Journal of Antimicrobial Agents
ISSN: 2472-1212

Vazini and Esboei, J Antimicrob Agents 2018, 4:2
DOI: 10.4172/2472-1212.1000172

Research Article Open Access

The Investigation of Nanoparticles of Gold's Fatality Effect on *Toxoplasma gondii* Parasite in an *In vitro* Study

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Received date: June 02, 2018; **Accepted date:** June 18, 2018; **Published date:** June 25, 2018

Abstract

Background: Toxoplasmosis caused by *Toxoplasma gondii* which is an obligate intracellular parasite with world-wide distribution. The use of nanoparticles is of the methods which have recently been applied in the anti-parasitic studies and have showed the acceptable results.

Objective: The aim of this study was to evaluate the anti-toxoplasma ability of gold nanoparticles *in vitro*.

Materials and Methods: In this experimental study, the concentrations of 100, 200, 400, 800 and 1000 ppm of gold nanoparticles at different times of 30, 60, 120 and 180 minutes were separately used to evaluate the effect of nanoparticles on the RH strain of tachyzoite of *Toxoplasma gondii*. The number of live tachyzoites at each concentration as well as at positive and negative controls were determined using microscopy method and trypan blue 1%. The data obtained were analyzed by SPSS 18 software using ANOVA test.

Results: The percentage of live tachyzoites was decreased with increasing of time from 30-180 minutes and increasing of the concentration of gold nanoparticles from 100 to 1000 ppm. The best results were obtained in a concentration of 1000 µg/mL; so that it was able to destroy 100% of tachyzoite in this concentration.

Conclusion: The results showed that gold nanoparticles are able to effectively reduce the tachyzoites of *T. gondii* and it can be considered as a viable alternative to treat the toxoplasmosis.

Keywords: *Toxoplasma gondii*; Tachyzoites; Gold nanoparticles; *In vitro*

Introduction

Toxoplasmosis is an infection caused by *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular parasite with world-wide distribution [1]. This parasite can cause infection in humans and animals. Transmission can be done in adventitious or congenital forms [2]. In adventitious infections eating raw or uncooked meat consisting of tissue cysts, eating vegetables or drinking water with oocytes are the main route of transmission [3]. In congenital infection, the transference of parasite through placenta causes toxoplasmosis infection [2]. Toxoplasmosis in most of cases is asymptomatic, but in immune deficiency especially, it is recognized as an important opportunist that can cause some clinical manifestations such as encephalitis [4]. Based on the published reports, this infection causes the death of 10% of people who suffer from AIDS in Europe, and 30% of people who suffer from AIDS in America [4].

Abortion, retiniocorticoide, microcephaly, hydrocephalus, cerebral calcification, or chorioretinitis are the clinical manifestations of congenital infection [2]. These damages can appear in forms which cause severe complications like mental retardation, deafness, blindness, etc. gold standard treatment of toxoplasmosis is using pyrimethamine-sulfadiazine compound, although using of these drugs has some risks and side effects [5,6]. Some side effects like trombositopeni, leukopenia, and megaloblastic anemia have been reported in prolonged use of pyrimethamine [6,7]. Furthermore, using of pyrimethamine-sulfadiazine compound in people who suffer from AIDS has many complications and is toxic [8]. The above mentioned items impel scientists and researchers to look for a proper substitution for treatment of this infection. Nanotechnology and using of nanoparticles in different sciences and expertise have become important in recent decades and it has provided an important setting for scientists to study on. Nanoparticles are some materials that their sizes are less than 100 nanometer [9,10].

Different methods of synthesizing have been introduced for these materials, but the method of chemical decreasing has been introduced as the most applicable method of synthesizing of these kinds of materials [11]. The nanoparticles of gold are among nanoparticles that are applied effectively in medical science and treatment, for instance among its operations: anti HIV, anti-malaria, anti-joints pain, and etc. can be mentioned [12-15]. Bavend et al. showed the anti-giardia effects of gold's nanoparticles and discovered that these nanoparticles can favorably extirpate this parasite. In this study gold's nanoparticles in concentration of 0.3 mg/ml increased from 62% in 5 minutes to 96% in 180 minutes of tachyzoites. Furthermore, by increasing the time of adjacency, the highest fatal effect of gold's nanoparticles was from 78% in density of 0.05 mg/lit and up to 96% in density of 0.3 mg/ml [15]. Therefore, because of the importance of toxoplasmosis and the lack of

a study about anti-toxoplasmosis properties of gold's nanoparticles, the present study have been conducted with the purpose of investigation of nanoparticles of gold's effect on *T. gondii* parasite in an *in vitro* study.

