Iraqi Journal of Biotechnology, December 2018, Vol. 17, No. 3, 1-16

Using of Hybrid Nanoantibiotics as Promising Antimicrobial Agent

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Received: July 1, 2018 / **Accepted**: November 28, 2018

Abstract: Nanoparticles are particles with size 1-100 nm, which exhibit different shapes like spherical, triangular, rod, etc. Research on synthesis of nanoparticles is the current area of interest due to the unique visible properties (chemical, physical, optical) of nanoparticles compared with the bulk material. Owing to the wide range of applications offered by nanoparticles in different fields of science and technology, different protocols have been designed for their synthesis. The nanoparticles can be synthesized using the top-down (physical) approach which deals with methods such as thermal decomposition, diffusion, irradiation, arc discharge, and bottom-up (chemical and biological) approach,which involves seeded growth method, polyol synthesis method, electrochemical synthesis, chemical reduction, and biological entities for fabrication of nanoparticles. Different synthesis methods involve the use of different types of chemical, physical, and biological agents to yield nanoparticles of different sizes and shapes. The most often used method for the chemical synthesis of nanoparticles is the chemical reduction method, which deals with the reduction of metal particles to nanoparticles using chemical reducing agents like sodium borohydride or sodium citrate.

Keywords: Nanoparticles, antibacterial, antiviral, antiparasitic.

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Nanoantimicrobial agents:

1. Layered double hydroxide (LDH):

Layered double hydroxides (LDHs) as hydrotalcite-like materials or anionic clays, can be found in nature as minerals that are readily synthesized in the laboratory (1,2,3). In nature, they are formed from the weathering of basalts or precipitation in saline water sources (Figure 1) (10). LDH nanocomposite is a class of inorganic material with chemical composition represented by the general formula, [M2+1-xM3+x (OH) 2] x+ [An-] x/n·mH2O] where M2+ and M3+ are divalent and trivalent metal cations respectively (11). LDHs, also known as one of the inorganic delivery nanoparticles have recently been receiving considerable attention, they possess some promising advantages such as low cost, good biocompatibility, low toxicity to mammalian cells, controlled release property, and full protection for loaded drugs (4,5,6).

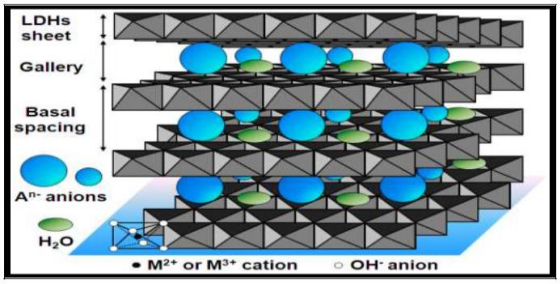

Figure (1): The structure of layered double hydroxides (LDHs) (10).

Many LDH compounds intercalated with beneficial organic anions, such as DNA (7,8,9,10,11,12,13,14), amino acids, anionic polymers, (15,16), pesticides (17) and drugs (18, 19, 20. 21) have been prepared successfully, furthermore, the antioxidant intercalated into LDH delivery can be used as a nanomaterial antioxidant supple mentation, thereby expanding its range of applications (22).

Cellular delivery involving the transfer of various drugs and bioactive molecules (peptides, proteins, and DNA) through the cell membrane into cells via some delivery vehicles has attracted increasing attention due to the difficulty and inefficiency for bioactive molecules and drugs to go across the cell membrane. Therefore, searching for efficient and safe transport vehicles (agents) to cellularly deliver biomolecules and drugs has been a challenging yet exciting task for scientific researchers (23).

LDH drug particles are adhered to the cell membrane surface, some are internalized into the cell. The possible pathway for such nanohybrids to be internalized into cells is phagocytosis or endocytosis. Phagocytosis generally involves the uptake of particles larger than 500 nm. In this connection, slightly agglomerated LDH aggregates (several hundreds of nm) may be taken up via phagocytosis while individual LDH crystallites (50-300 nm) via endocytosis. Obviously, two pathways give rise to a different internalization efficacy, and endocytosis can lead to a quicker uptake of LDH nanoparticles. Although endocytosis has not been well understood, microscopic observations indicate that cell morphology and cytoskeleton structure gradually change during the process, which is presumed to take place even with more difficultly for phagocytosis (24).

The endocytosis may be a receptor-mediated process that facilitates the uptake of LDH–drug nanohybrid particles. The cellular uptake is one key step for drug delivery. LDH nanoparticles, as potential delivery agents, provide the basic prerequisites to maximize the efficacy of the cellular uptake by tailoring the LDH particle size, adjusting the zeta potential, and conjugating the ligands to enhance the receptor- mediated endocytosis (25).

2. Antibiotics:

Antibiotics are a substance or compound that kills, or inhibits, the growth of, bacteria (26). Antibiotics belong to the broader group of antimicrobial compounds, used to treat infections caused by microorganisms, including fungi and protozoa. The term antibiotic was coined by Selman Waksman in 1942 (27). Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes (28). Those that target the bacterial cell wall (penicillins and cephalosporins) or the cell membrane (polymyxins), or interfere with essential bacterial enzymes (rifamycins, lipiarmycins, quinolones, and sulfonamides) have bactericidal activities. Those that target protein synthesis (macrolides, lincosamides and tetracyclines) are usually bacteriostatic

(with the exception of bactericidal aminoglycosides) (29).

Further categorization is based on their target specificity. Narrowspectrum antibacterial antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria. Following a 40-year hiatus in discovering new classes of antibacterial compounds, four new classes of antibacterial antibiotics have been brought into clinical use in the late 2000s and early 2010s: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), oxazolidinones (such as linezolid), and lipiarmycins (such as fidaxomicin) (30, 31).

Ciprofloxacin (CIP):

Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class. It is active against both Gram-positive and Gram-negative bacteria, (Figure 2) (32).

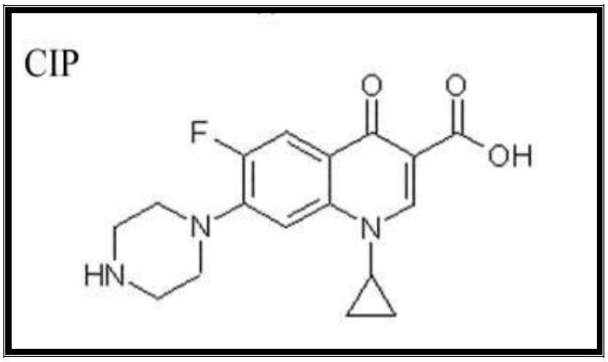

Figure (2): Chemical structure of ciprofloxacin (CIP) (32).

