The Impact of Gold and Silver Nanoparticles on The DNA Yield of *Mycobacterium Tuberculosis* MTB

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Abstract

The objective of this research study whether nanoparticles of gold (Au NP) and nanoparticles silver (Ag NP) have an effect on the yield of DNA from Mycobacterium tuberculosis (MTB) in this experiment when it was extracted DNA from MTB using gold and silver nanoparticles at different concentrations (l0µl, 50µl and 100µl) then showed a significant impact on the yield of DNA, especially gold nanoparticles compared with DNA extracted without any of the nanoparticles.by Using a graph obtained from the REALTIME qPCR, which became very clear when the control sample (MTB without any nanoparticles) showed a high value for the Ct (cycle threshold), which meant that low quantities of DNA. While samples containing nanoparticles were low in Ct which meant a high yield of DNA was shown. Ct values also decreased when the concentration of nanoparticles increased, eventually we conclude from the above experiment that nanoparticles showed high yield of MTB DNA. These results are likely due the interaction of the nanoparticles utilized with the cell wall and cell membrane of bacterium, which has facilitated the yield of DNA. Further refinement can be to the above said method and the nanoparticles could possibly use as an effective component of Nucleic acid extraction kits for molecular diagnosis and research so that a superior yield is obtaine.

***Keywords**: gold nanoparticles; silver nanoparticles; MDA.*

Introduction

Nanotechnology is expectant to open new methods to stop disease using atomic scale tailoring of materials, between the most auspicious nanomaterials with features of antimecrobial are nanoparticles, which show evelated the activity of chemical because of their big surface to size ratio and crystallographic surface body [(1).] the examination of bactericidal nanomaterials is especially timely considering the recent elevated of novel resistant races of bacteria to the stronger antibiotics [(2),] This has propelled look into in the notable action of silver and gold particles and their mixes, including silver and gold nanoparticles. The measure of nanoscale Gold and Silver is expanding rapidly in purchaser items the capacity to combine these particles for an immense scope improves [(3).] Gold and Silver nanoparticles (NPs) are poisonous to microscopic organisms, and are starting at now used in everything from restorative devices to display socks and garments washers to hinder microbial development. Silver is a particularly poisonous overwhelming metal as it meddles with the electron transport chain and ties to DNA [(4).] Certain microscopic organisms has appeared to amass or accelerate silver into silver metal or other insoluble structure. Bactericidal lead of nanoparticles is ascribed to the nearness of electronic effects that are achieved because of changes in neighborhood electronic structures of the surfaces as a result of smaller sizes [(5).]Ionic silver determinedly associates with thiol gatherings of indispensable compounds and inactivates them, It has been prescribed that DNA loses its replication capacity once the bacterium are treated with silver ions [(6).] Two dimensional electrophoresis and proteins ID investigation of antibacterial activity of

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silver nanoparticles have unveiled gathering of envelope proteins antecedents. Silver nanoparticles destabilize plasma layer potential and exhaustion of levels of intracellular adenosine triphosphate (ATP) by focusing on bacterial film bringing about bacterial cell death [7].

Mycobacterium tuberculosis: Mycobacteria are Gram-resistant (waxy cell wall), non-motile, pleomorphic poles, identified with the Actinomyces. Most Mycobacteria found in environments, for example, water or soil. By and by, a couple are intracellular pathogens of creatures and people. Mycobacterium tuberculosis, alongside M. bovis, M. africanum, and M. microti all explanation the contamination known as tuberculosis (TB) and are people from the tuberculosis species complex [6].Every individual from the TB complex is pathogenic, yet M.tuberculosis is pathogenic for people, anyway M.tuberculosis is pathogenic for individuals while M. bovis is commonly pathogenic for animals. There are known occurrences of this bacterium being found in the mummies of antiquated Egypt [7].

There are four imperative sorts of mycobacterium: Mycobacterium tuberculosis, which is the most unavoidable sort, mycobacterium bovis, mycobacterium africanum, and mycobacterium microti. This particular sort of minuscule living beings is interesting a direct result of the thicker degree of lipids on the cell divider [8]. Given that, the Mycobacterium tuberculosis gram recolor is insufficient, the most ideal approach to know whether a patient has mycobacteria in his blood is to take a gander at it under a magnifying lens. Under [9]. Mycobacterium tuberculosis disease transmitted through the sputum, which is also the primary spot that you can obviously perceive the contamination [10]. Essentially having the microscopic organisms in your blood isn't sufficient to turn out to be sick be that as it may. Regularly, the microscopic organisms will be unmistakably conspicuous in the blood a long time before the injured individual shows any side effects of the dynamic tuberculosis. This inactive tuberculosis contamination, or LTBI, in like manner can't be transmitted to some other individual as the microscopic organisms are to a great extent kept up in granulomas which shield them from influencing the body [11].