Methods

Preparing of gold's nanoparticles

Gold's nanoparticles in different concentrations of 100, 200, 400, 800 and 1000 ppm were used in the present study for investigation of their effect of anti-toxoplasmosis. Gold's nanoparticles were prepared by standard method in pharmaceutics group of pharmacy faculty of Mazandaran's Medical Sciences University. First of all the *Penicillium citrinum* was cultured in fluid medium of Czapek dox broth and it was incubated for 10 days at 28°C with 200 rpm. Then, the grown fungi were parted from the medium. Afterwards, the amount of 100 ml of 1 millimolar of gold's chloride was added to 100 ml of fluid medium (supernatant) and again it was incubated for 24 hours at 28°C. After the production of gold's nanoparticles, the sizes of particles were confirmed by electronic microscope. Furthermore, pyrimethamine-sulfadiazine compound and dimethyl sulfoxide (DMSO) was investigated as positive and negative control, respectively [16].

The culture of toxoplasmosis tachyzoites

Rh standard strain was provided from parasitology group of public health faculty of Tehran University of Medical Science and it was intra-peritoneally injected in mice. Tachyzoites were collected by peritoneum ablution by use of normal saline and were centrifuged for 2 minutes in 200 rpm in order to obtain pure tachyzoites without cells. Tachyzoites were washed three times by sterilized PBS. The number of takizoyites was counted by Neobar Lam. Finally 107 Tachyzoites per a milliliter were used for the anti-toxoplasmosis effects of gold's nanoparticles [17].

The assessment of anti-toxoplasmosis effects

In current work, 200 microliter of gold's nanoparticles in different densities of 100, 200, 400, 800, and 1000 ppm was added to 200 microliter suspension containing of tachyzoites. After incubation at 37°C, the percentage of parasites' survival in intervals of 30, 60, 120, and 180 minutes were measured by microscope method and trepan blue 1% as a vital staining. All phases of this study were done in triplicate. Finally the obtained data of the study were analyzed by SPSS 18 software and X2 statistical test [18].

Cytotoxicity assay

The cytotoxicity of the different concentrations of gold's nanoparticles (0.3 to 100 ppm) was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay on Vero cells as described by Mosmann T. In current study, from the suspension of 1 × 104 Vero cells/mL, 100 µL were added to each well of a 96-well plate and incubated at 37°C. Consequently, 100 µL of each concentration of gold's nanoparticles were added. Negative control was cell suspension without and treatment and positive control was cell suspension and pyrimethamine and conventional drug. After 24 hours of incubation, 100 µL of MTT-PBS solution in ratio of 1:9 were added to each well, aluminium foil was used as cover and left it for 4 hours of incubation. After incubation time, 100 µL of DMSO was used to solubilize the purple MTT formazan [19]. Plates were shaken and the absorbance was measured at 540 nm and the growth inhibition (GI) was considered using formula below:

A treatment=Absorbance of treatment

A control=Absorbance of control

Results

According to Table 1, the percentage of alive takizoyites decreased by increasing the time of incubation from 30 minutes to 180 minutes and increasing the concentration of gold's nanoparticles from 100 to 1000 ppm. The concentrations of 800 and 1000 ppm gold's nanoparticles are more effective, and by increasing the time of contact, all tachyzoites were killed (Figure 1).

Nonetheless, the best results were seen in density of 1000 ppm of gold's nanoparticles after 120 and 180 minutes. The comparison of our results to negative and positive controls (pyrimethamine) showed the ability of gold's nanoparticles in decreasing the number of alive tachyzoites ($p<0.05$).

Time and concentrations	30 minutes	60 minutes	120 minutes	180 minutes
100 ppm	85	63	49	17
200 ppm	61	54	31	6
400 ppm	47	36	19	0
800 ppm	34	14	7	0
1000 ppm	28	9	0	0
NC	100	100	100	100
PC	23	0	0	0
PC: positive control (pyrimethamine), NC: negative control.				

Table 1: The effects of nanoparticles of gold on *T. gondii* parasite at different time and concentrations in an *in vitro* study.