The primary functional route of this class of antibiotic is the inhibition of the enzyme, DNA gyrase, in bacterial cells (bacterial topoisomerase II). This enzyme is responsible for negative supercoiling of DNA during the replication process. The inhibition of this enzyme‘s activity will, naturally, lead to a number of adverse and bactericidal effects as a result of inhibition of DNA replication. These effects include interfering with the DNA replication, transcription and the separation of the bacterial chromosomes, along with the damage of DNA and other cellular processes (33). Ciprofloxacin is recognized as one of the most effective antibiotics of the quinolone drug class (34) and has been used for the treatment of urinary tract infections (35), sexually transmitted diseases (36), bacterial prostatitis (37), and infections of the respiratory and digestive tract (38).

Nalidixic acid (NA):

Nalidixic acid is the first of the synthetic quinolone antibiotics. In a technical sense, it is a naphthyridone, not a quinolone: its ring structure is a 1,8- naphthyridine nucleus that contains two nitrogen atoms, unlike quinoline, which has a single nitrogen atom (Figure 3) (39). Synthetic quinolone antibiotics were discovered by George Lesher and coworkers as a by-product of chloroquine manufacture in the 1960s (40).

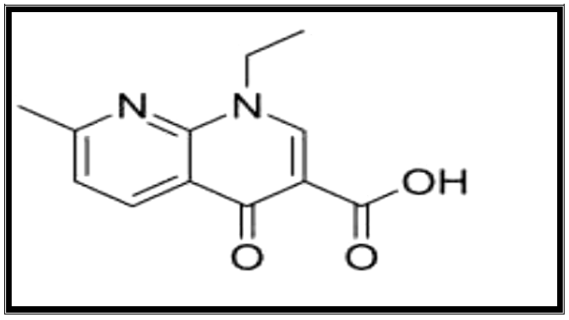

Figure (3): The chemical structural of nalidixic acid (NA) (39).

Nalidixic acid is effective primarily against gram-negative bacteria, with minor antigram- positive activity. In lower concentrations, it acts in a bacteriostatic manner,that it inhibits growth and reproduction. In higher concentrations, it is bactericidal, meaning , that it kills bacteria instead of merely inhibiting their growth. It has historically been used for treating urinary tract infections, caused, for example, by *Escherichia coli*, *Proteus*, *Shigella*, *Enterobacter*, and *Klebsiella*. It is no longer clinically used for this indication in the USA as less toxic and more effective agents are available. It is also a tool in studies as a regulation of bacterial division. It selectively and reversibly blocks DNA replication in susceptible bacteria. Nalidixic acid and related antibiotics inhibit a subunit of DNA gyrase and topoisomerase IV and induce formation of cleavage complexes. It also inhibits the nickingclosing activity on the subunit of DNA gyrase that releases the positive binding stress on the supercoiled DNA(41).

Nanoparticles and drug delivery:

The primary goals for research of nano-bio-technologies in drug delivery include: i) more specific drug targeting and delivery, ii) reduction in toxicity while maintaining therapeutic effects, iii) greater safety and biocompatibility, iv) faster development of new safe medicines, v) reduce the dosage required by more efficient, localized targeting of the drug (42).

Nanoparticles (NPs) in general have many properties that are different from those of traditionally used materials (43, 44, 45). They have dimensions typically below 100 nm, which allows them to reach specific sites inside the body and even to be permeable to tissues and cells. Therefore, they can deliver the drugs in active forms at sites that conventional drugs may not reach by themselves and thus minimise the undesirable side effects (46). Additionally, they have high surface to volume ratio, therefore by using a small amount of drug, the exposure of it at the point of interest will be maximized and thus any toxicity issues of the incorporated drug can be avoided (47).

Moreover, by integrating different coatings with drugs, non-acute and time controlled drug delivery can be achieved. This is very important for treating infectious diseases since the delivery of the drug should be controlled both in time and in quantity. Many nanosized structures can be used as drug carriers such as liposomes, synthetic or natural polymers, inorganic and metallic NPs, dendrimers, silicon and others. Iron oxide magnetic NPs

(MNPs) have many advantages and are considered very promising drug carriers (48). They can be handled by using an external magnetic field, thus directed to a specific area in the body where they then excrete the medicine, avoiding delivery to unwanted tissues. MNPs can be formed either from pure metals (cobalt, nickel, manganese and iron) or alloys and their oxides (Figure 4) (49).

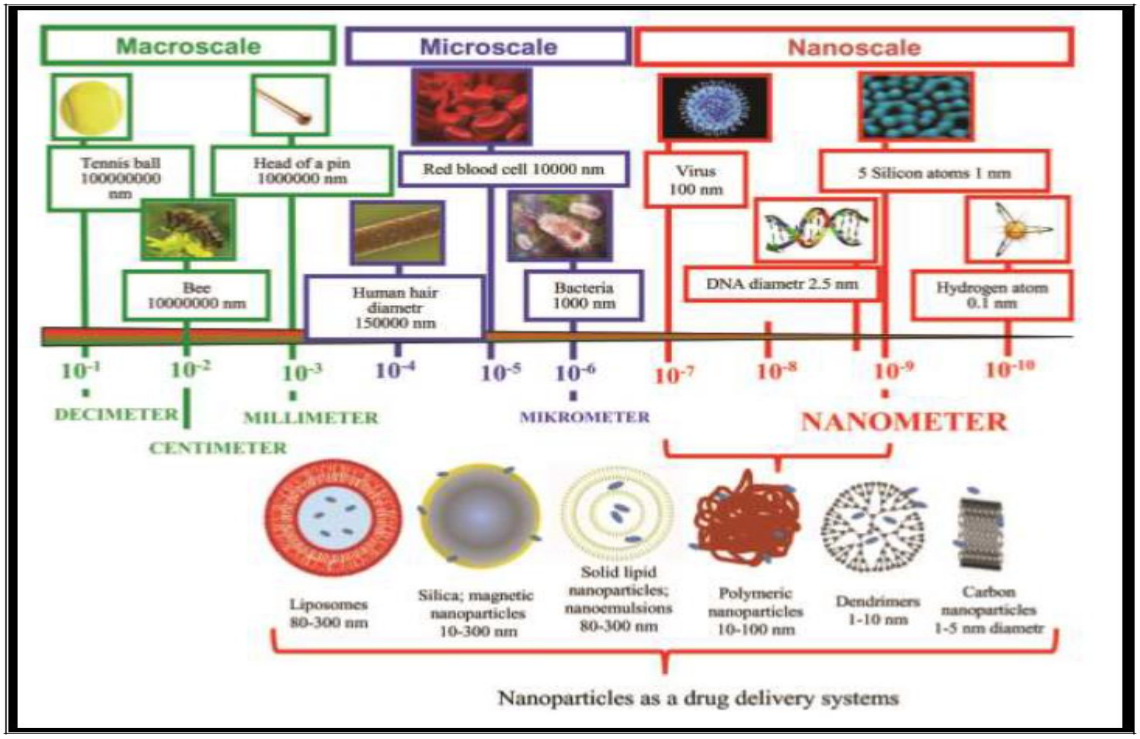

Figure (4): Nanoparticle drug delivery systems with relation to other scales (49).

