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EFFECT OF SYNTHETIC GOLD NANOPARTICLES ON IMMUNE PROTEINS OF TOXOPLASMOSIS PATIENTS

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ABSTRACT

Toxoplasma gondii is an obligate intracellular protozoan that causes toxoplasmosis in human and animal. This parasite is worldwide spread and about one third of people are seropositive. Toxoplasmosis in immune compromised patients causes serious symptoms. A total of 14 antenatal women were included in the study. The study group comprised of antenatal women with bad obstetric history (BOH) in the age group of 32-38 years and a total of 10 blood samples and 6 lung lavage samples from Kidney transplanted patients admitted to the infectious wards and the patients undergoing bronchoscopy were also collected. All the samples were screened by ELISA for Toxoplasma specific Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies. Of the 14 antenatal women in the study group, 6 (42.3%) were seropositive for *Toxoplasma* specific IgG antibodies ($P < 0.005$), 5 (35%) were seropositive for Toxoplasma specific IgM antibodies ($P < 0.005$) and 4 (28.5%) were seropositive for both IgG and IgM antibodies ($P < 0.005$). Gold nanoparticles were purchased directly from Sigma Aldrich, further characterized with XRD, FTIR, SEM-EDX techniques. It was identified that purchased products containing 54 nm particles and used against isolated *T. gondiii* samples. Results clearly indicate the Au NPs significantly reduced the parasite Growth. The Au NPs significantly reduced the parasite viability by 94% with EC50 values of <7µg/mL for AuNPs. Accordingly, Au NPs can be considered as a promising alternative to the standard therapy for treating toxoplasmosis.

INTRODUCTION

Toxoplasmosis is caused by a protozoan parasite, *Toxoplasma gondii*. Humans and other warm-blooded animals are its hosts and the disease has a worldwide distribution. Depending on the age at the time of primary infection, the virulence of the strain of T.gondii, and the immune status of the host, symptoms vary from no symptoms to death [1]. There is no vaccine against toxoplasmosis for use in humans at present. Three recent studies, however, indicated that the prevalence may be a lot higher than previously reported. Of 123 serum samples obtained from 27 villages surrounding a hospital in Kulapur, U.P., 18% had IHA antibodies to T. gondii at a titre of 1:64. Prevalence increased with age. Of importance is that 4 of 41 (10%) children below 9 years of age were seropositive suggesting exposure to contaminated food or congenital infection [2]. In another survey, of 100 asymptomatic women (83 of them 16 to 50 years old) from villages in Almora district, 77% had IHA antibodies in titres of 1:162 or more (20 of them had titres of 1:18 to 1:54) [3]. Even if one discounted lower IHA titres as nonspecific, this is a high prevalence of *T. gondii* in women of child bearing age. In another study of children from a residential tribal school in Maharashtra, 35.1% of 194 persons had *T.gondii* antibodies. Of particular interest is a 30% prevalence rate in 10 to 12 year old children [4]. Rawal [5] found that the prevalence of *T.gondii* in vegetarians (37.8% of 141) and nonvegetarians (37.4% of 246) was similar. This study is important because results were based the dye test. Epidemiologic studies from other parts of world indicate that the ingestion of undercooked meat is an important means of transmission of *T. gondii*. For example, the very high (70 to 90%) seroprevalence of *T. gondii* in Paris, France is attributed to eating undercooked meat [6]. For Treatment of the parasite, there are no effective drugs in the market to kill *T.gondii* cysts in tissues. Although spiramycin, clindamycin, azithromycin, and sulfanomides in general are antitoxoplasmic, sulfadiazine (SD) and pyrimethamine (PY) still remains the drug of choice [7]. Current treatment options for toxoplasmosis patients are limited. They include the use of antimalarial drugs or antibiotics, which often cause significant side effects, including bone marrow suppression and rash [8]. The need for nontoxic medicines is further emphasized by the prolonged courses of therapy required for treatment and suppression of infection. 5

Consequently, toxoplasmosis represents a large global burden that is further enhanced by the shortcomings of the current therapeutic options. These factors underscore the urgent need for better anti-Toxoplasma drugs and/or new approaches in the treatment of toxoplasmosis. An ideal anti-Toxoplasma drug would be potent, nontoxic, and able to eliminate latent infection. Immunosuppressed patients infected with Toxoplasma gondii show symptoms such as diffuse encephalopathy, meningoencephalitis, extensive brain lesions and pneumonia. Toxoplasma pneumonia in immunosuppressed individuals caused55% of the mortalities. Patients received organ transplant administrate immunosuppressive drugs to prevent the rejection of the organ. Immunosuppression influences the patient resistance to a variety of opportunistic pathogens like *Toxoplasma gondii.* Early diagnosis of toxoplasmosis along with proper treatment can prevent serious consequences of infection [9].