Materials and methods

Materials

Various concentrations of Au and Ag nanoparticles such as l0µl, 50µl and 100µl were used in the DNA extraction process of MTB culture cells. The extraction procedure was carried out as per the protocol explained and following materials were used:

- MTB culture cells(The MTB culture was obtained from the patient's sputum, suffering from Tuberculosis).
- Nanoparticals: gold nanoparticle (Au NP) and Silver nanoparticle (Ag NP), in different concentrations (l0µl, 50µl and 100µl) of each one respectively,

Table1: Showing the product data of (Au ,Ag) Nanoparticles

Nanoparticals Formula	Average Particle Size nm	Appearance Form	CAS Number	Purchased From
Au NP	50 nm	Suspension	753645	Sigma-Aldrich Us
Ag NP	40 nm	Liquid Yellow	730807	Sigma-Aldrich Us

- DNA extraction solution
- Seegene Anyplex (MTB/NTM Real Time PCR kit).
- Real Time PCR (Cepheid Smart Cycler).

Methods

DNA quantification by real-time PCR:

In molecular biology, real-time polymerase chain reaction, likewise called quantitative continuous polymerase chain response (qPCR) or dynamic polymerase chain response is a lab procedure dependent on the PCR, which is use to enhance and at the same time measure a focused on DNA particle. For at least one explicit arrangements in a DNA test, Real Time-PCR empowers both discovery and evaluation [11]. The methodology follows the general guideline of polymerase chain response; its key component is that the enhanced DNA distinguished as the response advances progressively [12]. Two normal strategies for identification of items continuously PCR are: (l) vague fluorescent colors that intercalate with any twofold stranded DNA, and (2) grouping explicit DNA tests comprising of oligonucleotides that are named with a fluorescent journalist which grants location simply after hybridization of the test with its correlative DNA target (13).

Quantification:

Real-time PCR can used to evaluate nucleic acids by two normal strategies: relative measurement and outright evaluation. Relative measurement dependent on inward reference qualities to decide crease contrasts in articulation of the objective quality. Outright evaluation gives the specific number of target DNA atoms by correlation with DNA norms. A usually utilized strategy for DNA evaluation by continuous PCR depends on plotting fluorescence against the quantity of cycles on a logarithmic scale. An edge for location of DNA-based fluorescence is set somewhat above foundation. The quantity of cycles at which the fluorescence surpasses the edge called the cycle edge, Ct. During the exponential enhancement stage, the grouping of the DNA target copies each cycle. Be that as it may, the productivity of enhancement is regularly factor among groundworks and templates [14].

The cycle edge strategy makes a few presumptions of response component and has a dependence on information from low sign to-commotion areas of the intensification profile that can present significant difference during the information examination. To evaluate quality articulation, Ct of RNA/DNA separates the Ct for a RNA or DNA from the quality of enthusiasm from a housekeeping quality in a similar example to standardize for variety in the sum and nature of RNA between various examples. This standardization methodology is ordinarily called the ΔΔCt-strategy and grants examination of articulation of a quality of enthusiasm among various samples [15]. Component based PCR measurement techniques as proposed, and have the bit of leeway that they don't require a standard bend for evaluation. An expansion of this methodology incorporates a precise model of the whole PCR response profile, which takes into consideration the utilization of high sign to-commotion information and the capacity to approve information quality preceding analysis [16].

Procedure:

The reaction mix was prepared according to the table2 below, 15µl of these mix reagents was added into each sterile vial. 5 µl of diluted DNA template (*Mycobacterium tuberculosis* DNA) was added into these vials. Water was added up to 25µl to all the vials. The PCR amplified product was analyzed by the obtaining graphs (Ct values) as it shown in the result.

