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Antiparasitic Effect of CdO NPS Synthesized by Simple Chemical Method SCM on Leishmania donovani in vivo and in vitroherbicide glyphosate

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Abstract

In this study, the effect of cadmium oxide nanoparticles synthesized by a chemical method on Leishmania donovani In vitro and In vivo was evaluated. In this study, (34-51) nm Cadmium Oxide Nanoparticles were prepared by Simple Chemical Method SCM . The structural properties of the synthesized nanoparticles were investigated by using ransmission electron microscopy ,X-ray diffraction and UV-VIS spectrophotometer,.Viability percentage of promastigotes after adding CdO nanoparticles to the culture of parasite was estimated by MTT assay, then 18 mice were infected by 1×105 promastegotes/ml to study the effect of CdO nanoparticles on parasite in vivo.After 21days post inoculation all the mice were scarified, liver and intestine were removed for studying histopathological changes. The treatment resulted in promastigote of Leishmania parasites with CdO nanoparticles 100% lead to highly decrease occurs in viability rate reached to(80.92%;34.14%, and 7.51%) after (30min,1hr and 24hr) respectively. The parasite viability rate treated with CdO nanoparticles (50%),which was (85.37%,46.48% and 8.21%) respectively after same incubation period .Comparison with promastigote treated with pentostam showed significant decrease occur in parasite viability rate reached to (88.63%,78.37% and 63.08%) respectively, but less than CdO nanoparticles ,but untreated parasites which recorded only (95.87% ,94.12% and 88.82%) respectively ,there was significant (P<0.05) difference between all treatments.Conclusion: CdO NPs had antiparasitic effect on viability rate of Leishmania donovani in vivo and in vitro.

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Keywords: Leishmania donovani , Cadmium Oxide Nanoparticles , XRD, TEM

Introduction

. Visceral leishmaniasis (VL or kalaazar) is a systemic disease that is lethal without treatment (1). It is the most severe form of leismaniasis caused by Leishmania infantum , Leishmania donovani, and Leishmania infantum chagasi (2). Leishmania have a dimorphic lifecycle divided between the amastigote stage in mammalian hosts and promastigote stage in the sand fly, parasite is transmitted by sand flies bite (3).There are assessed event of VL somewhere around 500,000 and 1,000,000 cases all around(4).In Iraq, generally found in the center and the south eastern parts after the Gulf War that causes by Leishmania Infantum and Leishmania donovani (5).The start of illness is generally gradually as; sporadic fever, accompanied by sweating, weakness, weight reduction and dynamically gets to be observable(6).The present administration of leishmaniasis is medication of patients treatment, to ease illness and vector control to decrease its Transmission(7,8,9). Pentavalentantimonials (sodiumstibogluconate (SSG) and meglumin-eantimoniate) are the pillar of anti-leishmanial treatment (10,11). Despite the fact that glucantime is generally utilized for the treatment of leishmaniasis, it has some reactions counting expanded liver catalysts and electrocardiogram changes. Likewise, the medication is costly, the infusion is agonizing, and look into demonstrating that parasite resistance to glucantime is developing in various area of the world (12,13).In this respect, synthesis of nanoparticles with antimicrobial effect is essential, and has good promising applications(14). Nanotechnology field has a good potential in medical applications such as therapy and medical diagnostics (15). Metal oxide NPS have different usage in the various sciences (16). The current investigation was aimed to evaluate the antileishmanial effect of CdO NPS on Leishmania donovani in vitro and in vivo .

Material and Methods

Parasite culture

Leishmania dononvani parasite obtained from the Department of Biology \ College of Sciences \ University of Thi-Qar, Iraq. The promastigote was cultivated in NNN media and incubated at 26 C° then serial passage in NNN medium done each 5 days.

