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RESEARCH ARTICLE

Antibacterial and Anticancer Activity of Silver Nanoparticles Synthesized from *Cynodon dactylon* Leaf Extract

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Abstract

Silver nanoparticles were synthesized using *Cynodon dactylon* leaf extract and this study demonstrates the efficacy of biologically synthesized Silver nanoparticles (AgNPs) as anticancer and antibacterial agent. Anticancer activity of the biologically synthesized silver nanoparticles was studied using HEpG-2 cell line. The AgNPs showed dose-dependent cytotoxicity against HEpG-2 cells. Antimicrobial activity of the synthesized nanoparticles was tested against *Escherichia coli, Staphylococcus aureus, Micrococcus lutues and Salmonella typhimurium.* The zone of inhibition increased with the increase in the concentration of silver nanoparticles. Antimicrobial activity and anticancer activity of the green synthesized silver nanoparticles proves the potential of silver nanoparticles in the area of nanomedicine.

Keywords: *Cynodon dactylon*, silver nanoparticles, anticancer, antibacterial, nanomedicine.

Introduction

Noble metal nanoparticles have been the subject of intense research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. These properties are attributed to their small sizes and large surface areas. Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses (Forough and Farhadi, 2010). Research in the silver nanoparticles has become vital owing to their applications in various areas. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology and distribution (Awwad *et al*., 2013). Silver nanoparticles have potent antibacterial, antifungal and larvicidal properties (Gnanadesigan *et al*., 2012). Resistance of bacteria to bactericides and antibiotics has increased and hence there is a growing need to formulate safe and cost-effective antimicrobial agents. Previous studies show that antimicrobial formulations of silver in the form of nanoparticles could be used as effective bactericidal materials (Rajawat and Qureshi 2012). Cancer is an abnormal type of tissue growth in which the cells exhibit and uncontrolled division leading the increase in the number of dividing cells (Kanchana and Balakrishna, 2011). The discovery and identification of new anticancer drug with low side effects on immune system has become an active area of research in the field of immunotherapy (Xu *et al.,* 2009). Multi-drug resistance is still considered as a major drawback in chemotherapy of cancer which has been the subject of many experiments recently. Silver nanoparticles have been investigated for the cytotoxic activity and the silver nanoparticles showed different degrees of *in vitro* toxicity (Devi *et al*., 2012).

Considering the above facts, this study was aimed to investigate the efficacy of nanoparticles synthesized using *Cynodon dactylon* leaf extract as a potent antimicrobial and anticancer agent under *in vitro* conditions.

Materials and methods

Preparation of leaf extract by homogenization method: The extract used for the synthesis of silver nanoparticles was prepared by taking 20 g of thoroughly washed and finely cut *Cynodon dactylon* leaves (Fig. 1) with 200 mL of distilled water. The suspension was homogenized. The homogenized suspension was centrifuged and the supernatant was collected. The extract obtained was filtered through Whatman No. 1 filter paper. The filtrate was collected and stored at 4°C for further use.

Fig. 1. *Cynodon dactylon.*

Table 1. Antibacterial activity by disc diffusion method.

Organisms	Zone of inhibition (mm)			
	250 µg AgNP	500 µg AgNP	750 µg of AgNP	1000 µg of AgNP
Escherichia coli	12	15	19	20
Staphylococcus aureus	10	11	13	16
Micrococcus lutues	15	18	20	21
Salmonella typhimurium	20	21	22	23

Table 2. Anticancer effect of silver nanoparticles synthesized from *C. dactylon* leaf extract on HEpG-2 cell line.

S.No.	Conc. (µg/mL)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.09	19.5
2	500	1:1	0.13	28.2
3	250	1:2	0.21	**45.6**
4	125	1:4	0.26	56.5
5	62.5	1:8	0.32	69.5
6	31.2	1:16	0.35	76.0
7	15.6	1:32	0.41	89.1
8	Cell control	-	0.46	100

Synthesis of silver nanoparticles: Silver nanoparticles were synthesized using *Cynodon dactylon* leaf extract based on Supraja *et al*. (2013). Different optimization studies were carried out for the synthesis of silver nanoparticles from *Cynodon dactylon* leaf extract. The optimization studies indicated that sunlight exposure method and homogenized extract solution were best for the production of silver nanoparticles.