Table2 showing the component and volume of the reaction mix

Component	Volume (µl)
2 x Precision TM Master Mix	10 µl
TB Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	15 µl

Results

Table3 showing the gold nanoparticle (Au NP) and Silver nanoparticle (Ag NP), in different concentrations (l0µl, 50µl and 100µl) of each one respectively with control sample and Ct value for all sample

Nanoparticle	Conc.(µl)	Ct Values
Au	10	17.10
Au	50	17.08
Au	100	16.73
Ag	10	17.66
Ag	50	17.54
Ag	100	17.12
Normal Control	Nil	21.77

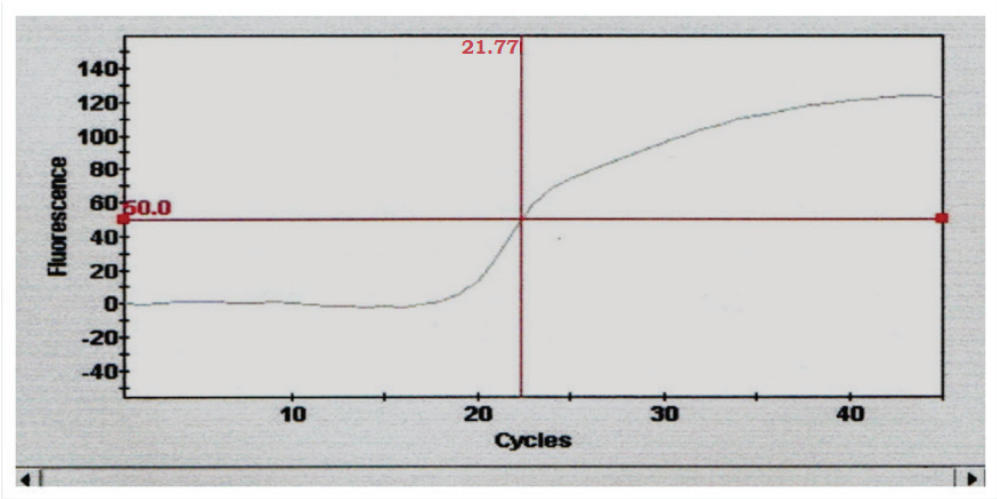

Figure1: The above graph obtained from real time PCR shows high Ct value (21.77) for control sample i.e. culture sample incubated without any nanoparticle. High Ct value corresponds to less amount of DNA.

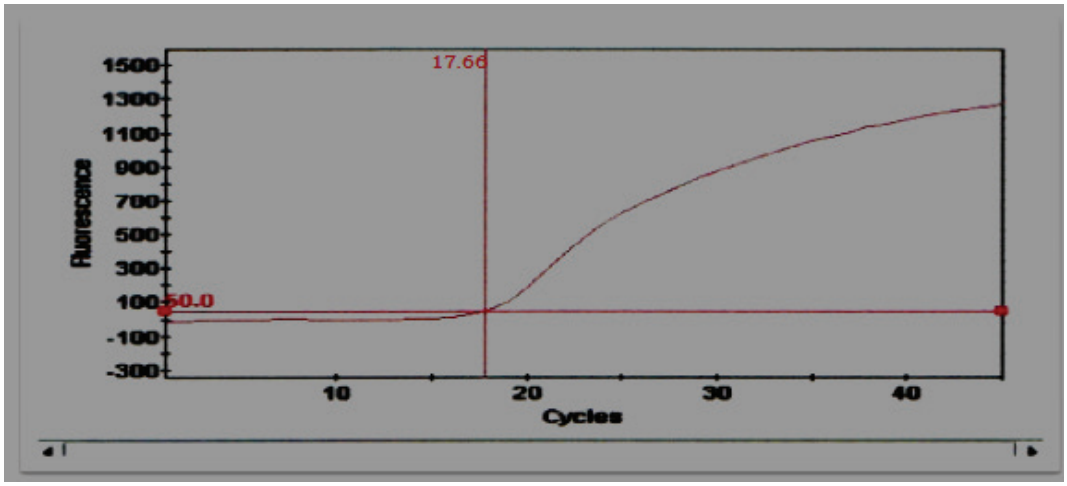

Figure 2: The above graph obtained from real time PCR shows Ct value (17.66) for culture sample incubated with 10 µl of silver nanoparticle. Low Ct value corresponds to more amount of DNA compared to control.