Preparation of cadmium oxide (CdO) nanoparticles

Distilled water was used during the experiment. In a typical procedure, 1.5 g of cadmium nitrate (BDH Chemicals Ltd Pool England) was dissolved in 50 mL Poly Vinyl Pyrrolidone (Sigma Aldrich USA) 1 WT. %. The solution was added in a round flask with flipping. The color of the mixture was blue. About 15 mL of sodium hydroxide (1M) was added to the mixture, and the nano suspension was formed. The suspension was maintained at 75 ° C for one hour. A large amount of black deposit was produced. After cooling to room temperature, the particles were separated by centrifugation and washed with distilled water to remove any contamination . The particles were then dried in an oven at 80 ° C. The structure of droplets dropping the CdO nanoparticles on a glass layer was examined by X-ray measuring device (XRD-6000, Shimadzu, X-ray, refractometer) with Cukα radiation at (wavelength = 0.154056 nm). The optical absorption of colloidal NPs CdO was measured using the optical spectrometer (Cary, 100 Conc Plus, UV-Vis-NIR, beam split optics, double detectors). The shape and size of CdO nanoparticles was studied using an atomic force microscope and TEM (Angstrom AA 3000).

Evaluation of CdO nanoparticles on promastigotes viability *in vitro*

Prepared 1 x10^5cell /ml by using haemocytometer slide, then added one milliliter of this number in tubes which divided into groups each one had 6 repeaters .Then added to first group 1 ml of pentostam drug , second group 1ml of CdO 100%,third group CdO nanoparticles 50% and left fourth group without addition consider as control ,all tubes incubated at 25 C° and after (30 min, 1 hr ,24 hr), put 100 µl from each tube in microplate wells .10 µl of the MTT solution **was added to the well**,and I**ncubated** of microplate for 4 hrs at 25 °C.The media were removed, then added 100 µl of the solubilization solution into each well checked for complete solubilization of the purple formazan crystals, then measured of absorbance by using ELISA reader at 600 nm.

Animals:

Thirty-Four male albino mice aged 12-13 weeks, weighing 15-17gm were gain from the animal house in collage of Medicine -Baghdad University and housed under standard conditions.

Measuring the toxicity of CdO nanoparticles in mice

Firstly, before beginning to experiment, ten of mice inoculated with (0.1ml\day) CdO nanoparticles orally by stomach tube for (30 days) to determine whether cadmium toxicity to mice.

Effect of CdO nanoparticles on parasite *in vivo*

Then 18 mice were infected by 1×10^5 promastegote/ml. After 14day the infected mice were divided into 4 groups, each group contains 6 mice, the last 6 mice non infected remain as negative control. Then each group inoculated as a follow: Group one: inoculated with (0. 1ml/day) from CdO nanoparticles every day, group two: injected with (0. 1ml/day) from pentostam by intraperitonial each day, group three: inoculated with (0. 1ml/day) normal saline consider as control positive and group four (none infected): inoculated with (0. 1ml/day) normal saline considers as control negative. After 21days post inoculation all the mice were scarified, liver and intestine were removed.

Liver parasite burden

Impression smears from liver were stained by using Giemsa stain to assess parasite burden. The no. of amastigote per nucleus of host cell was investigated by counting 1000 host cells as described by Bardiy *et al*.(17). The relative total no. of parasites / organ (Leishman- Donovan units (LDU)) and total LDU (total Leishman- Donovan units) were determined according to the equation :

$$\text{LDU} = \frac{\text{number of parasites}}{\text{1000 host cell nucleus}} \qquad \ldots (1)$$

Histopathological changes

Liver and intestine were removed and fixed in 10% formalin processed stain with hematoxylin and eosin for studying histopathological changes.

Statistical Analysis

The date of this study was subjected to statistical analysis using SPSS software program and t test analysis.

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Results and Discussion

The XRD patterns of synthesized CdO NP_S thin films and deposited on a glass substrate as shown in Fig.1. XRD spectrum for CdO thin film, which showed in Fig.1 have four sharp and narrow peaks of cubic face-centered (FCC) CdO corresponding to (111), (200) ,(220) and (311) and planes which have been compared with standard XRD data file (JCPDS file No. 75-0594) (18). In the current investigation, the films showed a preferential orientation along the (200) diffraction plane, which were grown CdO thin film on a glass substrate by the a simple chemical method . Peak density is the full width value at half the maximum (FWHM) of the main diffraction peaks, indicating the formation of smaller particles. No diffraction peaks associated with the other stages were observed in the XRD spectra of the CdO thin film.