Nanotechnology and the treatment of viral infection:

Certain challenges exist for the treatment and subsequent eradication of viruses in the infected host. One major example is the establishment of reservoirs in cellular and anatomically rich sites such as the blood-brain barrier (BBB) (50) and blood-testis barrier. This leads to low-level replication in these compartments, which are inaccessible to conventional therapeutics. Nanoparticulate drug carriers are, however, able to traverse these membranes and are therefore promising tools to be investigated for circule this barrier (51). Other challenges in viral treatment include the use of RNA interference (RNAi) technology–a popular molecular approach for the treatment of many infectious diseases. The inability of RNA to cross the cell membrane, due to the large molecular weight and anionic charge, rapid renal clearance, uptake by phagocytes, and toxicity due to stimulated immune response, all present limitations which prohibits their clinical utility (52). The incorporation of siRNA onto nanocarriers, however, can also overcome this limitation to achieve successful inhibition of viral replication Moreover. There have been many attempts for improving the physico-chemical properties of antiviral drugs by chemical modifications (53,54). Another alternative for the delivery of

antiviral drugs is the use of controlled-release delivery vehicles in the form of tablets and patches. Such formulations reduce the administered dose and aim to overcome problems of non-compliance and loss of drug activity. The ideal delivery platform would release the antiviral drug at a constant dose over a long time (55).

The design of nanomaterials-based delivery systems has several advantages. Nanomaterials have the characteristics of high surface-to-volume ratios, enabling the packaging of multiple antiviral agents onto the same nanoparticles. Using these nanomaterials, it might be possible to overcome problems associated with the use of high doses of antiviral drugs. Moreover, this type of approach also provides the possibility of targeting specific biological sites actively or passively. Because of their unique features, such as nanometric size and controllable hydrophobicity/lipophilicity, such antiviral nanocarriers can target drugs to specific tissues or organs, while the modification of nanocarrier surfaces enables them to reach particular sites to deliver the drug at the specific target. This helps local and systematic delivery of antiviral drugs with the results of minimizing side effects of healthy cells and tissue. With respect to intravenous administration, due to their small size, nanoparticles can circulate in the bloodstream without being retained by the pulmonary capillaries or uptaken by the reticolo-endothelial system (RES). Indeed, the most frequently used approach to increase the longevity of nanocarriers to avoid the RES uptake is to modify their surface with hydrophilic polymers such as polyethylene glycol (PEG) units (56). Various nanocarriers for antiviral agents have been proposed over the years and some of the most currently considered are listed in (Figure 5) (57).

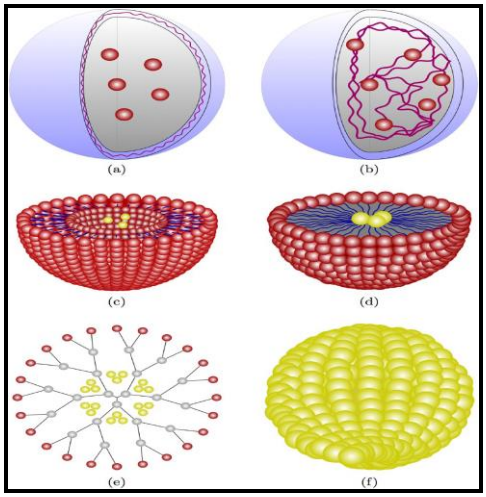

Figure (5): Different types of nanocarriers used for antiviral drug delivery: (a) nanocapsules (b) nanosphere (c) liposome (d) micelle (e) dendrimers and (f) gold nanoparticle (57).

Dendrimers are polymeric nanostructures with unique morphological features. They exhibit three-dimensional tree-like structures connected to a central core .They can be synthesized with precise physico-chemical and biological properties depending on the type of polymer and functional groups used. One of the most widely known antiviral dendrimeric

structure is VivaGel, the first topical nanomicrobicid (58). Liposomes are spherical carriers ranging from 20 to 30 nm in size. They are composed of a phospholipid bilayer (which can mimic cell membranes and directly fuse with microbial membranes) (59), containing an aqueous core. The surface of liposomes can be easily engineered with various functions to enhance their recognition as well as uptake by macrophages or other components of the immune system. They are mainly explored for delivering HIV vaccines as well as siRNA. While commercially feasible technologies are available for large-scale production, these systems have been shown to aggregate and the active ingredient degraded during storage, thereby reducing the performance of these molecular architectures over long periods of time. Inorganic nanoparticles, such as gold, silver, iron oxide, *etc.*, have found their application in antiviral therapy. Elichegurra *et al.* were the first to demonstrate the effect of silver nanoparticles (Ag NPs) on HCV-1. Several studies showed that Ag NPs interfered with several stages of the viral replication cycle (60).

Microemulsions, isotropic systems of oil, water, and a surfactant, are droplets in the nanometer range (61). These thermodynamically stable mixtures allow the entrapment of a wide range of active drugs and have great potential as drug delivery systems. Microemulsions can be classified into solid lipid nanoparticles (SLN) and lipid nanocapsules (LNC). SLN are made of a solid hydrophobic core (triglycerides, fatty acids, steroids) surrounded and stabilized by emulsifiers such as lecithin or polymers.. LNCs consist of an oily core surrounded by a crown of triglycerides composed of a lipophilic surfactant such as lecithin and a nonionic one such as Solutol HS15. The LNC have the advantage of being formulated in the absence of solvents, with biocompatible constituents with a size varying between 20 nm and a few hundred nanometers (62).

Types of nanomaterials used in HIV therapeutics:

Treatment for HIV/AIDS is based on the use of drugs that target the various stages in the life cycle of the virus. The current antiretroviral (ARV) includes six classes of drugs, that is, nucleoside/nucleotide reverse transcriptase inhibitors (N(t) RTIs), non-nucleoside inhibitors (NNRTIs), protease inhibitors (PIs), entry/fusion inhibitors (FIs), CCR5 antagonists, and integrase inhibitors. The combination of three or more drugs, known as highly active ARV therapy (HAART) has significantly improved the expectancy and quality of life of HIV-infected individuals (63).

Nanotechnology can impact HIV therapy at several levels (1). Nanoparticles by themselves have therapeutic effects since they can penetrate and neutralize the virus, by structural interference with viral assembly and thereby inhibit viral replication (2). Nanotechnology allows improved delivery platforms for systemic delivery of antiretroviral drugs, by allowing controlled release of antiretroviral drugs in circulation, thereby enhancing their half-lives and effectiveness, all of which can have a major implication in improving adherence to drugs in HIV infected patients (3). Gene immunotherapy can be significantly improved using various nanomaterials. Nanotechnology-based vaccines have the ability to target

specific immune cells, eliciting a controlled and sustained HIV-1 specific antibody and cellular immune response. Nonviral delivery of siRNA has tremendous translational potential and although delivery of siRNA to HIV-specific cells has been demonstrated (64).