There is expanding interest in deploying nanotechnology for biomedical purposes, and some reports suggest that nanoparticles (NPs) could form the bulk of future treatment strategies for various diseases. As NPs are recognized as foreign structures, they are eliminated by phagocytising cells. Thus, it should be easier to treat microorganisms which multiply within macrophages, because the nanoparticles would be concentrated automatically in the desired target cells. Metallic NPs are already being exploited for biomedical applications, due to their nanoscale size and other remarkable properties, including surface reactivity [10]. These particles can produce reactive oxygen species (ROS) that have the ability to kill infectious agents. Furthermore, the small size of NPs allows them to transverse membrane barriers, leading to greater reactivity. In addition, NPs could be accumulated in tissues, thereby presenting a formidable platform to target *T. gondii* cysts in host tissues [1]. Of special interest are the metal NPs such as gold and silver, which have antimicrobial activity ant parasitic activity and other bioactivities, including the selective inhibition of some enzyme activities. The versatility of metal NPs makes them an attractive choice to be explored further as antiparasitic agents, particularly against toxoplasmosis. Here, we explored the potential of metal NPs as novel anti-T. gondii agents using in vitro experimental infection models. Gold coatings are generally used for the preparation of bimetallic particles, containing a magnetic

core as platform for surface functionalization. Some strategies reported in literature describe different core-shell NPs covered with this metal [12]. Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also common in lateral flow immunoassays, a common household example being the home pregnancy test. Gold-coated nanoparticles (Au-shelled NPs) have been particularly designed for biomedicine, especially for drug delivery [13] and cancer applied hyperthermia treatments [14]. There are controversial studies concerning the toxicological effects of engineering gold nanoparticles. A study by Hashimoto *et al.* compared the exposure of cultured macrophages RAW264.7 to AuNPs with AgNPs. Although an inflammatory response was observed for both the Au- and Ag-NPs, the harmful cytotoxic effects of AuNPs were smaller than those of the Ag-NPs [15]. However, as described for other kinds of nanomaterials, the interaction between cells and Au nanoparticles could be mediated also by unspecific adsorption of serum proteins onto the gold surface [16]. The aim of this study was to identify and determine the prevalence of *Toxoplasma gondii* and an attempt was made to test the use of gold nanoparticles against the *T. gondii* cysts isolated from the clinical samples along with AgNPs characterization studies.

EXPERIMENTAL

Toxoplasmosis patient identification and sample collection

A total of 14 antenatal women were included in the study. The study group comprised of antenatal women with bad obstetric history (BOH) in the age group of 32-38 years and 16 patients selected for study, 6 were female (37.5%) and 10 male (62.5%). The mean age was 31.5 years. All participating women were administered a questionnaire (Adopted from Singh S et al., 2014) [17] before collection of blood sample, which provided information about their age, marital status, education, socioeconomic level, type of accommodation, eating habits, contact with pet and stray animals, exposure to soil and present/past obstetric history. Women having severe metabolic and autoimmune disorders, such as rheumatoid arthritis or immune deficiencies including AIDS, cancer and those on immune suppressive therapies etc., were excluded. Through venipuncture, 5 mL of blood sample was collected in a sterile container and serum was separated at the participating centers. The separated serum samples were stored locally at $4^{o}C$ for a maximum period of 72 hours during the

period of September 2017 to October 2018 from the infectious hospital (ID), Hyderabad. Data from the questionnaire was entered in an excel sheet. The laboratory technician was blinded on participant clinical or socio-demographic details. The study coordinator (author) evaluated the exclusion and inclusion criteria. However, for the present study only 14 women were selected.

Ethical aspects: Informed written consent was obtained from all participating women who agreed to participate in the study. The study followed the STROBE guidelines (Singh S et al., 2014).

Anti-Toxoplasma IgG and IgM antibody detection assays: Serum samples were assayed for anti-Toxoplasma IgG and IgM antibodies by a commercially available Vitek Immuno-Diagnostic Assay System, strictly following the manufacturer's instructions. All IgG and IgM positive samples were tested for IgG avidity using the same technology , as published previously [18].

Statistical analysis: Data was entered in Excel sheet and imported into SPSS statistical program for analysis.

Gold Nanoparticle: Gold Nanoparticles Purchased from Sigma-Aldrich (via Online) [19] in 100ml pack as per the products specifications λ_{max} 536-538 nm, diameter 100 nm product is ideal for the detection of proteins, pollutants, and other molecules label-free.