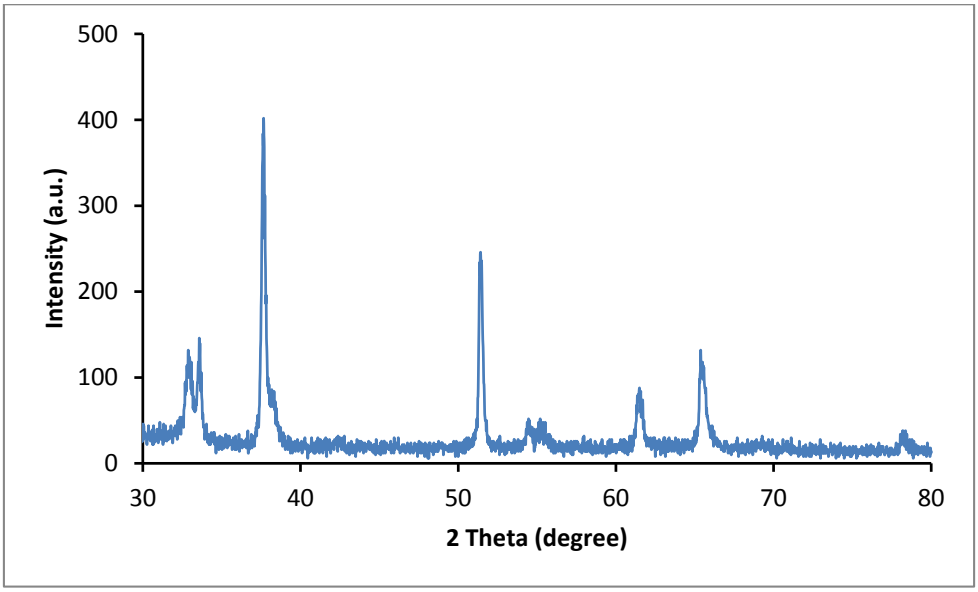

Fig. 1: XRD patterns of CdO thin film .

The crystallites size G_S of (111 plane was calculated using the following Debye–Scherrer relation (19) and listed in Table 1., (1) where λ is the x-ray wavelength, θ is the diffraction angle and β is the FWHM.

$$G_S = \frac{0.9\,\lambda}{\beta COS\,\theta} \ldots\ldots \quad (2)$$

The narrow and sharp XRD peaks indicate that the particles structure were polycrystalline, and there is no trace of another structre . The microstrain (ɲ) and the dislogation density (σ) can be calculated using the following equation (20) , and listed at Table (1):

$$\text{ɲ} = \frac{\beta COS\,\theta}{4} \ldots\ldots \quad (2)$$

$$\delta = \frac{1}{D^2} \ldots\ldots\ldots\ldots (4)$$

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Table1: summery of X-ray characterization

2 Theta (deg)	(deg) β	(*hkl*) *planes*	D (nm)	ɲ x 10-4 $lines^{-2}.m^{-4}$	δ $x10^{14}$ lines . m-2
33.57	0.33	(111)	24.91	16.11	13.90
38.92	0.06	(200)	126.46	0.62	2.73
55.25	0.42	(220)	21.34	21.95	16.23
65.78	0.28	(311)	33.77	8.76	10.25

The TEM image of CdO NP_S are shown in Fig. 2, The TEM micrographs confirm the formation of well-defined CdO NP_S having a diameter of about (34 - 51) nm which prepared by a simple chemical method.

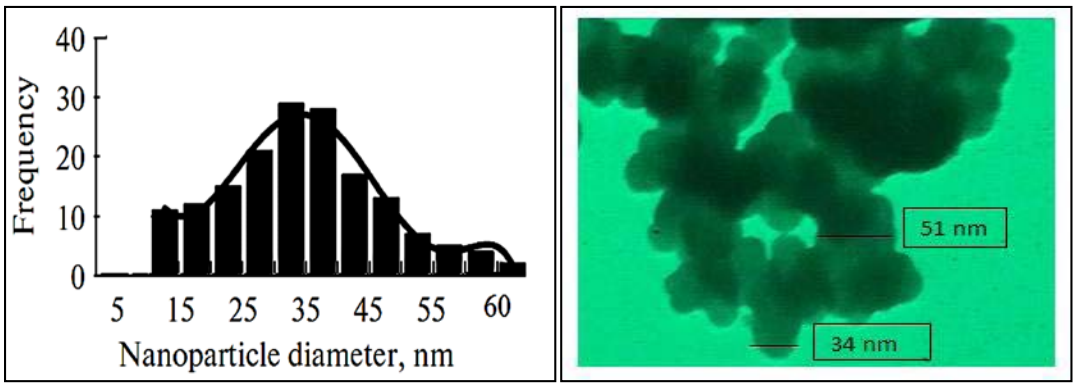

Fig. 2 : TEM images of CdO nanoparticles and their size distribution histograms.

