A Cytotoxic evaluation by HET CAM on Chick embryo and Antimicrobial assay of Silver Nanoparticles synthesized from Green Tea Extract

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Abstract

Nanotechnology is a type of technology that allows for the control, manipulation, research, and formation of structures and devices with "nanometer" scale dimensions. The burgeoning field offers the opportunity to design and develop a multifunctional device that can be used to target, diagnose, and treat lethal diseases. These silver nanoparticles can be made in a variety of ways. Green tea extracts-based silver nanoparticles have higher stability and here green tea also act as capping agent it means; green tea may produce coating upon silver nanoparticles. These green tea extract reduced silver nanopartilcleshave been widely used in various pharmaceutical applications and as a carrier due to their non-reactive nature, stability, high disparity, non-cell toxicity, and biocompatibility. Because silver has antibacterial efficacy, and green tea also then nanopartilces synthesized from green tea extract may produce better anti-bacterial effect

Keywords

Nanoparticles, Reducing agent, NPs, Biocompatible, Antibacterial

Introduction

These nano-structured objects, such as "nanoparticles (NPs)," acquire unique properties and functionalities not found in items made of identical materials[1,2]. Because of their smaller size, higher surface area, increased solubility, and numerous functions, nanoparticles (NPs) continue to open new biological doors and can be used in new applications[2,3,4]. Green nanotechnology is defined as a technique used to create clean and environmentally friendly technologies with the goal of reducing human health and environmental risks[5,6,7]. Medicinal, preservative, and antibacterial, action of silver have been known for many years. Since the nineteenth century, silver-based formulations have been used for wound healing, burns and bactericidal property [8,9]. Catechins are polyphenolic flavanoids that are found in both green and black tea and act as a reducing agent. There are many types of catechins found

in tea, including epicatechin(EC), epicatechin gallate(ECG), and epigallocatechin gallate(EGCG), all of which have antioxidant and anti-inflammatory properties, so whole tea extract can be used for their medicinal value as well as a green reducing agent in the synthesis of a variety of nanoparticle[8,9,10]. Being green and having larger surface area silver nanopartilces can be used in taergeted drug therapy with less side effects. Silver has antibacterial property and when it combines with green tea polyphenols it may enhance the antibacterial efficacy because in this type of synthesis green tea work as reducing agent as well as capping agent. Silver nitrate solution turns milky white to, this color change indicates that silver nanoparticles has been prepared. Zeta sizer and SEM used for particle size determination as well as size and shape morphology of nanopartilces[11,12,13]. There are several methods are available for determination of antibacterial efficacy of formulation one of them is Disc diffusion method, it is economical and less interpretation get easy by using this method. HET-CAM is an alternate assay of draize test or Rabbit eye test for irritation testing, it is also economical and less time consuming process. In this method chik embryo is used to check the irritancy potency of formulation for about 300 sec after application of formulation [14,15]

Material and Methods:

Green method is used for synthesis of silver nanoparticles, Silver nitrate purchased from Loba Chemicals and green tea purchased from local market of shimla, H.P.

Following step are involved for synthesis of silver nanoparticles are given below:

- Prepare 0.05M solution of silver nitrate in distilled water and divide it in 13 test tube contains 1ml in each dilute silver nitrate solution with distilled water up to 10 ml
- Take 100 ml of distilled water in beaker and place it on heating mantle, heat the water and diffuse tea bags for 10 mins and then remove it from beaker
- Add different volume of green tea extract in silver nitrate solution to get desired silver nanopartilces
- Solution turns from milky white to brown indicates nanoparticles has been prepared

Characterization of Nanoparticles

Particle size: Using a zetasizer, the particle size and zeta potential of nanoparticles were determined (Malvern, U.K). A sample solution of nanoparticles in a cuvvette was taken after being treated with an ultrasonc water bath to break down the aggregrates present in the formulation. All measurements were taken in triplicate[16,17].