Characterization: Characterization of obtained AuNPs in the colloidal solution was performed by using UV-Visible spectroscopy. A small aliquot from Ag NPs was taken in a quartz cuvette and observed for wavelength scanning between 200 to 900 nm with distilled water as a reference. The UV-Vis absorption spectrum of the sample was performed in Perkin Elmer Spectrophotometer, at different time 2hrs, 12hrs, 24 hrs and 48 hrs min. Infrared spectra (IR) were obtained with a FTIR spectrophotometer (IRAffinity, Shimadzu) from 400 to 4000 cm^{-1}. The SEM images and the elemental analysis were recorded using the Scanning Electron Microscopy (SEM) model FEI Quanta 200 FEG high microscopy resolution with EDAX Energy Dispersive Spectroscopy (EDS) and X-ray diffraction measurements were carried out on the Philips Xpert pro XRD system (DY 1650).

The assessment of anti-toxoplasmosis effects

In current work, 200 microliter of gold's nanoparticles in different densities of 50,100, 150, 200, and 500 ppm was added to 500 microliter suspension containing of *T. gondii*. After incubation at 37°C, the percentage of parasites' survival in intervals of 1hr, 2hr,

3hr, and 4hrs were measured by microscope method and trepan blue 1% as a vital staining. All phases of this study were done in triplicate. Finally, obtained data of the study were analyzed by SPSS 10 software.

RESULTS AND DISCUSSION

Au NPs Characterization

UV-Vis Studies: A UV-vis analysis for the synthesized gold nanoparticles is shown in Figure 1. The concentration and stability of AuNPs was indicated from the high absorbance value, smallest maximum wavelength (□max), and sharpest peak shape. Absorbance indicates the amount of substance to absorb light. Wavelength indicates the amount of energy needed by nanoparticles to conduct surface plasmon resonance (SPR). The great size of nanoparticles results in a smaller bandgap. Therefore, the energy required to conduct electronic transition was getting smaller and shifted towards a higher value. Sharp absorption peaks indicate that the formed nanoparticles have a homogeneous size. The UV-Vis absorption spectra of AuNPs at different time intervals are shown in Figure 1. AuNPs had a good stability for 24 hours observed at ʎ max of 532–534 nm as shown in Figure 2. The observation of stability after 24 hours showed larger ʎ max shift and a significant decrease of absorbance, indicating the occurrence of agglomeration [20].

SEM-EDX technique is used to study the size and morphology shape of the nanoparticles. An SEM image of AuNPs is displayed in Figure 2(a) and particle size of nanoparticles was ranged (56-60) nm and gold nanoparticles have clear and smooth circular surfaces. The presence of gold was confirmed by EDS (Figure 2b).

XRD analysis is used to evaluate of a crystallite size of the aggregate nanoparticles. Figure 3 shows the XRD pattern of AuNPs nanoparticles purchased from Sigma Aldrich Company. The diffraction peaks of Au NPs correspond to (100), (002), (101), (102), (110), (103), (112) and (201) planes [21]. The 2-theta values of (100), (002) and (101) lines of Figure 2 of the crystal planes are located at 32.01, 33.6 and 35.4°. Some peaks were also noticed which associated with impurity on Au NPs. The average crystallite size of the Au NPs calculated using Debye Scherer formula was about 56 nm.

FTIR is an important study for the quick identification of functional group identification. For Au NPs, The FTIR peaks (Figure 4) were observed at 3304 cm^{-1} that assigned to the stretching vibration of OH (may be moisture, Amide etc.,); at 2925 cm^{-1} that approximate assigned C–H and C–H

(methoxy compounds) stretching vibration; at 1654 cm^{-1} and at 1104 cm^{-1} that assigned to having approximate vibration of (-NH) -C=O group and for C–C and C–N stretching respectively [22].

The assessment of anti-toxoplasmosis effect of AuNPs

According to Table 1a (blood Samples) and Table 1 b (lung lavage), the percentage of alive *T. gondii* parasite decreased by increasing the time of incubation from 1hr to 4hrs and increasing the concentration of gold's nanoparticles from 50 to 500 ppm. The concentrations of 200 and 500 ppm AuNPs are more effective, and by increasing the time of contact, all *T. gondii* parasite were killed (Figure 5). Nonetheless, the best results were seen in density of 500 ppm of gold's nanoparticles after 12 hrs. The comparison of our results to negative and positive controls showed the ability of gold's nanoparticles in decreasing the number of alive parasite ($p<0.05$).