The optical transmittance at arrange of wavelength (350 – 1100) nm.. The optical transmittance of the CdO thin film at 15 min the deposit time was 20% at the wavelength 350 nm then it is increases to 60% at the wavelength 1100 nm as shown in fig. 3,a. Also, it is observed that the optical transmittance spectra shift towards shorter wavelength as particle size decrease due to increase in energy band gap E_g. Figure 3,b shows that the graph between $(\alpha h\upsilon)^2$ versus photon energy ($h\upsilon$) gives the value of the direct band gap .The extrapolation of the straight line to $(\alpha h\upsilon)^2 = 0$, gives the value of the band gap . From the UV spectra shows the absorbance decreases with increasing wavelength and the energy gap increase from 2.3 eV to 2.45 eV from bulk to the thin film via quantum size effect.

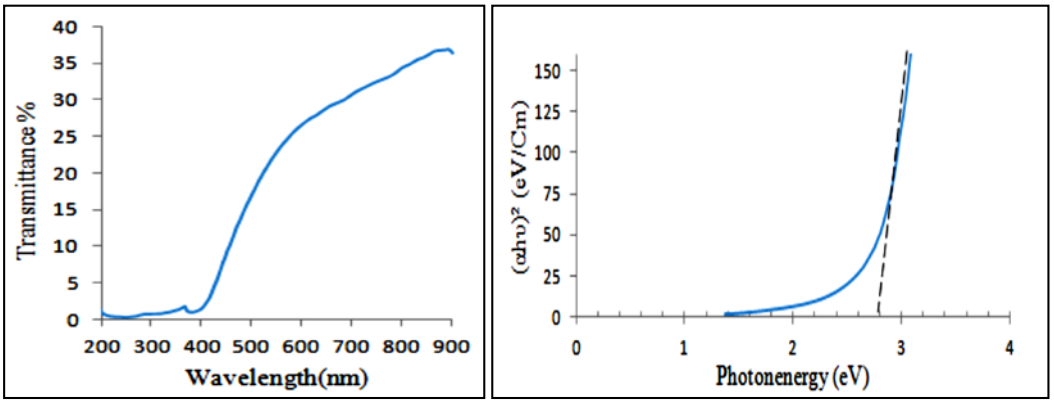

Fig.3: Transmittance Spectra of CdO thin film and $(\alpha h\upsilon)^2$ versus photon energy gap of CdO thin film continuously

Nanoparticles have many applications in medicine as antibacterial eliminate different type of pathogens (21)

Effect of CdO nanoparticles on promastigote viability *in vitro*

The treatment resulted in promastigote of *Leishmania* parasites with CdO nanoparticles 100% lead to highly decrease occur in viability rate reached to (80.92%, 34.14%, 7.51%) after (30min,1hr and 24hr) respectively , while the parasite viability rate treated with CdO nanoparticles 50% which was (85.37%,46.48% and 8.21%) respectively after same incubation period .Compration with promastigote treated with pentostam showed significant decrease occur in parasite viability rate reached to (88.63%,78.37% and 63.08%) respectively, but untreated parasites(control) which recorded only (95.87% ,94.12% and 88.82%) **respectively there was difference (P<0.05) among all treatments.**

The results have shown for clear decline in the viability of promastigote forms of Leishmania parasites treatment with CdO nanoparticles (100% and 50%)for a period (30min ,1hr and 24 hr) that statistically significant difference compared with pentostam and control, and increasing this reduction is proportional to the increasing Cd concentration (Table 2).

Table(2):Compare between CdO nanoparticles and Pentostam treatments effect in promasitgoteviability percentage.