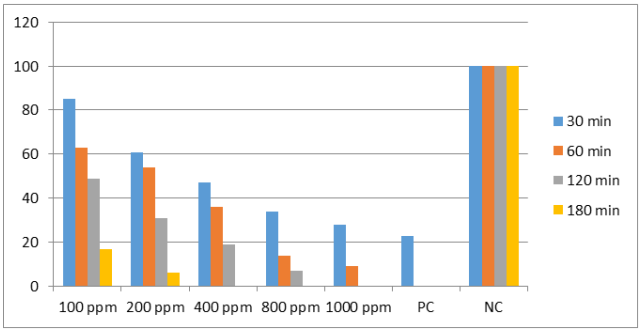

Figure 1: There is no significant difference between the concentration of 1000 ppm and positive control; PC: Positive Control (pyrimethamine), NC: negative control.

The results showed that gold's nanoparticles had very low cytotoxicity effects on Vero cells with IC50 at 12.36 ppm.

Discussion

In this study different concentrations of gold's nanoparticles were used in different times of adjacency against *T. gondii*. It was found that the percentage of alive tachyzoites decreased by increasing the time of encountering from 30 minutes to 180 minutes and by increasing the density of gold's nanoparticles from 100 to 1000 picomole.

The decreasing rate of tachyzoites' percentages was better in densities of 400, 800, and 1000 ppm gold's nanoparticles; however, in times of more adjacency with these densities, all tachyzoites were killed. The density of 1000 ppm was more effective than other concentrations and the percentage of survival in this concentration reached from 23% in 30 minutes to 0% in 120 minutes that were approximately in accordance with the positive control ($P>0.05$).

The application of gold's nanoparticles in the present period turns into a popular option in scientific and industrial contexts; medical science is not an exception among them and different explorations are conducted in this regard in medical science [20,21]. Different materials have been used till now for nanoparticles synthesis and their applicability have been utilized in medical science and for treatment of some infections that we can point to silver, chitosan, gold, etc. among them [15,22-25]. Gold's nanoparticles have been used against different parasites in several studies. This particle was utilized against leishmaniasis in Mohebali and his colleagues' study. In their study, they investigate two densities of gold's nanoparticles (4 and 40 micro grams on milliliter) against the cutaneous leishmaniasis, the Iranian *L. major* strain was investigated in 61 mice. The obtained results showed the significant decrease of the number of Amastigotes parasite in wound. Moreover, it was found that the rate of mice's fatality decreased [26].

Bavend and his colleagues in 2013 conducted a study about the properties of anti-giardia of gold's nanoparticles and it was discovered that these nanoparticles can favorably extirpate this parasite. In this study the mean of fatal percent of gold's nanoparticles in density of 0.3 mg/ml increased from 62% in 5 minutes to 96% in 180 minutes. Furthermore, by increasing the time of adjacency, the highest fatal effect of gold's nanoparticles was from 78% in density of 0.05 mg/L. The above mentioned studies showed that gold's nanoparticle has the ability to properly eradicate the parasites that the results are a confirmation to the present study, and the effect of gold's nanoparticle on toxoplasma [15]. Also, other cases and studies have been conducted about the anti-parasite strength of gold's nanoparticles. Among them we can point to the comparison of the effects of selenium and silvers' nanoparticles in amendment of cutaneous leishmaniasis due to *L. major* in mouse which was conducted by Allahverdiyev and his colleagues [27]. The results showed that the wounds' size of the group which was under treatment of nano selenium did not differ significantly with the control group, but the wounds' size of the group which was under treatment of Nano silver differed significantly with the negative control but not equal as glucantime. Moreover, based on the study conducted by Boakye and his colleagues about the fatal effect of silver's nanoparticles against visceral leishmanisis, it was found that this nanoparticle had significant effect in eradicating of parasite [28].

Furthermore, the anti-parasitic effect of nanoparticles on helminths was investigated in the study of Mamoon et al. They produced silver's nanoparticles by method of decreasing AgNO3 in presence of NaBH4 and investigated its anti helminths activity. Their results showed the favorable strength of nanoparticles in annihilating of parasites as they could destroy helminths in an almost short time.

Conclusion

It can be understood that the applicability of nanoparticles in treatment of infections and diseases is increasing and this is because of their high effects that was observed in this study and previous ones. As it was found in the present study, gold's nanoparticles showed favorable effects against toxoplasma parasite in comparison to pyrimethamine-sulfadiazine compound and were able to eliminate it completely. Moreover, it was found that they showed their efficacy in an almost short time (180 minutes). The findings showed that gold's nanoparticle can have significant effects as anti-toxoplasmosis and also it can be used as a proper substitution for usual treatment of this infection in conducting the supplementary examination in an *in vivo* situation.

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