Nanotechnology in parasitology:

Parasites can be affect millions of people worldwide and are involved with many limitations in treatment and control methods. Despite the rapid and remarkable developments in health care and health advancement in most regions of the world, parasite infections remain as one of the most main health problems affecting the economy, especially in developing countries. According to the world health organization (WHO), around two-thirds of the world's population Moreover, about 16 million of the whole deaths each year that happen in developing countries are associated with parasitic infections (65).

Nanotechnology have been used the materials and systems at atomic scales (1–100 nm), where their properties differ significantly from those at a larger scale. The use of nanotechnology and nanomaterials in medical research was grow (66). Nanotechnology in microbiology have gained importance in the field of chemotherapy. Nanotechnology are associated with modern science. Nanomaterials have admitted more attention as antiparasitic agents (67). The size nanomaterials of is alike to that of most biological structures and molecules; therefore, nanomaterials can be helpful for both *in vivo* and *in vitro* parasitical studies and applications (68). The percentage 30% of the world's population have been affected of parasitic infections. The parasitic infections caused morbidity round in the developing countries. The antiparasitic chemotherapy residue is the only weapon for fighting parasitic infections. The side effects of antiparasitic drugs and the severity of parasitic diseases, it is necessary to investigate on new antiparasitic compounds with high activity, low toxicity that are cheaper and have more efficacies. Therefore, in this review we prepared a list of all nanomaterials, which were used against parasites (69).

The unique properties of NPs including AgNPs, AuNPs, chitosan, selenium oxide, and other metallic oxide NPs that have shown excellent inhibitory effects against parasitic infections including insect larvae (70). The rate of discovery in the anti-parasitic segment was seen in last few decades and has necessitated effective management of existing drugs by modulating their delivery. The NPs may not have the recognizable antimicrobial activity compared to the mass formulations of the metal oxide or solutions of metal salts. But, the stability and slow release of metal ions from NPs are the main characteristics during usage of them (71). The antimicrobial effective of NPs depends on the particle size. The smallest sized NPs showed the antimicrobial activity. NPs are killed parasites by cytotoxic and inhibitory effects. The table 1 described the antiprotozoal of NPs against diverse genera of protozoa and the antihelminth of NPs against diverse genera of helminth (72).

In the early of 1980s, NPs are synthetic and complex molecules with specified chemical structures that were synthesized firstly. These nanomaterials are nanosized polymers and are assembled from branch units. The surface of a synthetic nanomaterial has

numerous chain ends, which can be tailored to complete specific chemical functions. This property could also be helpful for catalytic uses. Nanomaterials show some remarkably improved chemical and physical properties compared to traditional polymers (73).

Table (1): Antiprotozoa and helminthes of NPs against diverse genera of protozoa and helminthes (72).

Antiprotozoal activates of NPs	
Giardia lamblia	**References**
Silver	(70)
Chitosan	(70)
Curcumin	(70)
Gold	(74)
E. histolytica	
Copper	(75)
Silver	(75)
Leishmania	
Gold	(76)
Poly(D, L -lactide-co-glycolide)	(77)
Titanium	(78)
Silver	(79)
Selenium	(80)
Chitosan	(81)
Cryptosporidium parvum	
Nano-Nitazoxanide	(82)
Silver	(70)
Toxoplasma gondii	
Chitosan	(80)
Silver	(80)
Plasmodium	
Copper	(83)
Chitosan	(84)
Curcumin	(78)
Silver	(85)
Echinococcus multilocularis Albendazole–Chitosan	(86)
Trichinella spiralis Chitosan	(87)
Fasciola Silver	(88)

Nanotechnology diagnosis in parasite infections:

In the diagnosis of malaria, recombinant heat shock protein 70 (HSP70) of P. *Plasmodium falciparum* conjugated with gold NPs and functionalized with anti-HSP70 monoclonal antibodies are sensitive in the detection of malaria antigen (89). Polyclonal IgG antibodies specific conjugated polystyrene NPs with to P. falciparum showed sensitive results (90). *P. falciparum* biomarkers (histidine-rich protein-2 and pan-Plasmodium lactic dehydrogenase, Pf-HRP2/ PAN-pLDH) linked gold NPs in an immunochromatographic (ICT) assay rapid diagnostic test (91). Specific agglutination of antigen-coated gold NPs uses of detection in *Toxoplasma* of the corresponding antibody gave

satisfactory agreement with ELISA results (92).The gold NPs conjugated HSP7 of cryptosporidiosis to target HSP70 mRNA from C. parvum oocysts (93). The assay that used probes of oligonucleotide-functionalized gold NPs (complementary to the 18s rRNA sequence on *C. parvum*) proved its ability to detect the nucleic acids of *C. parvum* oocysts in stool samples (94). The fluorescent silica NPs were synthesized and conjugated with monoclonal anti-*E. histolytica* IgG1 for diagnosis of *E. histolytica*. It showed high sensitivity results without cross reaction with other protozoa (*E. dispar*, *E. moshkovskii, G. lamblia and Blastocystis spp.*) (95).

Synthesized iron oxide NPs were functionalized with CS and used to capture and remove *Entamoeba spp.* cysts after application of an external magnetic field. Shukla *et al*. (96) reported that the synthesized NPs were well dispersed and suitable for water treatment. They suggested that cyst wall components (lectins and chitin) might interact with CS NPs, and recommended further studies to validate the use of NPs in water treatment, with a special emphasis on possible NPs toxicity. For *leishmania*, gold NPs conjugated with four oligonucleotide probes, targeting DNA of *Leishmania* kinetoplastid, were used this mothod. The gold NPs were also conjugated with labeled *Leishmania* spp. primers and magnetic beads for isothermal amplification of *Leishmania* DNA in blood samples of infected dogs. It was found that NPs have electrocatalytic activity for the rapid detection of the amplified DNA. This approach was found to be more sensitive and less expensive than the traditional PCR methods used in the diagnosis of visceral leishmaniasis (VL) (97).