Treatment	Time		
	30min	1hr	24hr
*Control	95.87%	94.12%	88.82%
*Cd(100%)	80.92%	34.14%	7.51%
*Cd(50%)	85.37%	46.48%	8.21%
*Pentostam	88.63%	78.37%	63.08%
*P<0.05			

Effect of CdO nanoparticles on promastigotes *in vivo*

There have been no deaths in mice over a period of 30 days after inoculated with (0.1ml\day) from CdO nanoparticles daily, this means that CdO nanoparticles nontoxic on animals.

Liver parasite burden:

To determined the parasitism degree in the liver , engraves from this organ were assessed by microscope following 21 day of infection,as can be seen in Table(3).**The parasitic burden was higher in the mice liver** which treated with pentostam (600parasites /1,000 host cell) than mice treated with CdO nanoparticles (521.5parasites /1,000 host cell) compared with control group was (3075parasites /1,000 host cell),the statistically significant (P<0.05). was showed among three groups .

Table (3): Number of parasites, LDU (Leishman- Donovan units) and total LUD in liver of mice treated with CdO nanoparticles and pentostam compared with positive control.

Groups	No of parasites /1000 host cell	LDU
***Positive control**	3075±95.7	3.075
***Cd**	521.5±1.29	0.52
***Pentostam**	600±0.51	0.6
*significant difference P<0.05 between two groups and control group		

Histological Study

The study included a study of the effect of CdO nanoparticles on the tissue of the intestine and liver after dosing mice at (0.1ml\day) for 21 days,and compared with the petostam drug , the results showed that CdO nanoparticles may impact clearly on the tissue of the intestines, it led to the occurrence of several changes included mucosal damage, increasing in numbers of goblet cell and necrosis compared with control group Figures (4:a,b) .

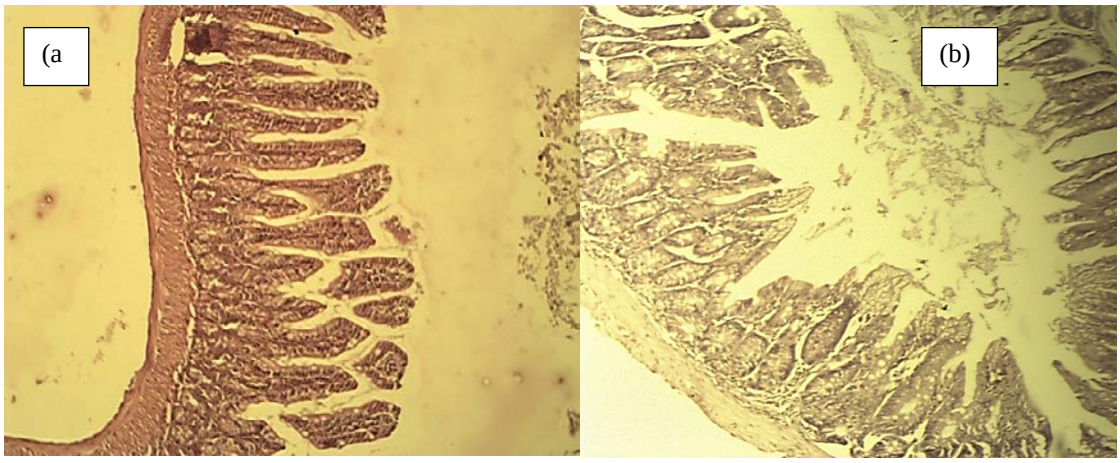

Figure 4:a) section of control intestine mice appearance of normal mucosa tissue. H&E.(10X). b):section of mice intestine treated with CdO nanoparticles showing damage in mucosa tissue,increasing in number of goblet cell and necrosis.H&E.(10X).

The histological section of liver for infected mice with leishmamial parasites showed haemorrhage, infiltration of lymphocytes, heperplasia for kupffer cell and depletion of glycoprotein as a result of the presence of parasites ,compared with control group liver showing normal structure. Figures(5:a,b).