Antibacterial activity: Test pathogens viz., *Escherichia coli, Salmonella typhii, Staphylococcus aureus* and *Micrococcus luteus* were obtained from Armats Biotek Training and Research Institute, Chennai, Tamil Nadu, India. Nutrient agar was prepared and poured in petri dish, 24 h growing culture were swabbed on it. The wells (10 mm dia) were made by using cork borer and different concentrations of the silver nanoparticles (250, 500, 750 and 1000 µg) were loaded in the wells. The plates were then incubated at 37ºC for 24 h. The inhibition diameter was then measured.

In vitro assay for cytotoxicity activity (MTT assay): The cytotoxicity of samples on HEpG-2 was determined by the MTT assay cells (1×10^5/well) were plated in 1 mL of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 h incubation, the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48 h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200 µL/well (5 mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4 h incubation, 0.04 M HCl/isopropanol was added. Viable cells were determined by the absorbance at 570 nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically.

The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HEpG-2 was expressed as the % cell viability, using the following formula:

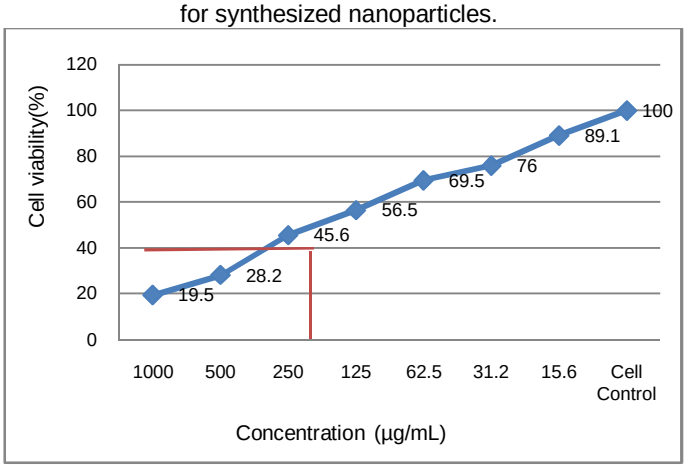
% cell viability = A570 of treated cells/A570 of control cells × 100%.

Results and discussion

Antibacterial activities of silver nanoparticles evaluated against the microorganisms are presented in Table 1. The maximum zone of inhibition was recorded for *Salmonella typhimurium.* From the Table 1 it can be observed that the zone of inhibition increased as the concentration of the silver nanoparticles increased. The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well known. The different mechanism by which the silver nanoparticle inhibits cell wall synthesis is by interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis and inhibition of a metabolic pathway. As the concentration of the silver nanoparticles increased the zone of inhibition increased (Guzman *et al*., 2012). It has been reported the greater the zone of inhibition, greater the antibacterial properties of the silver nanoparticles and that antibacterial effect was size and dose dependent (Devi *et al*., 2012). The inhibition of bacterial growth reported in this study is dependent on the concentration of silver nanoparticles in the medium.

Cytotoxicity of the silver nanoparticles was evaluated *in vitro* against HEpG-2 cell line. Cytotoxic analysis of the sample showed a direct dose response relationship and cytotoxicity increased at higher concentrations. The sample demonstrated a considerable cytotoxicity against HEpG-2 cell line. The concentration necessary to produce 50% death rate was 45.6 µg/mL. Anticancer activities of the silver nanoparticles evaluated against HEpG-2 cell line are presented in Table 2 and Fig. 2.

Fig. 2. Cell proliferation using MTT for synthesized nanoparticles.



In this study, it was observed that the synthesized AgNPs induces a concentration dependent inhibition of HEpG-2 cells. Cytotoxic effects of silver nanoparticles was probably due to the fact that SNPs may interfere with the proper functioning of cellular proteins and induce subsequent changes in cellular chemistry provide a relatively high hydrophobicity inside bovine hemoglobin which causes a transition from alpha helixes to beta sheets and leads to partial unfolding and aggregation of the protein. Other reports suggest that SNPs are likely to interact with thiol-rich enzymes. Therefore, it is possible that once penetrated into cells, SNPs may attack functional proteins of cells which results in partial unfolding and aggregation of proteins as it is the case in the bovine hemoglobin (Shawkey *et al*., 2013).

Conclusion

Biosynthesized silver nanoparticles showed excellent antimicrobial activity and possessed considerable cytotoxic effect against HEpG-2. This study explores the potential use of nanoparticles as alternative medicine. Therefore, further studies are needed to fully characterize the toxicity and the mechanisms involved with the antimicrobial and anticancer activity of these particles.

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