs against *L. tropica* and *L. donovani*, determined by MTT assay:

Concentrations (mg/ml)	Cytotoxicity (%) against *L. tropica*	Mean ± SD	*L. donovani*	Mean ± SD
2.4	38.20	0.202± 0.01	51.31	1.139 ± 0.02
1.2	37.10	0.233±0.02	47.80	1.194 ± 0.01
0.6	31.42	0.240±0.02	39.97	1.440 ± 0.01
0.3	27.91	0.259±0.00	37.00	1.290 ± 0.03
0.15	24.90	0.183± 0.03	34.65	1.280 ± 0.01
Control	0	0.281± 0.00	0	2.285 ± 0.00

The characteristics of CNSs make them suitable for various potential applications such as therapeutic applications. The effects of CNSs on parasite strains induced by their solubility, size and shape. The weak solubility of CNSs in water leads to aggregate and then prevents the penetration inside the cells. Moreover, the effects of CNSs may be decreased due to the reducing in the attachment area between nanoparticles and cell surface. Therefore, the CNSs in this study was suspended in DMSO and cells exposed to different doses of CNSs . Good distribution of CNSs and their solubility in DMSO make the fullerenes expose overall the cell surface and lead to increase in cytotoxicity.

On the other hand, the shape and size of prepared carbon spheres were characterized by SEM and TEM. The product revealed fluffy, spongy, black, and light weight carbon spheres (CSs) with regular shapes of 200-400 nm. The carbon structure was characterized by using SEM and TEM, as a nanospheres (Fig. 4a, 4b).

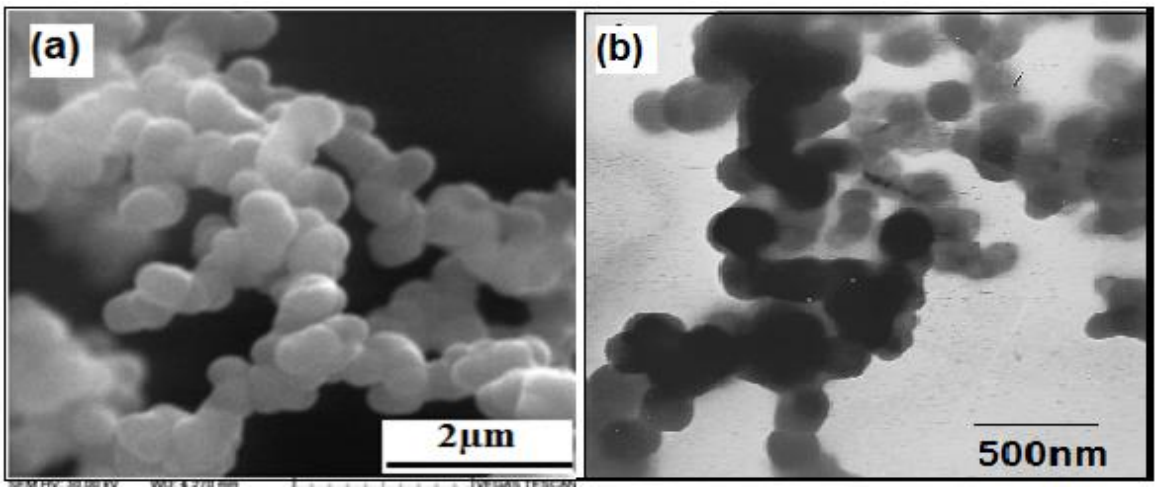

Figure 4. SEM and TEM images for carbon nanospheres as corresponds in (a) and (b) respectively.

The obtained CNSs have a spherical shape with sizes of 200-400nm which is supposed to be smaller than the used cells. So, the CNSs might cross the cell membrane, interacting with different sites in the cell, and preventing the cell from proliferation. Large surface area per unit volume and high proportion of atoms in the different surface layers makes the carbon nanoparticles more active and useful in the treatment of diseases. In physiological environment, nanomaterials are encapsulated with a layer of proteins, known as corona proteins. The protein corona changes the size of nanomaterials and may modify their distribution, toxicity and uptake by cells. It appears to be unique for each nanomaterial (22,23,24). The size of nanomaterials effects on their distribution and cellular uptake (25). When the size of nanoparticles decreased, the surface area of particles increase and higher surface area will facilitate the diffusion of particles into cells (26). By decreasing in nanoparticles size, cellular uptake of nanoparticles increases via cancer cell surface (27,28). On the other hand, nanoparticles shapes possess high effect on their uptake, penetration, cell targeting, biodistribution and duration time inside the cell (29,30,31,32).

Conclusion:

In the present work, the used carbon nanospheres (CNSs) was previously synthesized without catalyst via chemical vapor deposition (CVD) technique. The products revealed a successful antibacterial activity against Gram positive and negative bacteria, as prokaryotes. On the other hand, the cytotoxicity effects of CNSs against examined parasites, as eukaryotes, were type depend. Moreover, the effect of CNSs on cytotoxicity without functionalization is an obvious finding. It is also suggested that the shape and size of the prepared CNSs may enhanced their toxicity against infectious agents.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Technology.

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فعالية الكاربون النانو كروي المحضر محليا ضد بعض انواع الممرضات البكتيرية والطفيلية

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الخلاصة:

تحتوي المواد الكاربونية النانوية الكروية (CNSs) على خصائص يمكن استغلالها في علاج العوامل المعدية المختلفة. تم في هذه الدراسة اعتماد جسيمات الكاربون النانوية الكروية التي حضرت في دراستنا السابقة، حيث قمنا بتوصيف حجم وشكل الكاربون النانوي الكروي بواسطة المجاهر الإلكترونية (SEM و TEM) . يتميز المنتج من الكاربون النانوي الكروي الذي تم تشخيصه على أنه رقيق، إسفنجي ، أسود ، وخفيف الوزن ويتميزبأشكال كروية منتظمة ذات احجام تتراوح بين 200-400 نانومتر. لقد أجري تقدير تاثير الكاربون الكروي النانوي المحضر على البكتيريا والطفيليات. وكانت فعاليته ضد بكتريا المكورات العنقودية الذهبية والإشريكية القولونية من خلال قياس منطقة تثبيط لنمو البكتريا بمقدار 18 و 20 ملم ضد البكتيريا العنقودية الذهبية والإشريكية القولونية ، على التوالي عند تركيز 2 ملغم / مل من الكاربون النانوي الكروي بعد 24 ساعة من الحضانة عند 37 م°. أجريت تجارب قياس السمية ضد اثنان من السلالات الطفيلية من *Leishmania donovani* و *Leishmania tropica* باستخدام اختبار MTT بعد تحضينها مع الكاربون النانوي الكروي في غضون 24 ساعة عند 37 م°. من ناحية أخرى ، لوحظ ان طفيلي *L. donovani* أكثر حساسية للكاربون المحضر من *L. tropica* ،حيث ظهر مستوى متوسط من السمية الخلوية وبمقدار 51.31 ٪ عند حضن 2.4 ملغ / مل من الكاربون المحضر مع *L. donovani* ، في حين كانت السمية الخلوية ضعيفة (37.20 ٪) عندما تم استخدام نفس التركيز ضد *L. tropica* في غضون 24 ساعة في 37 م°.

الكلمات المفتاحية : كاربون نانو كروي، الفعالية ضد البكتريا ، السمية الخلوية، بكتريا، طفيليات.