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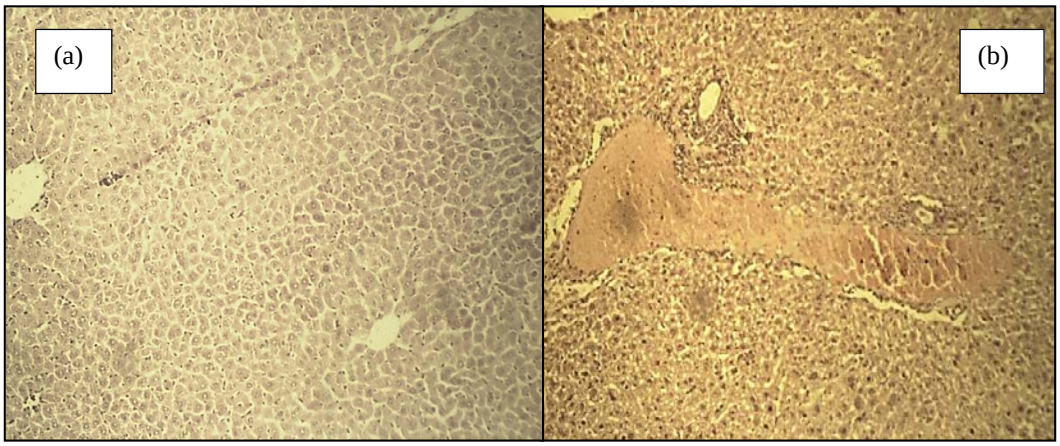

Figure5: a) Liver of non infected mice shows normal liver tissue. H&E (200X).b) Liver infected mice showed hemorrhage, infiltration of lymphocytes, hyperplasia for kupffer cell and depletion of glycoprotein H&E (200X).

Liver section of treatment infected mice with Cdo nanoparticles for three weeks appeared hyperplasia for kupffer cell , depletion of glycoprotein and hydropic degeneration but noticed reduce presence of parasites and approximately mild reaper occur figure (5.a),while the liver section for mice treated with pentostam drug showed repair occurred for parenchymal liver with mild infiltration of lymphocytes and mild activation of kupffer cells figure(5,b).

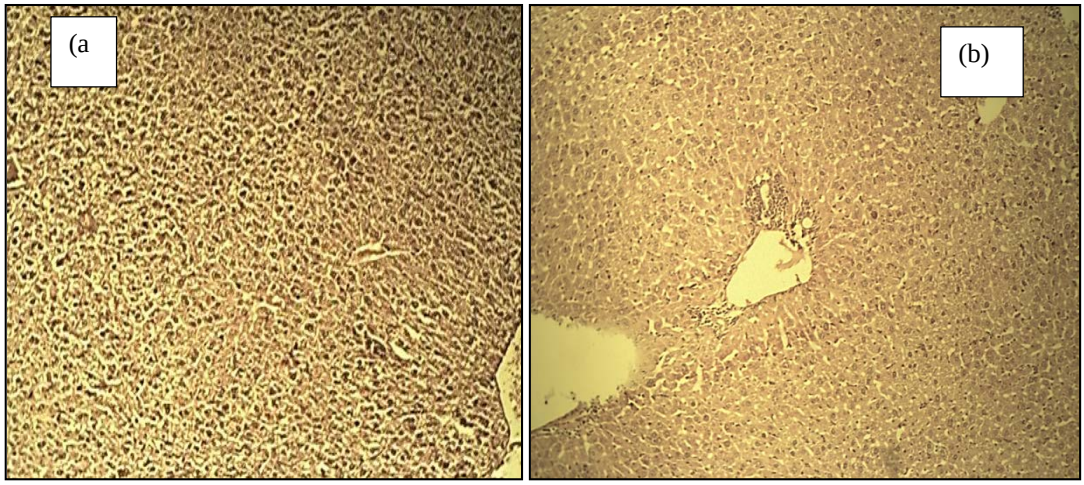

Figure6:a) liver section of infected mice treated with Cdo nanoparticles shows hyperplasia for kupffer cell and depletion of glycoprotein H&E (200X).b) liver section of infected mice treated with pentostam shows tissue repaired with mild infiltration of lymphocytes and mild activation of kupffer cells .H&E(200X).