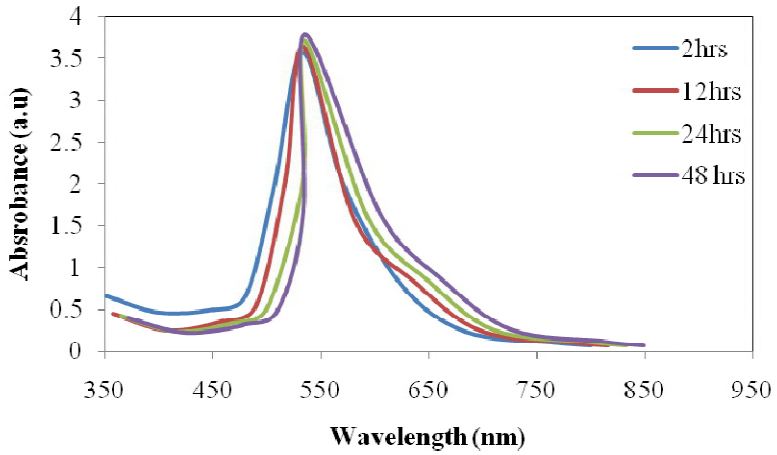

Figure 1: UV-Vis absorption spectra of AuNPs

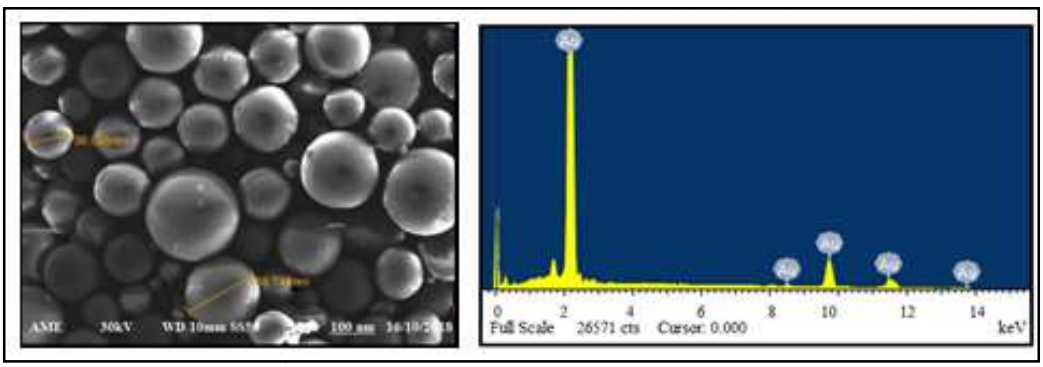

Figure 2 (a): SEM image of AuNPs (left) (b) EDX spectra of AuNPs (right)