Other nanotechnology applications uses in parasite:

One of the important applications that used as a drug delivery system, for example quercetin conjugated with gold NPs was established for treatment of visceral leishmanisis caused by wild-type resistant strains (98). CS proved to be a suitable drug delivery mean for several drugs used in VL treatment. A Doxorubicin displayed significant reduction in *Leishmania* amastigotes *(in vivo*) and promastigotes (*in vitro*), while amphotericin B and rifampicin gave significant results compared with control drugs without CS. Amphotericin B was also encapsulated in PLGA NPs and gave significantly effective results in comparison with the drug alone (99). Chavez-Fumagalli *et al.*, (100) discussed all the studies that employed nanotechnology in drug delivery systems for amphotericin B in the treatment of VL. The reviewers concluded that CS and chondroitin sulfate NPs are the best ones nowadays due to their lower costs. In cutaneous leishmaniasis, glucantime formulated with liposomes was effective in the topical treatment of leishmanial ulcers caused by L. major in mice. It resulted in a significant decrease in lesion size and spleen parasite burden. *Trichoderma harzianum* Isolated from the soil conjugated with silver NPs increased the efficacy of triclabendazole in the treatment of fascioliasis (88). Polyvinyl alcohol conjugated with CS was proven to suppress the attachment of *Cryptosporidium* sporozoites to enterocytes in vitro. In *E. multilocularis*, Albendazole bound to CS was effective in the treatment of alveolar echinococciosis (101). In visceral larva migrans caused by *T. canis*. In the *schistosomiasis mansoni*,

praziquantel (300mg/kg) encapsulated in liposomes showed a significant reduction in worm burden and stool and intestinal egg counts as well as in the number of hepatic granulomas. Diminazene aceturate also encapsulated into liposomes, showed *in vitro* and *in vivo* significant results in treatment of Suda, caused by *T. evans*. Miltefosine, an anticancer therapy, was enclosed in lipid nanocapsules and administered to *S. mansoni*-infected mice as a single oral dose (20 mg/kg), and its efficacy was compared with that of praziquantel. The results proved its potentiality in schistosomiasis mansoni, and the ability of nanomedicine (102).

Conclusions:

In our conclusions, the possible ability of hybrid nanoantibiotics preparation from the free antibiotics that used in treatment of human infections caused by different pathogenic bacteria. The hybrid nanonalidixic acid had more inhibition activity comparing with hybrid nanocirofloxacin against several bacteria. Using of hybrid nanoantibiotics resulting from loaded of different antibiotics on LDHs carrier could be represented a promising future tool to treat the human bacterial infections. On other hand several aspects still need to be considered and optimized for a successful translation of nanomaterials from the laboratory to the clinical setting. In fact, most of the studies using anti-viral nanostructures are limited to either cell culture or *in vivo* models.

References:

1. Rai, M.; Yadav, A. and Gade, A. (2008). Current trends in phytosynthesis of metal nanoparticles. *Crit. Rev. Biotechnol*. 28(4): 277-284.
2. Huang, J.; Chen, C.; He, N.; Hong, J.; Lu, Y. and Qingbiao, L. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology*. 18: 105-106.
3. Ferrari, M. (2005). Cancer nanotechnology: opportunities and challenges. *Nat. Rev. Cancer*. 5: 161-171.
4. Rai, M.; Yadav, A. and Gade, A. (2009). Silver nanoparticles: as a new generation of antimicrobials. *Biotechnol Adv*. 27: 76-83.
5. Sau, T. and Rogach, A. (2010). Nonspherical noble metal nanoparticles: colloid-chemical synthesis and morphology control. *Adv. Mater*. 22(16): 1781-1804.
6. Reddy, V. (2006). Gold nanoparticles: synthesis and applications. *Synlett*. 11: 1791-1792.
7. Jana, N.; Pal, T.; Sau, T. and Wang, Z. (2000). Seed- mediated growth method to prepare cubic copper nanoparticles. *Curr. Sci*. 79(9): 1367-1370.
8. Cao, J. and Hu, X. (2009). Synthesis of gold nanoparticles using halloysites. *J. Surf. Sci. Nanotechnol*. 7: 813-815.
9. Braterman, P.; Xu, Z. and Yarberry, F. (2004). Layered double hydroxides. In: Auerbach SM, Carrado KA, Dutta PK, ed. Handbook of layered materials. New York: Marcel Dekker, Inc. p. 373-474.
10. Bi, X.; Zhang, H. and Dou, L. (2014). Layered double hydroxide-based nanocarriers for drug delivery. *Pharmaceutics*. 6: 298-332.
11. Wang, Q. and O'Hare, D. (2012). Recent advances in the synthesis and application of Layered Double Hydroxide (LDH) nano-sheets. *Chem. Rev*. 112: 4124-4155.
12. Xu, Z. and Lu, G. (2006). Layered double hydroxide nanomaterials as potential cellular drug delivery agents. *Pure and Applied Chemistry*. 78(9): 1771-1779.
13. Xu, Z.; Zeng, Q.; Lu, G. and Yu, A. (2006). Inorganic nanoparticles as carriers for efficient cellular delivery. *Chem. Eng. Sci*. 61(3): 1027- 1040.
14. Xu, Z.; Walker, T.; Liu, K.; Cooper, H. and Lu, G. (2007). Bartlett PF. Layered double hydroxide nanoparticles as cellular delivery vectors of supercoiled plasmid DNA. *Int. J. Nanomedicine*. 2(2): 163-174.