Since the nanoparticles have potency in the study for new agents against different microbes ,in the current study, we tested activity against leishmania of CdO Nanoparticles on the Iraqi isolate of *L. donovani.* To our results, depend on a the literature result, no research have been conducted on the cytotoxicity activity of CdO NPs on *L. donovani in vitro* and *in vivo*.In current study, a relevant viability test (MTT) was used to determine the cytotoxic activity of ZnO Nanoparticles on promastigotes of *L. donovani*.

The results of current study revealed that CdO Nanoparticles have activity against leishmania. Counting number of leishmania showed a significant difference between control and tests groups. Other nanoparticles such as nanogold ,nanosilver and nanoselenium have been possess antileishmanial activities on *L. infantum* and *L. major* (22,23).

Some other drugs against *Leishmania* can induce apoptosis in *Leishmania* like Miltefosine. Miltefosine induces apoptosis in *Leishmania donovani* by increasing release of cytochrome C, followed by reduction permeability of mitochondrial membrane (24,25).But mechanism of CdO NPs against leishmania is yet unknown. In research done by Salehi *et al*.(26) they studied the antibacterial activity of Cdo NPs in 20 µg/ml concentration and they showed antibacterial activity on the antibiotic resistance bacteria. Hateet *et al*.(27) recorded that the probable mechanism of action is the metal nanoparticles are have the +ve charges and the microbe cells are having the -ve charges which produce the electromagnetic attraction between the microbes and nanoparticles.

Conclusions

CdO NPs had antiparasitic effect against viability rate of Leishmani donovani in vivo and in vitro . Study for the mechanism of CdO NPs against Leishmani can be an interesting field for research in the future.

Acknowledgment

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References

[1]-Chappuis,F. ; SundarS.; Hailu ,A.; Ghalib,H.; Rijal,S.; Peeling,R.W.; Alvar,J. and Boelaert ,M. (2007).Visceral leishmaniasis: what are the needs for diagnosis, treatment and control. Nat Rev Microbiol.,5(11):873-82.

[2]-Banuls, A.L.; Hide, M. and Prugnolle, F. (2007). Leishmaniaand the leishmaniases: A parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. AdvaParasitol., 64:1-109.

[3]-Mougneau, E.; Bihl, F. and Glaichenhaus, N. (2011) .Cell biology and immunology of Leishmania. Immunol Rev., 240(1):286-296.

[4]-Alvar, J.;Velez, I.D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J. and den Boer, M.(2012). Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE. , 7(5): p. e35671.

[5]-Stoops, C.A.; Heintshcel, B.; El-Hossary, S.; Kaldas, R.M.; Obenauer, P.J.; Farooq, M. and Villinski, J.T. (2013). Sand fly surveillance and control on Camp Ramadi, Iraq, as part of a leishmaniasis control program. J Vector Ecol, 38: 411-414.

[6]Balana-Fouce,R.; Reguera,R.M. ; Cubrıa,J.C. and Ordonez,D.(1998). The Pharmacology of Leishmaniasis , Gen. Pharmac., 30(4) :435–443 .

[7]-. Chaurasia, M.; Pawar ,V.K.; Jaiswal, A.K.; Dube ,A.; Paliwal, S.K; et al. (2015) Chondroitin nanocapsules enhanced doxorubicin induced apoptosis against leishmaniasis via Th1 immune response. Int J BiolMacromol., 79: 27-36.

[8] Ibrahim, F.; Thio, T.H; Faisal, T.; Neuman, M. (2015) The application of biomedical engineering techniques to the diagnosis and management of tropical diseases: a review. Sensors (Basel) 15: 6947-6995.

[9] De Carvalho, R.F; Ribeiro, I .F; Miranda-Vilela, A.L; de Souza Filho, J. Martins, O.P; et al. (2013). Leishmanicidal activity of amphotericin B encapsulated in PLGA-DMSA nanoparticles to treat cutaneous leishmaniasis in C57BL/6 mice. ExpParasitol .,135: 217-222.

[10]-. Zucca, M; Savoia, D. (2011) Current developments in the therapy of protozoan infections. Open Med Chem J .,5: 4-10.

[11]- Mohan ,S.; Srivastava, P.; Maheshwari, S.N.; Sundar, S.; Prakash, R .(2011) Nano-structured nickel oxide based DNA biosensor for detection of visceral leishmaniasis (Kala-azar). Analyst., 136: 2845-2851.