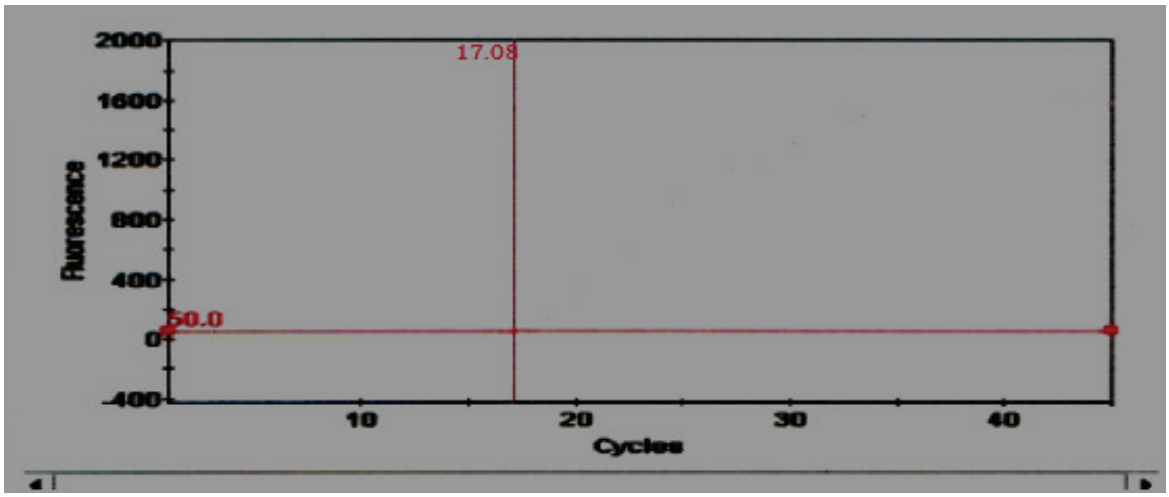

Figure 3: The above graph obtained from real time PCR shows Ct value (17.08) for culture sample incubated with 50 µl of gold nanoparticle. Low Ct value corresponds to more amount of DNA compared to 10 µl concentration of gold nanoparticle.

Discussion

It is notable that nanoparticles of inorganic metals have solid antimicrobial impacts and numerous specialists are keen on utilizing these inorganic nanoparticles as antimicrobial operators The system of the inhibitory impacts of nanoparticles on microorganisms is in part known. A few examinations have revealed that the positive charge on the nanoparticle particle is vital for its antimicrobial action through the electrostatic fascination between negative charged cell film of microorganism and positive charged nanoparticles. Conversely, Sondi and Salopek-Sondi detailed that the antimicrobial movement of silver nanoparticleson Gram-negative microscopic organisms was reliant on the convergence of Ag nanoparticle, and was firmly connected with the development of "pits" in the cell mass of bacteria [18]. The antimicrobial impact of silver is subject to shallow contact, in that silver can repress enzymatic frameworks of the respiratory chain and change DNA blend. Thus, the mechanism of inhibition is not clearly understood. Since the observed Ct value in the samples where nanoparticles were used is low compared to normal values, there could be a possible effect of nanoparticle in the yield of DNA in MTB. This possible could be because of the interaction of the nanoparticles used with the cell wall and cell membrane of bacterium which has facilitated the yield of DNA. Further refinement can be done to the above said method and the nanoparticles could possibly be used as an effective component of Nucleic acid extraction kits for molecular diagnosis and research so that a better yield is obtained.

Conclusion

In this experiment, when Au and Ag NanoParticles showed considerable effect on the DNA yield of *Mycobacterium tuberculosis* (MTB), especially gold nanoparticles. When DNA of MTB was extracted, using nanoparticles high yield of DNA was obtained when compared to the DNA extracted without any of nanoparticles. With the graph obtained from real time PCR it became very clear as the control (MTB without any nanoparticle) showed high Ct value i.e. 21.77 which means very low amount of DNA was obtained. Whereas for samples containing nanoparticle Ct values were low showing high yield of DNA. Further, the Ct values decreased when concentration of nanoparticles increased. When compared between Ag and Au nanoparticles, Au nanoparticles showed better result with low Ct value compared to Ag nanoparticle. In this experiment, gold nanoparticle was most effective at 100µl concentration with least Ct value i.e. 16.73. At last to conclude from above experimen it's very clear that nanoparticles are showed high yield of MTB DNA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of

both MOH and MOHSER in Iraq

Conflict of Interest: Non

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