15. Li, A.; Qin, L. and Wang, W. (2011). The use of layered double hydroxides as DNA vaccine delivery vector for enhancement of antimelanoma immune response. *Biomaterials*. 32(2): 469-477.
16. Hayashi, A.; Fujimoto, Y.; Ogawa, Y.; Nakayama, H. and Tsuhako, M. (2005). Adsorption of gaseous formaldehyde and carboxylic acids by ammonium-ion-exchanged alpha-zirconium phosphate. *J. Colloid Interface Sci*. 283(1): 57-63.
17. Zhang, H.; Zou, K.; Guo, S. and Duan X. (2006). Nanostructural druginorganic clay composites: Structure, thermal property and in vitro release of captopril-intercalated Mg–Al-layered double hydroxides. *J. Solid State Chem*. 179: 1792-1801.
18. Tyner, K.; Schiffman, S. and Giannelis, E. (2004). Nanobiohybrids as delivery vehicles for camptothecin. *J. Control Release*. 95: 501-514.
19. Li, B.; He, J.; Evans, D. and Duan, X. (2004). Enteric-coated layered double hydroxides as a controlled release drug delivery system. *Int. J. Pharm.* 287: 89-95.
20. Qin, L.; Xue, M.; Wang, W.; Zhu, R.; Wang, S. and Sun, J. (2010). The in vitro and in vivo anti-tumor effect of layered double hydroxides nanoparticles as delivery for podophyllotoxin. *Int. J. Pharm*. 388(1-2): 223-230.
21. Xiao, R.; Wang, W. and Pan L. (2011). A sustained folic acid release system based on ternary magnesium/zinc/aluminum layered double hydroxides. *J. Mater. Sci*. 46(8): 2635-2643.
22. Qin, L.; Wang, S.; Zhang, R.; Zhu, R.; Sun, X. and Yao, S. (2008). Two different approaches to synthesizing Mg-Al-layered double hydroxides as folic acid carriers. *J. Phys. Chem. Solids*. 69: 2779-2784.
23. Azzam, T. and Domb, A. (2004). Current developments in gene transfection agents. *Curr. Drug. Deliv*. 1(2): 165-193.
24. Rolland, A. (1999). Advanced Gene Delivery: From Concepts to Pharmaceutical Products, Harwood, Amsterdam. 25. Kauwe, L.; Xie, J. and Alvarez, S. (2005). Intracellular trafficking of nonviral vectors. *Gene Therapy*. 12: 1734-1751.
25. Kauwe, L.; Xie, J. and Alvarez, S. (2005). Intracellular trafficking of nonviral vectors. *Gene Therapy*. 12: 1734-1751.
26. Davey, P. (2000). Antimicrobial chemotherapy. in Ledingham JGG, Warrell DA. Concise Oxford Textbook of Medicine. Oxford: Oxford University Press. pp. 1475.
27. Waksman, S. (1947). What is an antibiotic or an Antibiotic substance. *Mycologia*. 39(5): 565-569.
28. Ong, S.; Nakase, J.; Moran, G.; Karras, D.; Kuehnert, M. and Talan, D. (2007). Antibiotic use for emergency department patients with upper respiratory infections: prescribing practices, patient expectations, and patient satisfaction. *Annals of emergency medicine*. 50(3): 220-213.
29. Ian, C. and Marilyn, R. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. Microbiol Mol Biol Rev. 65(2): 232-260.
30. Wiegand, I.; Hilpert, K. and Hancock, R. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*. 3(2): 163-175.
31. Dalhoff, A.; Ambrose, P. and Mouton J. (2009). A long journey from minimum inhibitory concentration testing to clinically predictive breakpoints: deterministic and probabilistic approaches in deriving breakpoints. *Infection*. 37(4): 296-305.
32. Black, M.; Stachyra, T.; Platel, D.; Girard, A.; Claudon, M. and Bruneau, J. (2008). Mechanism of action of the antibiotic NXL101, a novel nonfluoroquinolone inhibitor of bacterial type II topoisomerases. *Antimicrobial Agents and Chemotherapy*. 52(9): 3339-3349.
33. Drlica, K.; Zhao, X. and Zhao, X. (1997). DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Mol. Biol. Rev*. 61(3): -377-392.
34. Oates, J.; Wood, A.; Hooper, D. and Wolfson, J. (1991). Fluoroquinolone antimicrobial agents. *New England Journal of Medicine.* 324: 384-394.
35. Verbrugh, H.; Verkooyen, R.; Van der, E.; Hustinx, W.; Oostinga, J. and Mintjes-De, J. (1992). Prophylactic ciprofloxacin for catheterassociated urinary-tract infection. *The Lancet.* 339: 946-95
36. Wawer, M.; Sewankambo, N.; Serwadda, D.; Quinn, T.; Paxton, L. and Kiwanuka, N. (1999). Control of sexually transmitted

diseases for AIDS prevention in Uganda: a randomised community trial. *The lancet.* 353: 525-535.
37. Bundrick, W.; Heron, S.; Ray, P.; Schiff, W.; Tennenberg, A. and Wiesinger, B. (2003). Levofloxacin versus ciprofloxacin in the treatment of chronic bacterial prostatitis: a randomized double-blind multicenter study. *Urology.* 62: 537-541.
38. Fass, R. (1987). Efficacy and safety of oral ciprofloxacin in the treatment of serious respiratory infections. *The American Journal of Medicine.* 82: 202.
39. Baumann, M. and Baxendale, I. (2013). An overview of the synthetic routes to the best selling drugs containing 6-membered heterocycles. *Beilstein .J. Org. Chem*. 9: 2265-2319.
40. Emmerson, A. and Jones, A. (2003). The quinolones: decades of development and use. *The Journal of Antimicrobial Chemotherapy*. 51(1): 13-20.
41. Pommier, Y.; Leo, E.; Zhang, H. and Marchand, C. (2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. *Chem. Biol*. 17: 421-433.
42. Borm, P. and Muller-Schulte, D. (2006). Nanoparticles in drug delivery and environmental exposure: same size, same risks. *Nanomedicine*. 1: 235-249.
43. Chifiriuc, C.; Grumezescu, A.; Saviuc, C.; Croitoru, C.; Mihaiescu, D. and Lazar, V. (2012). Improved antibacterial activity of cephalosporins loaded in magnetic chitosan microspheres. *Int. J. Pharm.* 436: 201-205.
44. Filippousi, M.; Papadimitriou, S.; Bikiaris, D.; Pavlidou, E.; Angelakeris, M. and Zamboulis, D. (2013). Novel core-shell magnetic nanoparticles for Taxol encapsulation in biodegradable and biocompatible block copolymers: Preparation, characterization and release properties. *Int. J. Pharm.* 448: 221-230.
45. Liakos, I.; Rizzello, L.; Scurr, D.; Pompa, P.; Bayer, I. and Athanassiou, A. (2014). All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. *Int. J. Pharm.* 463: 137-145.
46. Wilczewska, A.; Niemirowicz, K.; Markiewicz, K. and Car, H. (2012). Nanoparticles as drug delivery systems. *Pharmacol. Rep.* 64: 1020- 1037.
47. Gelperina, S.; Kisich, K.; Iseman, M. and Heifets, L. (2005). The Potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. *Am. J. Respir. Crit. Care Med*. 172: 1487-1490.
48. Baumgartner, J.; Bertinetti, L.; Widdrat, M.; Hirt, A. and Faivre, D. (2013). Formation of magnetite nanoparticles at low temperature: From superparamagnetic to stable single domain particles. *PLoS One*. 8: e57070.
49. Nevozhay, D.; Kanska. U.; Budzynska, R. and Boratynski, J. (2007). Current status of research on conjugates and related drug delivery systems in the treatment of cancer and other diseases. *Postepy. Hig. Med. Dosw*. 61: 350-360.
50. Pereira de Oliveira M.; Garcion, E.; Venisse, N.; Benoit, J.P.; Couet, W. and Olivier, J.C. (2005). Tissue distribution of indinavir administered as solid lipid nanocapsule formulation in mdr1a (+/+) and mdr1a (−/−) CF-1 mice. *Pharm Res* .s 22: 1898–1905.
51. Mahajan, S.D.; Aalinkeel, R.; Law, W.C.; Reynolds, J.L.; Nair, B.B.; Sykes, D.E., *et al*. (2012). Anti-HI nanotherapeutics: promises and challenges for the future. *Int. J. Nanomedicine* .7: 5301–5314.
52. Chen, B.M.; Su, Y.C.; Chang, C.J.; Burnouf, P.A.; Chuang, K.H.; Chen, C.H., *et al*. (2016). Measurement of pre-existing IgG and IgM antibodies against polyethylene glycol in healthy individuals. *Anal Chem*; 88: 10661–10666.
53. Shen, W.; Kim, J.S.; Mitchell, S.; Kish, P.; Kijek, P. and Hilfinger, J. (2009). *5′-O*-D-valyl ara A, a potential prodrug for improving oral bioavailability of the antiviral agent vidarabine. *Nucleic Acids*, *28*: 43–55.
54. Zhang, Y.; Gao, Y.; Wen, X. and Ma, H. (2014). Current prodrug strategies for improving oral absorption of nucleoside analogues. *Asian J. Pharm. Sci. 9*: 65–74.
55. Johnson, T.P.; Frey, R.; Modugno, M.; Brennan, T.P. and Margulies, B.J. (2007). Development of an acyclovir implant for the effective long-term control of herpes simplex virus type-1 infection in Vero cells and in experimentally infected SKH-1 mice. *Int. J. Antimicrob. Agents*, *30*: 428–435.
56. Lee, C.C.; MacKay, J.A.; Frechet, J.M. and Szoka, F.C. (2005). Designing