Shape and surface Morphology: The surface morphology of nanoparticles was studied using scanning electron microscopy. The nanoparticles were mounted directly on the scanning electron microscopy stub with double-sided, sticking tape that was coated with platinum and scanned in a high vacuum chamber with a focused electron beam, secondary electrons emitted from the samples were detected, and the image was formed[18,19].

FTIR Spectra: Confirmation of the silver nanoparticles was done by using Fourier Transmisaion Infra Red spectroscopy. It was done to identify which functional group is responsible for stabilty of prepared nanoparticles from greent tea extract.

HET-CAM assay

A HET-CAM assay can be used to determine the toxicity or skin iriitation of formulation in place of Draize test, it is economical and quick test. In this test fertilized egg were purchased from a poultry farm, kept these eggs in an incubator at 37°C for proper incubation insuring that air sac is upright. Using sterile method albumin of the egg removed out from th egg at 3rd day of expermint and closed the egg opening by using parafilm. Allowed the development of the chorioallantoic memebrane(CAM). At 10th day when the membrane developed completely, made an casement of 2*2cm^2 and 0.5 ml of silver nanoparticles inserted over the CAM , after 20 sec rinsed the membrane with 5ml of warm saline. Examined the membrane vascular drainage, haemorrhagic damge and coagulation for upto 300 sec .

Scores= [301-H/300]*5 + [301-L/300]*7+[301-C/300]*9

Where H= Haemorrhage (Sec), L=Lysis Time(Sec), C= Coagulation Time(Sec)

Table:1 Scoring Chart for HET-CAM test

Score Range	Interfrence
0-0.9	Non irritant
1-4.9	Slighly irritant
5-8.9or 5-9.9	Moderate irritant
9-21or 10-21	Strong irritant

Microbial Strains and Culture Conditions.

Bacterial isolates were extracted from soil samples, which were collected from different geographical areas in Himachal Pradesh,such as Solan, Baddi, and all other pharmaceutical industrial area. Such areas have been known to be contaminated with metal, different acids etc. The bacteria were isolated by plating dilutions of soil samples in saline solution (0.9% solution of NaCl) on nutrient agar and incubated for 48 hours at 28°C. Individual colonies of bacteria which varied in shape and color were chosen and purified by streaking on nutrient agar. The bacterial isolates were identified using the VITEK 2 GN ID and VITEK 2 system. A total of 25bacterial isolates were collected, purified, and identified to the species level. Of the 25 bacterial isolates, three isolates were selected for the present study, namely, *Escherichia hermannii*(isolate SHE), *Citrobacter sedlakii* (isolate S11P), and *Pseudomonasputida* (isolate S5). *Pseudomonas* selective medium(cetrimide agar) was used in isolation and identification of *Pseudomonas* isolates. Ethylene Methylene Blue medium was used to isolate and verify the bacteria.

Antimicrobial Susceptibility Test.

Antimicrobial activities of biosynthesized silver nanoparticles from green tea extract were tested against the following strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC25922, and *Staphylococcus aureus* ATCC 29213, using the agar diffusion assay method using 10 μL of sample nanoparticles solution (0.0001mg). Briefly, bacterial lawns of bacterial test strains and hospital isolates were made on ready made Muller-Hinton agar and left to dry. The sterile discs were loaded with 10 μL silver nanoparticles solution containing

approximately 0.0001 mg silver nanoparticles and then placed on Muller-Hinton agar nutrient agar plate with bacterial lawns and allowed it for incubation at37°C for 24 hours. All experiments were done in aseptic condition in laminar air flow cabinet. Zones of inhibition for control, SNPs, and antibiotics were measured in millimeters

Results and Discussion

Particles size and PDI: Particle size of silver nanopartilcles was found 74.96 nm with PDI 0.261 It is reported in previous research that -30 to +30 mv zeta potential indicates stability due to presence of highly negatively charged particles as shown in the figure:

Zeta potential: The surface charge of the nanoparticles was measured by the electrophoretic mobility of noble metal nanoparticles in U type tube at 25ºC using Zetasizer.The zeta potential of optimized gold nanoparticle formulation was found to be – 12.8 mv which. Indicated formulation is stable.