[12]- FattahiBafghi ,A.; Shahcheraghi, S.H.; Nematollahi, S. (2015) Comparison of hematological aspects: Visceral leishmaniasis and healthy children. Trop Parasitol., 5: 133-135.

[13] Ayatollahi, J; FattahiBafghi A; Shahcheraghi SH. (2014) .Rare variants of cutaneous leishmaniasis presenting as eczematous lesions. Med J Islam Repub Iran., 28: 71.

[14]- Fayaz, A.M.; Balaji, K.; Girilal, M.; Yadav, R.; Kalaichelvan, P.T.andVenketesan, R.(2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. Nanomed. Nanotechnol. Biol. Med.,6(1):103–109.

[15]- Qing Jiao,1.; Liwen, L .1; QingxinMu,2,3 and Qiu Zhang (2014). Immunomodulation of Nanoparticles in Nanomedicine Applications., BioMed Research International., Article ID 426028, 19 pages

[16]- Ahamed, M.; Akhtar ,J.M.; Raja, M.; Ahmad ,I.; Siddiqui ,M.K.J; AlSalhi M.S.; Alrokayan ,S.A.(2011)ZnO nanorod induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: role of oxidative stress. Nanomedicine :NBM.; 7: 904-13.

[17]- Bradley,D .J.; Kirkley,J. (1977). Regulation of Leishmania populations within the host The variable Course of Leishmania donovani infections in mice . Clinical and Experimental Immunology., 30:119-129..

18- Koffyberg, F. P. (1971) “Diffusion of donors in semiconducting CdO,” Solid State Common., 9: 2187-2189.

[19] P. Scherrer, Go¨tt. Nachrichten Gesell. 2, 98 (1918).

[20] Chang ,E.L. ; Simmers ,C. and Knight, D.A.(2010). Cobalt Complexes as Antiviral and Antibacterial Agents. Pharmaceuticals ., 3: 1711-1728;

[21] Hernandez-Sierra, J.; Ruiz ,F.; Pena, D.; Martinez-Gutierrez, F.; Martinez, A.E.; Guillen, A.J, et al. (2008).The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine.,4:237–40.

[22] Torabi, N.; Mohebali ,M.; Shahverdi, A.R.; Rezayat ,S.M.; Edrissian, G.H.; Esmaeili, J. et al. (2011).Nanogold for the treatment of zoonotic cutaneous leishmaniasis caused by Leishmania major (MRHO/IR/75/ER): an animal trial with methanol extract of Eucalyptus camaldulensis. J Pharm Sci., 113-16.

[23]- Mohebali, M.; Rezayat, M.M.; Gilani ,K.; Sarkar ,S.; Akhoundi ,B.; Esmaeili, J. Satvat, T.; Elikaee, S.; Charehdar, S.; Hooshyar, H. (2009).Nanosilver in the treatment of localized cutaneous leishmaniasis caused by Leishmania major (MRHO/-IR/75/ER): an in vitro and in vivo study. Da-ru., 17(4):285-289.

[24]- Verma, N.K; Singh, G.; Dey ,C.S. (2007).Miltefosine induces apoptosis in arsenite-resistant Leishmania donovani promastigotes through mitochondrial dysfunction. Exp Parasitol., 116:1-13.

[25]- Delavr, M.; Dalimi,A. ; Ghaffarifar,F. and Sadraei ,J.(2014). In Vitro Study on Cytotoxic Effects of ZnO Nanoparticles on Promastigote and Amastigote Forms of Leishmania major (MRHO/IR/75/ER) . Iranian J Parasitol., 9(1):6-13.

[26]- Salehi,B.; Mehrabian,S. and Ahmadi,M.(2014). Investigation of the antibacterial effect of Cadmium Oxide nanoparticles on Staphylococcus aureus bacteria. J. of Nanobiotechnology. , 12:26

[27]- Hateet, R.R. ; Hachim,A.K. and Lazim,H.G.(2016) .Evaluation of antibacterial of cadmium oxide nanoparticles against some bacterial pathogens . World Journal of Pharmacy and Pharmaceutical Sciences., 5(9): 2167-2174.