dendrimers for biological applications. *Nat. Biotechnol. 23*: 1517–1526.
57. Lavanya, S; Hendrik, G.K.; Gleen, E.M.; Thavendran, G. and Raveen, P. (2017). The role of nanotechnology in the treatment of viral infection .*Ther.Adv.Infectious Dis.* 4 (4): 105-131.
58. Rupp, R.; Rosenthal, S.L. and Stanberry, L.R. (2007). VivaGel (SPL7013 Gel): A candidate dendrimer—Microbicide for the prevention of HIV and HSV infection. *Int. J. Nanomed., 2*: 561–566.
59. Amin, R. (2011). Nanotechnology in controlling infectious disease. In: Hunter RJ and Preedy VR (eds) *Nanomedicine in health and disease*. Enfield, NH: Science Publishers,: 167–183.
60. Elechiguerra, J.L.; Burt, J.L.; Morones, J.R.; Camacho-Bragado, A.; Gao, X.; Lara, H.H., *et al*. (2005). Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnol. 3.
61. Lawrence, M.J. and Rees, G.D. (2000). Microemulsion-based media as novel drug delivery systems. *Adv. Drug Deliv. Rev. 45*: 9–121.
62. Heurtault, B.; Saulnier, P.; Pech, B.; Proust, J.E. and Benoit, J.P. (2002). A novel phase inversion-based process for the preparation of lipid nanocarriers. *Pharm. Res .19*: 875–880.
63. Barbaro, G.; Scozzafava, A.; Mastrolorenzo, A. and Supuran, C.T. (2005). Highly active antiretroviral therapy: current state of the art, new agents and their pharmacological interactions useful for improving therapeutic outcome. Curr Pharm Des .11: 1805–1843.
64. Mahajan, S.D.; Aalinkeel, R.; Law, W-C.; Reynolds, J.; Nair, B.B.; Sykes, D.E., *et al*. (2012). Anti-HIV-1nanotherapeutics: Promises and challenges for the future. *Internt. J. Nanomed.*,7 :5301-5314
65. Malakotian, M.; Hosseini, M. and Bahrami, H. (2009). Survey of the parasites of vegetable in Kerman province. Medical Journal of Hormozgan University. 13(1): 55-62.
66. Quadir, M.A.; Morton, S.W.; Mensah, L.B.; Shopsowitz, K.; Dobbelaar, J.; Effenberger, N., *et al*. (2017). Ligand-decorated click polypeptide derived nanoparticles for targeted drug delivery applications. Nanomedicine: Nanotechnology, Biology and Medicine.
67. Rai, M.; Kon, K.; Ingle, A.; Duran, N.; Galdiero, S. and Galdiero, M. (2014). Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects. Applied microbiology and biotechnology, 98(5): 1951-1961.
68. Sarkar, S.; Osama, K.; Jamal, Q.M.S.; Kamal, M.A.; Sayeed, U.; Khan, M.K.A., *et al*. (2017). Advances and implications in nanotechnology for lung cancer management. Current drug metabolism,18(1): 30-38.
69. Datta, A.K.; Datta, R. and Sen, B. (2008). Antiparasitic Chemotherapy. Drug targets in Kinetoplastid parasites, 116-132.
70. Said, D.; Elsamad, L. and Gohar, Y. (2012). Validity of silver, chitosan, and curcumin nanoparticles as anti-Giardia agents. Parasitology research, 111(2): 545-554.
71. Heinlaan, M.; Ivask, A.; Blinova, I.; Dubourguier, H-C. and Kahru, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO 2 to bacteria Vibrio fischeri and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere, 71(7): 1308-1316.
72. Tanwar, J.; Das, S.; Fatima, Z. and Hameed, S. (2014). Multidrug resistance: an emerging crisis. Interdisciplinary perspectives on infectious diseases.
73. Krukemeyer, M.; Krenn, V.; Huebner, F.; Wagner, W. and Resch, R. (2015). History and possible uses of nanomedicine based on nanoparticles and nanotechnological progress. Journal of Nanomedicine & Nanotechnology, 6(6): 1.
74. Bavand, Z.; Gholami, S.; Honary, S.; Rahimi, E.B.; Torabi, N. and Barabadi, H. (2014). In vitro evaluation of the effect of gold nanoparticles on Giardia lamblia cyst.
75. Saad, H.; Soliman, M.I.; Azzam, A.M. and Mostafa, B. (2015). Antiparasitic activity of silver and copper oxide nanoparticles against *Entamoeba histolytica* and Cryptosporidium parvum cysts. J Egypt Soc Parasitol, 45(3): 593-602.
76. Sazgarnia, A.; Taheri, A.R.; Soudmand, S.; Parizi, A.J.; Rajabi, O. and Darbandi, M,S. (2013). ntiparasitic effects of gold nanoparticles with microwave radiation on promastigotes and amastigotes of *Leishmania major*. International Journal of Hyperthermia, 29(1): 79-86.
77. Venier-Julienne, M.; Vouldoukis, I.; Monjour, L. and Benoit, J. (1995). In vitro