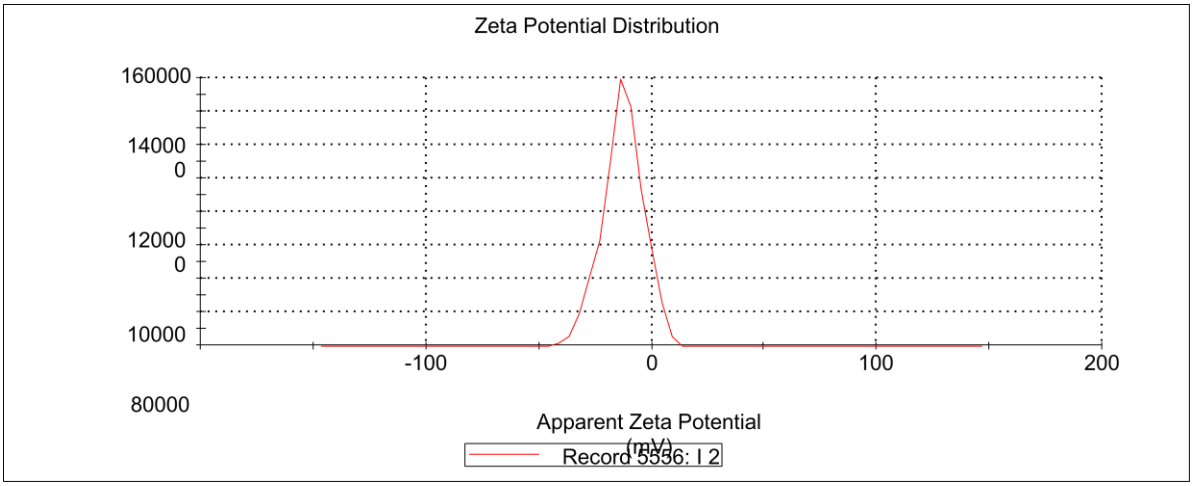


Figure 1: Zeta potential of Silver Nanoparticles

Shape and Surface morphology SEM analysis was used to determimme the shape and surface morphhology. They were spherical in size and have a smooth surface without pores and cavities.

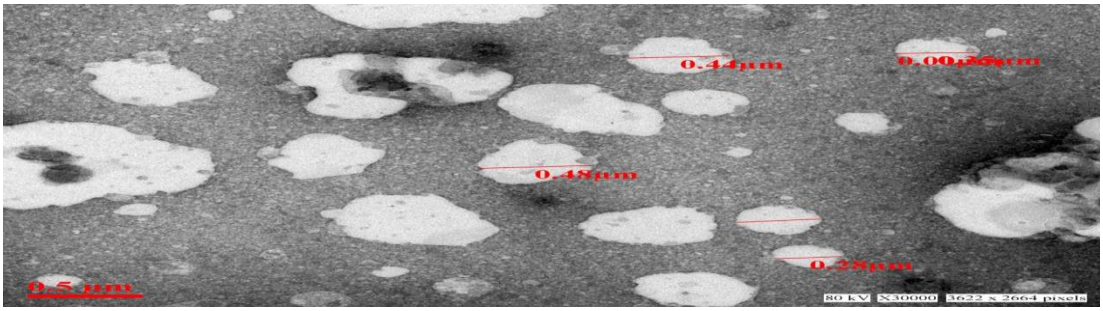


Figure 2: SEM analysis of Silver Nanoparticles

FTIR Spectra of Silver nanoparticles

The probable moieties involved in encapsulating and reducing agents for the Ag nanoparticles generated by green tea extract were identified using FTIR measurements. At 3319 cm-1, 1635.17 cm-1, and 576 cm-1, three distinct infrared signal may be seen. The O-H and N-H stretched modes in the protein connection are responsible for the extreme bandwidth at 3319 cm-1.The C=O bending pattern in the amine I class, which is usually located in proteins, causes the medium intensity band at 1635 cm-1, showing the existence of

proteins as a capping agent for silver nanoparticles, which enhances the stability of the nanoparticles generated. The powerful and wide peak at 575 cm-1, on the other hand, is related to the Ag metal as shown in the figure:

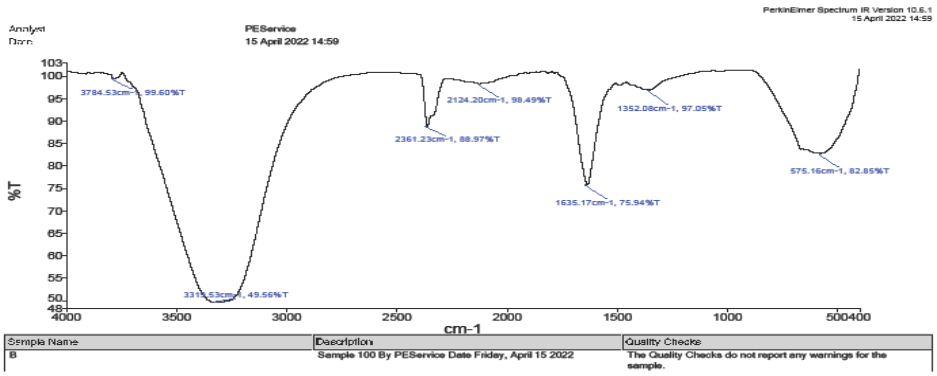
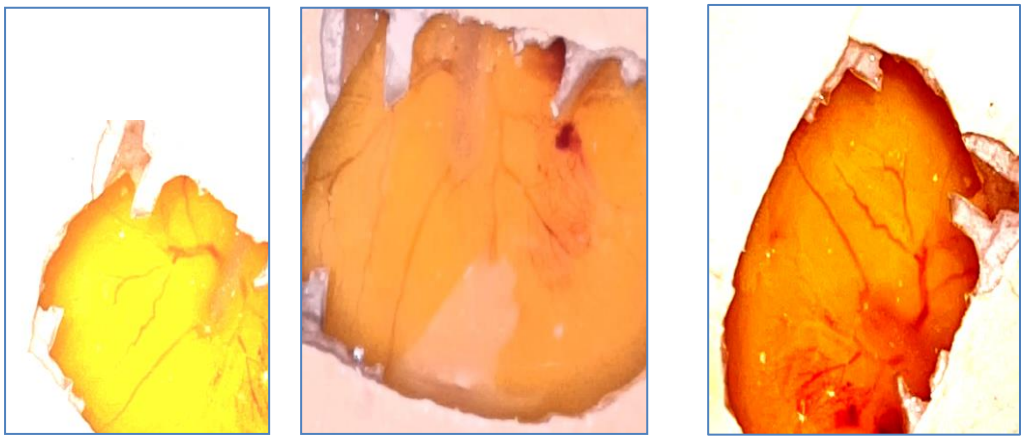


Figure 3: FTIR Spectra of silver nanopartilcles

HET-CAM Assay

A very quick method to determine the irritation of any pharmaceutical formulation intended for topical application, it is reliable and economical method. CAM of chick embryo consist, capillaries, veins and arteries, which respond for any inflammatory mediators. The irritation score was found to be in 0.45± 0.02 it is indicated that the silver nanoparticles are not irritant



(a) **(b)** **(c)**

Figure 4: HET-CAM images (a) normal saline, (b) Silver nanopartilces (15 seconds) , (c) Silver nanopartilcles (30 seconds)

Antimicrobial assay by disc diffusion method Antimicrobial assay of silver nanopartilcles was performed for three different bacterial cultures and it was found that silver nanopartilcles showing antibacterial efficacy in all three strains one drug named silver sulphadiazine is used as reference standard. Maximum antibacterial effect of silver nanoparticles was shown towards *Pseudomonas aeruginosa* bacterial strain. The table of zone of inhibition is given below:

Table 2: Antimicrobial assay Data

Pseudomonas aeruginosa		Escherichia coli		Staphylococcus aureus		STANDARD	
radius	area(mm)	radius	area(mm)	radius	area(mm)	radius	area(mm)
13.1	538.85	11.4	408.07	8.1	206	15.8	783.86
13.2	547.11	11.5	415.26	8.3	216.31		
13	530.66	11.2	393.88	8.1	206		
13.2	547.11	11.1	386.87	8.4	208		
13	530.66	11.2	393.88	8.2	211.13		
AVG	538.878		399.592		209.488		

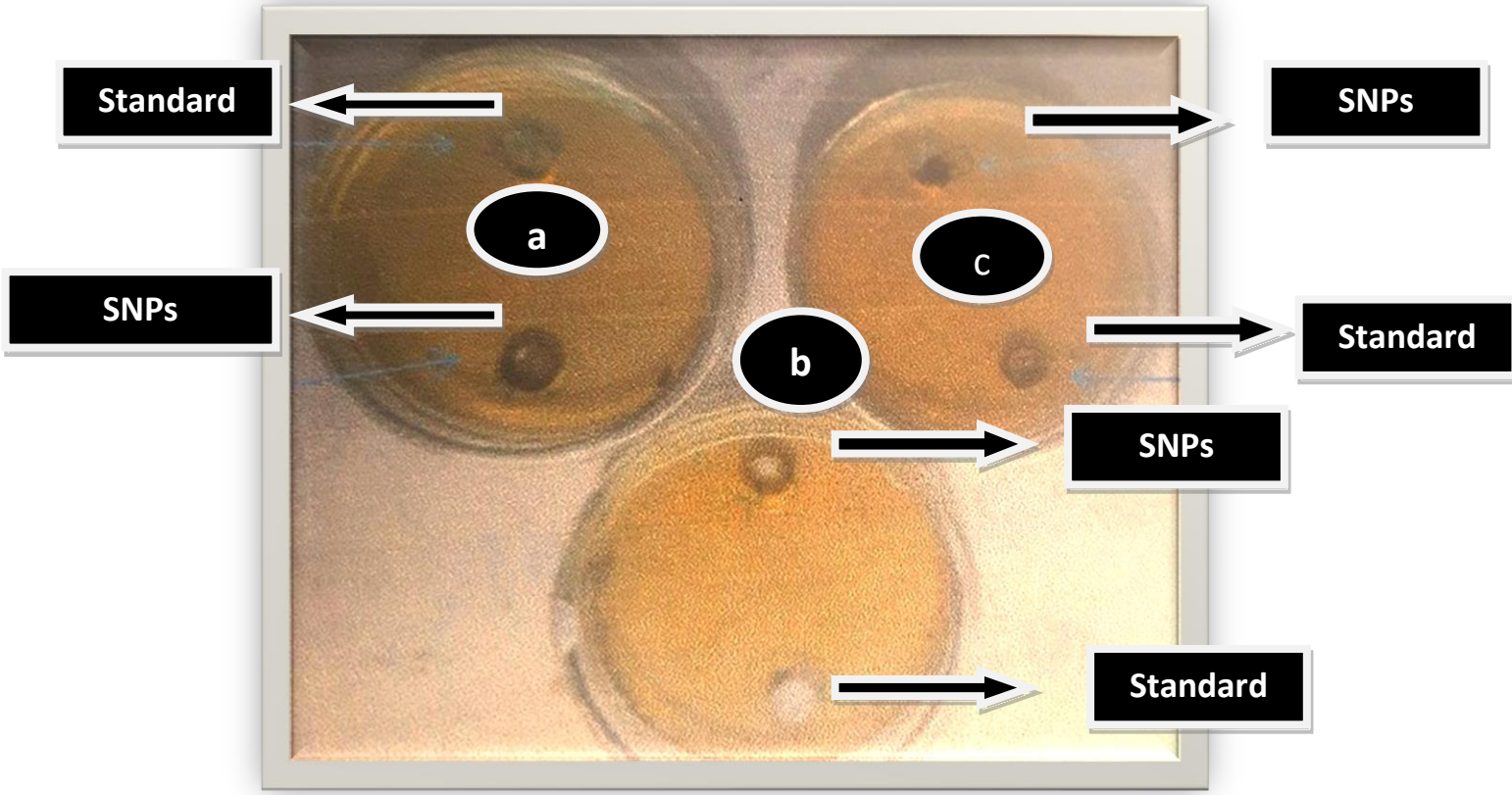


Figure 5: Zone of Inhibition data of SNPs (Silver nanopartilcles) and standard formulation in different bacteria medium a (Pseudomonas aeruginosa) b (Escherichia coli) c (Staphylococcus aureus)

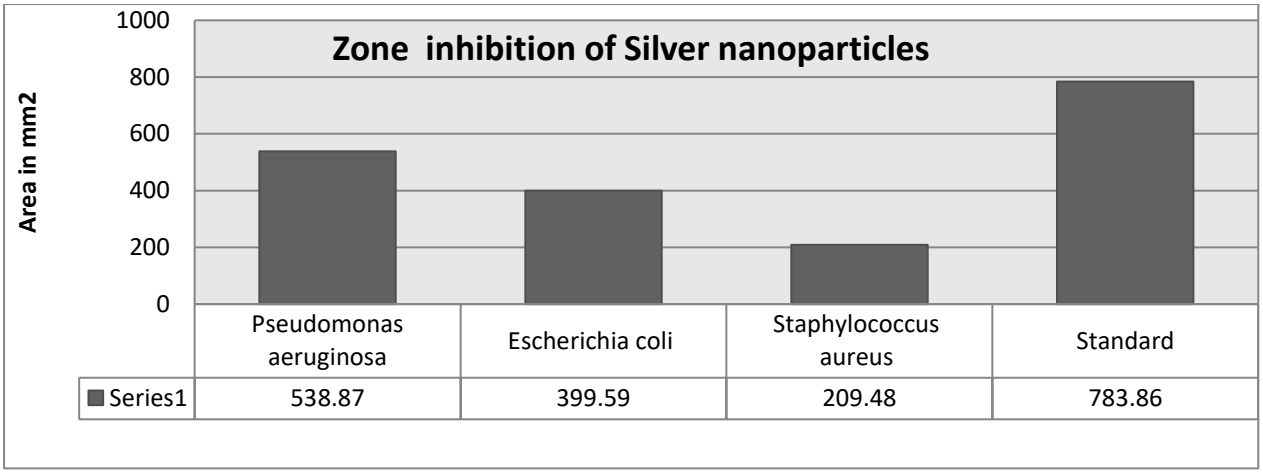


Figure 6: Histogram Showing Zone of Inhibition

Discussion

The best method for synthesis of silver nanoparticles was green method in which green tea extract used as reducing agent as well as capping agent, there is no need to add any stabilizing agent. It was economical, environmental friendly, less time consuming and one step synthesis. Silver nitrate is the precursor for synthesis of nanoparticles. Nanoparticles larger surface area. that's why they can reach to the small space or smaller tissue and can also be use in target drug delivery. Green tea extract reduced silver nanoparticles shown better antibactrial effect and also found smooth and equal nano sized particles on SEM analysis. In HET-CAM assay it was found that green tea extract reduced silver nanoparticles are non-irritant. it was revealed that green tea extract reduced silver NPs have better antibacterial effect and nonirritant property so these can also use for further formulation development.

Conclusion

During this study we concluded that green tea synthesized silver nanoparticles produced good antimicrobial effect and it is very economical method to produce metallic nanopartilcles. HET-CAM study has proved beneficial in cytotoxic evaluation without using animal. In future these nanopartilcles would be beneficial in targetting of several fatal disease**.**

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