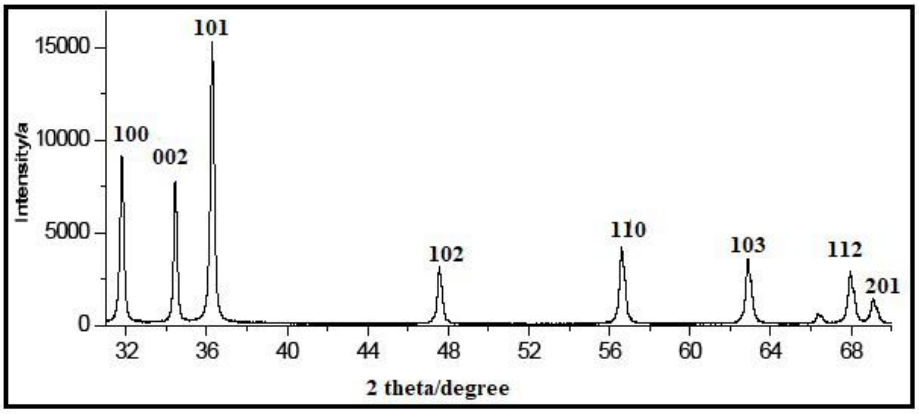

Figure 3 XRD pattern of Gold nanoparticles

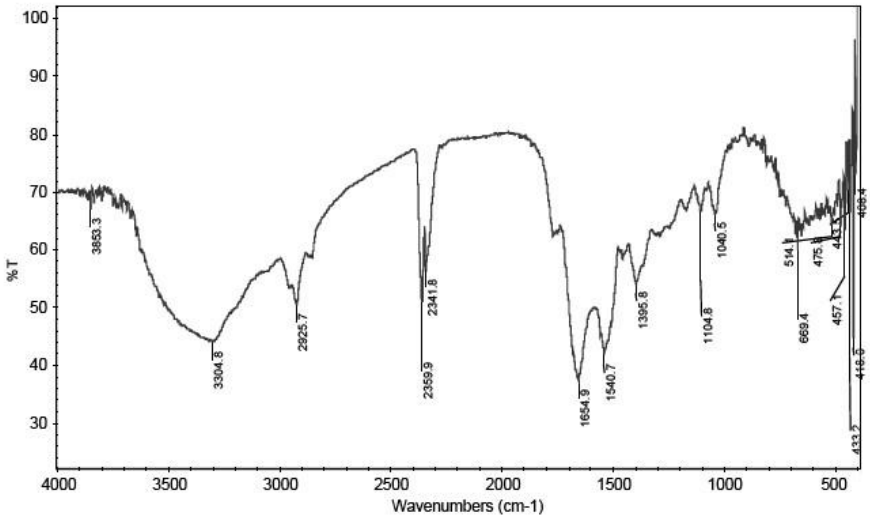

Figure 4: FT IR spectra of AuNPs

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

Time→ concentration of AuNPs	% of Toxoplasma gondii in blood samples			
	1hr	2hrs	3hrs	4hrs
50	81.40	68.38	34.19	3.42
100	74.10	32.60	7.82	0.63
150	22.70	9.99	2.40	0.19
200	12.40	1.24	0.01	0.00
500	2.10	0.01	0.00	0.00

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

	% of *Toxoplasma gondii*			
Time→ concentration of AuNPs	**1hrs**	**2hrs**	**3hrs**	**4hrs**
50	**83.19**	**69.88**	**34.94**	**3.49**
100	**75.73**	**33.32**	**8.00**	**0.64**
150	**23.20**	**10.21**	**2.45**	**0.20**
200	**12.67**	**1.24**	**0.01**	**0.00**
500	**2.15**	**0.01**	**0.00**	**0.00**

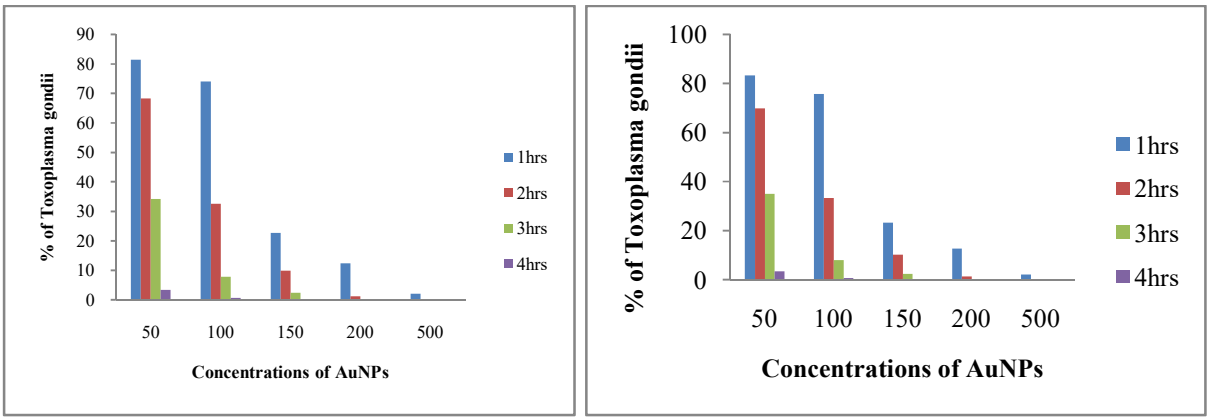

Figure 5: Schematic representation of concentration, time vs *T. gondii* in blood samples (left) and lung lavage (right) samples

In this study different concentrations of AuNPs were used in different times of adjacency against *T. gondii*. It was found that the percentage of alive parasites decreased by increasing the time of encountering from 3 hrs to 4 hours and by increasing the density of AuNPs from 200 to 500ppm. The decreasing rate of T. gondii percentages was better in AuNPs concentration of 200 and 500 ppm AuNPs; however, in times of more adjacency with these densities, all *T. gondii* were killed. The concentration of 500 ppm was more effective than other concentrations and the percentage of survival in this concentration reached from 81 to 2.1 % in blood samples, 83 to 2.15 % in lung lavage samples, finally at 4hrs it was zero percent in teo types of samples accrodance ith 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 [23] is not an exception among them and different explorations are conducted in this regard in medical science. 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 [24]. among them [25].

Gold's nanoparticles have been used against different parasites in several studies.

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 both blood and lung lavage samples.

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