study of the anti-leishmanial activity of biodegradable nanoparticles. Journal of drug targeting, 3(1): 23-29.
78. Allahverdiyev, A.M.; Abamor, E.S.; Bagirova, M. and Rafailovich, M. (2011). Antimicrobial effects of TiO2 and Ag2O nanoparticles against drug-resistant bacteria and *Leishmania* parasites. Future microbiology, 6(8): 933-940.
79. Karimi, M.; Dalimi, A.; Jamei, F.; Ghaffarifar, F. and Dalimi, A. (2015). The Killing effect of Silver Nanoparticles and Direct Electric Current Induction on *Leishmania major* Promastigotes In Vitro.
80. Jameii, F.; Dalimi Asl, A.; Karimi, M. and Ghaffarifar, F. (2015). Healing Effect Comparison of Selenium and Silver Nanoparticles on Skin Leishmanial Lesions in Mice. Scientific Journal of Hamadan University of Medical Sciences, 22(3): 217-223.
81. Gaafar, M.; Mady, R.; Diab, R. and Shalaby, T.I. (2014). Chitosan and silver nanoparticles: promising anti-toxoplasma agents. Experimental parasitology, 143: 30-38.
82. Sedighi, F.; Abbasali, P.R.; Maghsood, A. and Fallah, M. (2016). Comparison of therapeutic effect of anti-cryptosporidium nano-nitazoxanide (ntz) with free form of this drug in neonatal rat.
83. Mohapatra, S.C.; Tiwari, H.K.; Singla, M.; Rathi, B.; Sharma, A.; Mahiya, K., *et al*. (2010). Antimalarial evaluation of copper (II) nanohybrid solids: inhibition of plasmepsin II, a hemoglobin-degrading malarial aspartic protease from *Plasmodium falciparum*. JBIC Journal of Biological Inorganic Chemistry, 15(3): 373-385.
84. Tripathy, S.; Das, S.; Chakraborty, S.P.; Sahu, S.K.; Pramanik, P. and Roy, S. (2012). Synthesis, characterization of chitosan–tripolyphosphate conjugated chloroquine nanoparticle and its in vivo anti-malarial efficacy against rodent parasite: A dose and duration dependent approach. International journal of pharmaceutics, 434(1): 292-305.
85. Ponarulselvam, S.; Panneerselvam, C.; Murugan, K.; Aarthi, N.; Kalimuthu, K. and Thangamani, S. (2012). Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. Asian Pacific journal of tropical biomedicine, 2(7): 574-580.
86. Abulaihaiti, M.; Wu, X-W.; Qiao, L.; Lv, H-L.; Zhang, H-W.; Aduwayi, N., *et al*. (2015). Efficacy of albendazole-chitosan microsphere-based treatment for alveolar echinococcosis in mice. PLoS neglected tropical diseases, 9(9): e0003950.
87. Brodaczewska, K.; Wolaniuk, N.; Donskow-Lysoniewska, K.; Doligalska, M. editors. (2013). Chitosan stimulates lymphocyte proliferation during the muscle phase of *Trichinella spiralis* infection in mice. Front Immunol Conference Abstract: 15th International Congress of Immunology (ICI) doi: 103389/conf fimmu.
88. Gherbawy, Y.A.; Shalaby, I.M.; El-sadek, M.S.A.; Elhariry, H.M. and Banaja, A.A. (2013). The anti-fasciolasis properties of silver nanoparticles produced by *Trichoderma harzianum* and their improvement of the anti-fasciolasis drug triclabendazole. International journal of molecular sciences, 14(11): 21887-21898.
89. Guirgis, B.S.; Sá e Cunha, C.; Gomes, I.; Cavadas, M.; Silva, I.; Doria, G., *et al*. (2012). Gold nanoparticle-based fluorescence immunoassay for malaria antigen detection. Anal Bioanal Chem; 402: 1019–1027.
90. Thiramanas, R.; Jangpatarapongsa, K.; Asawapirom, U.; Tangboriboonrat, P. and Polpanich, D. (2013). Sensitivity and specificity of PS/AA-modified nanoparticles used in malaria detection. Microb Biotechnol, 6: 406–413.
91. Wu, J.; Peng, Y.; Liu, X.; Li, W. and Tang, S. (2014). Evaluation of Wondfo rapid diagnostic kit (Pf-HRP2/PAN-pLDH) for diagnosis of malaria by using nano-gold immunochromatographic assay. Acta Parasitol, 59:267–271.
92. Wang, H.; Lei, C.; Li, J.; Wu, Z.; Shen, G. and Yu, R. (2004). A piezoelectric immunoagglutination assay for *Toxoplasma gondii* antibodies using gold nanoparticles. Biosens Bioelectron, 19: 701–709.
93. Javier, D.J.; Castellanos-Gonzalez, A.; Weigum, S.E.; White, A.C.J. and Richards-Kortum, R. (2009). Oligonucleotide-gold nanoparticle networks for detection ofCryptosporidium parvum heat shock protein 70 mRNA. J. Clin. Microbiol., 47: 4060–4066.

94. Weigum, S.E.; Castellanos-Gonzalez, A.; White, A.C.J. and Richards-Kortum, R. (2013). Amplification-free detection of Cryptosporidium parvum nucleic acids with the use of DNA/RNA-directed gold nanoparticle assemblies. J. Parasitol., 99: 923–926.
95. Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S. and Alivisatos, A.P. (1998). Semiconductor nanocrystals as fluorescent biological labels. Science, 281(5385): 2013-2016.
96. Shukla, S.; Arora, V.; Jadaun, A.; Kumar, J.; Singh, N. and Jain, V.K. (2015). Magnetic removal of *Entamoeba* cysts from water using chitosan oligosaccharide-coated iron oxide nanoparticles. Int. J. Nanomedicine, 10:4901–4917.
97. Escosura-Muñiz, A.; Baptista-Pires, L.; Serrano, L.; Altet, L.; Francino, O.; Sánchez, A., *et al*. (2016). Magnetic bead/gold nanoparticle double-labeled primers for electrochemical detection of isothermal amplified *Leishmania* DNA. Small, 12: 205–213.
98. Das, S.; Roy, P.; Mondal, S.; Bera, T. and Mukherjee, A. (2013). One pot synthesis of gold nanoparticles and application in chemotherapy of wild and resistant type visceral leishmaniasis. Colloids Surf B Biointerfaces, 107: 27–34.
99. Kumar, R.; Sahoo, G.C.; Pandey, K.; Das, V. and Das, P. (2015). Study the effects of PLGAPEG encapsulated amphotericin B nanoparticle drug delivery system against *Leishmania donovani*. Drug Deliv, 22: 383–388.
100. 100. Chávez-Fumagalli, M.A.; Ribeiro, T.G.; Castilho, R.O.; Fernandes, S.O.; Cardoso V.N.; Coelho. C.S., *et al*. (2015). New delivery systems for amphotericin B applied to the improvement of leishmaniasis treatment. Rev Soc Bras Med Trop, 48: 235–242
101. Sadhasivam, S.; Shanmugam, P. and Yun, K. (2010). Biosynthesis of silver nanoparticles by Streptomyces hygroscopicus and antimicrobial activity against medically important pathogenic microorganisms. Colloids and Surfaces B: Biointerfaces, 81(1): 358-362.
102. El-Moslemany, R.M.; Eissa, M.M.; Ramadan, A.A.; El-Khordagui, L.K. and El-Azzouni, M.Z. (2016). Miltefosine lipid nanocapsules: intersection of drug repurposing and nanotechnology for single dose oral treatment of pre-patent *schistosomiasis mansoni*. Acta Trop, 159:142–148.